

Composition, propriétés et préservation des substances exopolymériques dans les sédiments estuariens : implications pour les cycles biogéochimiques et la diagenèse précoce

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Université Bordeaux Montaigne

École Doctorale Montaigne Humanités (ED 480)

THÈSE DE DOCTORAT EN SCIENCES DE LA TERRE

Composition, properties and preservation of Exopolymeric Substances in estuarine sediments: implications for biogeochemical cycles and early diagenesis.

Présentée et soutenue publiquement le 08 juillet 2022 par

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Résumé

Des biofilms benthiques se développent à la surface des zones intertidales dans les estuaires. Ces biofilms correspondent à une association de microorganismes principalement photoautotrophes, principalement des diatomées, associés entre eux et fixés à la surface des sédiments grâce à la sécrétion d'une matrice extracellulaire adhésive composée de substances exopolymeriques (EPS). Les EPS sont constituées d'un assemblage de molécules de différentes tailles, principalement des polysaccharides et des protéines, ayant des propriétés physicochimiques variables. Certaines de ces molécules possèdent une forte réactivité, une capacité à interagir avec des éléments (cations, molécule d'eau, etc.) de l'environnement, ce qui permet aux EPS d'assurer de nombreux rôles pour la communauté microbienne constituant le biofilm : protection contre la dessiccation, protection contre certains polluants et pathogènes, fixation au substratum et migration dans les sédiments, accumulation de nutriments issus de l'environnement, limitation de l'érosion, etc. Ainsi les diatomées à travers la production d'EPS sont de plus en plus considérées comme des bio-ingénieurs des écosystèmes. Les EPS sont produites principalement en surface et sont progressivement dégradées et consommées par des microorganismes hétérotrophes en profondeur. Bien que les propriétés des EPS des biofilms de surface soient maintenant bien connues, leur préservation et leur évolution durant l'enfouissement et au cours de la diagenèse précoce restent méconnus.

Dans cette thèse, j'ai caractérisé les propriétés physico-chimiques des EPS extraites de biofilms à diatomées, ainsi que de sédiments du point bar fluvio-estuarien de Bordeaux-Nord, dans l'estuaire de la Gironde. Les propriétés des EPS ont été ensuite analysées dans une carotte sédimentaire de 6 mètres de profondeur afin d'évaluer leur préservation. Ces résultats sont ensuite comparés (i) aux propriétés des sédiments (faciès, granulométrie, carbone organique total, etc.), (ii) à des mesures chimiques des eaux porales, ainsi qu'à (iii) des mesures des activités métaboliques et enzymatiques microbiennes. L'objectif principal est d'établir un modèle géomicrobiologique de l'évolution des sédiments estuariens au cours de la diagenèse précoce incluant pour la première fois le rôle des EPS. Une fois les EPS des biofilms de surface caractérisées, j'ai testé en laboratoire leur rôle dans l'agrégation des sédiments détritiques (minéraux argileux et sable).

Dans l'estuaire de la Gironde, des EPS réactifs (i.e., acides) sont produites abondamment en surface des sédiments par les biofilms à diatomées. Ces EPS sont composées de polysaccharides

et de protéines qui comportent des fonctions chimiques réactives comme des acides carboxyliques. La quantité et la réactivité des EPS diminuent dans les premiers décimètres du sédiment. Cette diminution coïncide avec le maximum d'activité métabolique microbienne, indiquant leur probable dégradation par des organismes hétérotrophes. Néanmoins, certains horizons profonds de la Long Core (à plus 4 mètres de profondeur) ont fourni des EPS en concentration et ayant une acidité comparable aux biofilms de surface. Cette préservation pourrait s'expliquer par des conditions sédimentaires particulières. Une période de sédimentation à dominante tidale pourrait ainsi permettre le développement du point bar ainsi qu'une stabilité des zones intertidales sur des échelles pluri-décennales. Cette zone étendue et stable permettrait le développement des biofilms et la production d'EPS. A l'inverse, une période dominée par les crues fluviales induirait une migration du point bar, une érosion plus importante des zones intertidales, impliquant une préservation limitée des EPS. D'importantes variations de la concentration d'éléments (oxygène, nitrate, sulfate, manganèse et fer) nécessaires au métabolisme microbien sont observées dans le premier mètre des sédiments ainsi qu'une activité microbienne élevée. Dans le sédiment, les métabolismes microbiens sont stratifiés selon un gradient de métabolites fournissant une énergie décroissante lors de leur réduction : oxygène, nitrate, sulfate, manganèse et fer. Dans les carottes étudiées, l'activité microbienne est fortement réduite en dessous du premier mètre, ce qui coïncide avec la diminution des accepteurs d'électrons potentiels. De ce fait, les EPS, qui servent de source de carbone organique aux microorganismes hétérotrophes, ont un plus fort potentiel de préservation à des profondeurs supérieures à un mètre. Les résultats des gradients géochimiques de l'eau porale expliquent donc probablement en partie la préservation des EPS. Les métabolismes microbiens impactent également la géochimie du sédiment et des eaux porales (pH, oxydo-réduction, etc.), créant les conditions pour la précipitation de phases minérales métalliques métastables, telles que des sulfures ou des oxydes. Des modélisations de précipitation-dissolution sous phreeQC montrent que de telles phases ont le potentiel de précipiter dans certains horizons sédimentaires : la mackinawite ou la pyrite dans la zone de réduction des sulfates (entre 4 et 18 cm de profondeur) ou la ferrihydrite ou l'hématite dans des zones plus profondes (entre 18 et 35 cm de profondeur) via des processus de ré-oxydation du sédiment. Dans les environnements carbonatés, les EPS sont connues pour favoriser (ex. silicates magnésiens) ou inhiber (ex. carbonates) la précipitation de phases minérales. Dans les sédiments étudiés, les seuls minéraux authigènes observés sont de rares pyrites, impliquant un potentiel rôle inhibiteur de ces EPS. En revanche, mes observations en cryo-microscopie électronique ont montré que les EPS forment des complexes organo-minéraux avec les

sédiments estuariens (sables, silt, mica et argile), et que ces complexes peuvent être préservés de la surface du sédiment jusqu'à au moins 6 mètres d'enfouissement. Nous avons mené une série d'expériences en laboratoire impliquant des mélanges entre des EPS purifiées de l'estuaire de la Gironde et des minéraux standards (argiles et quartz). Nos expériences ont résulté en la formation d'enveloppes d'argiles et d'EPS autour des grains de sables, autrement appelés clay-coats détritiques, et ont démontré le rôle de colle organique joué par les EPS dans les sédiments détritiques. Ces clay-coats ont été retrouvés dans de nombreux grès estuariens anciens.

Les EPS préservées en profondeur ont ainsi un fort potentiel pour la cohésion des sédiments, mais pourraient également jouer un rôle dans la précipitation de phases minérales pendant la diagenèse d'enfouissement. Les résultats de cette étude peuvent servir de base à de futures études sur les rôles des EPS dans d'autres environnements sédimentaires détritiques. Enfin, ces résultats apportent de nouvelles idées pour l'interprétation des processus sédimentaires anciens, des transformations minérales diagénétiques et dans la recherche de biosignatures.

Mots clés : biofilms à diatomées, substances exopolymériques, estuaire de la Gironde, carotte sédimentaire, clay-coat, sable, argile, caractérisation physico-chimique, eau porale, géochimie, métabolismes microbiens, activité microbienne, microbiologie

Abstract

Benthic biofilms develop on the surface of intertidal zones in estuaries. These biofilms correspond to an association of pirincipaly photoautotrophic microorganisms, mainly diatoms, associated with each other and attached to the surface of the sediment by the secretion of an adhesive extracellular matrix composed of exopolymeric substances (EPS). The EPS are composed of different sizes molecules, mainly polysaccharides and proteins, with variable physicochemical properties. Some of these molecules have a strong reactivity, a capacity to interact with elements (cations, water molecule, etc.) of the environment, which allows EPS to ensure many roles for the microbial community constituting the biofilm: protection against desiccation, protection against certain pollutants and pathogens, attachment to the substratum and migration in sediments, accumulation of nutrients from the environment, limitation of erosion, etc. Thus, diatoms, through the production of EPS, are more and more considered as ecosystem bioengineers. EPS are produced mainly at the surface and are progressively degraded and consumed by heterotrophic microorganisms at depth. Although the properties of EPS in surface biofilms are now well known, their preservation and evolution during burial and early diagenesis remain poorly understood.

In this thesis, I characterized the physico-chemical properties of EPS extracted from diatom biofilms, as well as from sediments of the fluvio-estuarine point bar of Bordeaux-Nord, in the Gironde estuary. The properties of the EPS were then analyzed in sediment cores up to more than 6 meters deep to assess their preservation. These results are then compared to (i) sediment properties (facies, grain size, total organic carbon, etc.), (ii) chemical measurements of pore waters, and (iii) measurements of microbial metabolic and enzymatic activities. The main objective is to establish a geomicrobiological model of the evolution of estuarine sediments during early diagenesis including for the first time the role of EPS. Finally, once the EPS of the surface biofilms were characterized, I tested in the laboratory their role in the aggregation of detrital sediments (clay minerals and sand).

In the Gironde estuary, reactive (i.e., acidic) EPS are produced abundantly on the sediment surface by diatom biofilms. These EPS are composed of polysaccharides and proteins that contain reactive chemical functions such as carboxylic acids. The amount and reactivity of EPS

decreases in the first few decimeters of the sediment. This decrease coincides with the maximum microbial metabolic activity, indicating their probable degradation by heterotrophic organisms. Nevertheless, some deep horizons of the long core (at more than 4 meters depth) provided EPS in concentration and with an acidity comparable to the surface biofilms. This preservation could be explained by particular sedimentary conditions. A period of predominantly tidal sedimentation could thus allow the development of the bar point as well as a stability of the intertidal zones on multi-decadal scales. This extended and stable zone would allow the development of biofilms and the production of EPS. Conversely, a period dominated by river floods would induce a migration of the bar point, a more important erosion of the intertidal zones, implying a limited preservation of the EPS. Important variations in element concentration (oxygen, nitrate, sulfate, manganese and iron) necessary for microbial metabolism are observed in the first meter of the sediment as well as a high microbial activity. In the sediment, microbial metabolisms are stratified according to a gradient of metabolites providing a decreasing energy during their reduction: oxygen, nitrate, sulfate, manganese and iron. In the cores studied, microbial activity is strongly reduced below the first meter, coinciding with the decrease in potential electron acceptors. As a result, EPS, which serve as a source of organic carbon for heterotrophic microorganisms, have a greater potential for preservation at depths greater than one meter. Thus, the results of pore water geochemical gradients probably explain in part the preservation of EPS. Microbial metabolisms also impact sediment and pore water geochemistry (pH, redox, etc.), creating conditions for the precipitation of metastable metal mineral phases, such as sulfides or oxides. Precipitation-dissolution modelling under phreeQC shows that such phases have the potential to precipitate in certain sedimentary horizons: mackinawite or pyrite in the sulphate reduction zone (between 4 and 18 cm depth) or ferrihydrite or hematite in deeper zones (between 18 and 35 cm depth) via sediment re-oxidation processes. In carbonate environments, EPS are known to promote (e.g. magnesian silicates) or inhibit (e.g. carbonates) the precipitation of mineral phases. In the studied sediments, the only authigenic minerals observed are rare pyrites, implying a potential inhibitory role of these EPS. On the other hand, my cryo-electron microscopy observations showed that EPS formed organomineral complexes with estuarine sediments (sands, silt, mica and clay), and that these complexes could be preserved from the sediment surface to at least 6 meters of burial. We conducted a series of laboratory experiments involving mixtures between purified EPS from the Gironde estuary and standard minerals (clays and quartz). Our experiments resulted in the formation of envelopes of clays and EPS around sand grains, otherwise known as detrital clay

coats, and demonstrated the role of organic glue played by EPS in detrital sediments. These clay-coats have been found in many ancient estuarine sandstones.

Thus, EPS preserved at depth have a strong potential for sediment cohesion, but could also play a role in the precipitation of mineral phases during burial diagenesis. The results of this study can serve as a basis for future studies on the roles of EPS in other detrital sedimentary environments. Finally, these results provide new insights into the interpretation of ancient sedimentary processes, diagenetic mineral transformations and in the search for biosignatures.

Key words: diatom biofilms, exopolymeric substances, Gironde estuary, sediment core, claycoat, sand, clay, physicochemical characterization, pore water, geochemistry, microbial metabolisms, microbial activity, microbiology

Table of contents

Thesis	plan		1
Chapto Substa	er 1: S nces i	State of the art on the Gironde estuary, benthic diatom biofilms and Exopolymen in sedimentary systems	ric 3
Resear	·ch qu	estions and objectives	3
1.1	The (Gironde estuary	6
1.1.1		Definition of estuaries	6
1.1.2	2]	Physiography and hydrology of the Gironde estuary	6
1.1.3	3	Physicochemical conditions in the Gironde estuary	7
1.1.4	1 9	Sedimentology of the Gironde estuary	9
1.1.5	5]	Bordeaux North Point Bar	11
1.1.6	5]	Mineralogy of clay minerals	15
1.1.7	7	Clay minerals reactivity	17
1.1.8	3	Properties of the clay mineral species found in the Gironde estuary	19
1.1.9)	Summary of the results of the ClayCoat Project	24
1.2	Bent	hic biofilm and Exopolymeric Substances in estuaries: state of the art	26
1.2.1		Generalities on diatoms	26
1.2.2	2 1	Microphytobenthic biofilms and exopolymeric substances	29
1.	2.2.1	Background on EPS	29
1.	2.2.2	Diatom EPS specificities	31
1.2.3	3	Benthic biofilms biological functions	33
1.	2.3.1	Adhesion to the sediment and substratum stabilization	33
1.	2.3.2	Frustule stabilization	35
1.	2.3.3	Cell motility in response to light and tidal cycles	35
1.	2.3.4	Cell protection	36
1.	2.3.5	Communication and interactions between cells	38
1.	2.3.6	Food and micronutrient accumulation source	40
1.	2.3.7	Sedimentary EPS cycling	40
1.	2.3.8	EPS-mineral interactions molecular mechanisms	41
1.3	Su	mmary and research questions addressed in this thesis	43
Refe	erence	S	47
Chapte	er 2: I	Material and methods	55
2.1	Me	ethodology and its specificities relative to the Gironde estuary	58
2.2	Co	mparison of EPS extraction procedures and quality of the purified material	62

2.3	Sediment sampling and measurements	. 67
2.3.1	Sedimentary core extraction	. 67
2.3.2	Reference mineral material	. 68
2.3.3	Surface sediment and core samples	. 68
2.3.4	FTIR spectroscopy (EPS and clay minerals)	. 69
2.3.5	Grain size analyzes	. 70
2.3.6	Measurements of microbial activity in the sediment	. 70
2.4	Water chemistry	. 71
2.4.1	Porewater sampling	. 71
2.4.2	Porewater chemical analyzes	. 72
2.4.3	Porewater geochemical modeling	. 73
2.5	EPS extraction and characterization	. 74
2.5.1	EPS extraction	. 74
2.5.2	EPS physico-chemistry: colorimetric assays	. 74
2.5.3	FTIR spectroscopy on EPS	. 75
2.5.4	EPS acid-base titration	. 76
2.5.5	EPS cation binding capacity	. 77
2.6	Microscopy	. 77
2.6.1	Cryo-scanning electronic microscopy	. 77
2.6.2	Optical microscopy: petrographic thin section and microbiological glass slides	. 78
2.6.3	Atomic force microscopy	. 79
Refere	nces	. 80
Annex	es	. 83
Ann	ex 1: EPS extraction protocol	. 83
Ann	ex 2: Alcian Blue assay protocol	. 84
Ann	ex 3: Phenol sulfuric assay protocol	. 85
Ann	ex 4 EPS acid-base titration protocol	. 86
Ann	ex 5 Fluorescein Diacetate (FDA) assay protocol	. 87
Ann	ex 6 Triphenyltetrazolium (TTC) assay protocol	. 88
Ann	ex 7 Sediments preparation for analysis and experiment	. 89
Ann	ex 8 Sediments preparation for Granulometry	. 90
Ann	ex 9 Diatom F/2 medium	. 91
Chapter 3 sediments	3: Production and preservation of Exopolymeric Substances in the Gironde estuary s	. 95
3.1	Characterization of microphytobenthic biofilm community	. 98
3.2	Preservation of exopolymeric substances in estuarine sediments	104

ABSTRACT	106
INTRODUCTION	107
Sedimentological settings	109
MATERIALS AND METHODS	112
DISCUSSION	
CONCLUSION	
ACKNOWLEDGEMENTS	
REFERENCES	
SUPPLEMENTARY MATERIAL (for Duteil et al. submitted in Frontier in	Microbiology)
3.3 Annovos	
3.3.1 Cryo-SEM and EDX complementary data for the RXN-Long Core	
3.3.2 Cryo-SEM and EDX complementary data for BXN-Box and BXN-	-Short Core, 163
Chapter 4: Impact of microbial activity on biogeochemical cycles and Exopolym	eric Substances
in estuarine sediments	
4.1 Manuscript article: Impact of microbial activity on biogeochemical cyc	les and
Exopolymeric Substances in estuarine sediments.	
Introduction	175
Materials and methods	
Results	
Discussion	204
Conclusion	220
References	
Supplementary material	231
Chapter 5: Role of diatom biofilm Exopolymeric Substances on clay coat format	ion in estuaries
5.1 Clay coats in the Bordeaux North Point Bar	241
5.1.1 Origin of detrital clay coats in modern sands	244
5.2 Experimental formation of clay-coated sand grains using diatom biofili	n exopolymers
249	
5.3 Specific EPS-clay mineral interactions	
References	
Annex	
Chapter 6: Discussion and way forward	271
6.1 Production, composition and preservation of Exopolymeric Substances	: a comparison
6.1.1 Structure and functioning of hypersoline microbial mate	۲/4
6.1.2 Composition of EDS in onon maxima and hyperseline microbial mats	
0.1.2 Composition of EFS in open marine and hypersaline microbial ma	ιτο Ζ//

6.1.3 sedimen	Comparison of EPS concentrations and physico-chemical properties in various tary environments	s 277
6.1.4	Sediment-EPS interactions: aggregation, attachment, trapping and binding	284
6.1.5	Evolution of EPS with depth in the sediment	285
6.2 EPS	S in the sediment to rock transition and their potential preservation	289
6.2.1. diagenes	EPS Relationships between EPS cycling, carbonate precipitation and early sis in hypersaline microbialites	289
6.2.2.	Role of EPS in silicate precipitation	290
6.2.3.	A potential role for EPS in the diagenesis of estuarine sandstones	292
6.2.4.	EPS-mineral interactions as biosignatures of past microbial activity	296
6.3 Per	spectives	298
6.3.1	Experiments on EPS from diatom cultures	298
6.3.2	Interactions between EPS, clay minerals and metals, and evolution through	
diagene	sis	302
References	§	303
Conclusion		313

This thesis is structured around 6 chapters:

- The first chapter "State of the art on the Gironde estuary, benthic diatom biofilms and Exopolymeric Substances in sedimentary systems. Research questions and objectives." presents the sedimentary context of the Gironde estuary, with a focus on the estuarine point bar of Bordeaux Nord. We also propose a state of art on diatom biofilms, Exopolymeric Substances (EPS) and their functions in estuaries. In a final part, we present the challenges and research questions of this thesis.
- 2. The second chapter "Material and methods" describes the workflow developed in this thesis.
- 3. In the third chapter "Production and preservation of Exopolymeric Substances in the Gironde estuary sediments", we characterize the physico-chemical properties and the preservation of EPS in a six-meter-long sedimentary core. This chapter is largely based on an article in revision in the journal *Frontiers in Microbiology*.
- 4. The fourth chapter "Impact of microbial activity on biogeochemical cycles and Exopolymeric Substances in estuarine sediments" aims to propose a conceptual geomicrobiological model of the estuarine sediments. To this aim, we characterized (i) pore water and sediment chemistry, (ii) metabolic and enzymatic activities and (iii) EPS physico-chemical properties, in three cores. This chapter includes a draft of a paper to be submitted to *Geobiology*.
- 5. The fifth chapter "Role of diatom biofilm Exopolymeric Substances on clay coat formation in estuaries" presents the experimental formation of detrital clay coats and

the role of EPS in the aggregation of clay and sand in estuaries. This chapter is based on an article published in *Geology* (Duteil et al., 2020).

6. The sixth chapter "Discussion and way forward" is a general discussion divided in three parts. First, we compare the production, composition and preservation of EPS in various sedimentary environments (e.g., rivers, estuaries, hypersaline lakes). Second, we discuss the role EPS in the sediment to rock transition. Finally, we present some potential perspectives for future EPS research.

Chapter 1: State of the art on the Gironde estuary, benthic diatom biofilms and Exopolymeric Substances in sedimentary systems. Research questions and objectives.

4 Chapter 1

Table of contents

Chapter 1:	State of the art on the Gironde estuary, benthic diatom biofilms and Exopolymeric	_
Substances	in sedimentary systems	3
Research q	uestions and objectives	3
1.1 The	Gironde estuary	6
1.1.1	Definition of estuaries	6
1.1.2	Physiography and hydrology of the Gironde estuary	6
1.1.3	Physicochemical conditions in the Gironde estuary	7
1.1.4	Sedimentology of the Gironde estuary	9
1.1.5	Bordeaux North Point Bar1	1
1.1.6	Mineralogy of clay minerals	5
1.1.7	Clay minerals reactivity1	7
1.1.8	Properties of the clay mineral species found in the Gironde estuary 1	9
1.1.9	Summary of the results of the ClayCoat Project	4
1.2 Ben	thic biofilm and Exopolymeric Substances in estuaries: state of the art	6
1.2.1	Generalities on diatoms	6
1.2.2	Microphytobenthic biofilms and exopolymeric substances	9
1.2.2.1	Background on EPS2	9
1.2.2.2	Diatom EPS specificities	1
1.2.3	Benthic biofilms biological functions	3
1.2.3.1	Adhesion to the sediment and substratum stabilization	3
1.2.3.2	Frustule stabilization	5
1.2.3.3	Cell motility in response to light and tidal cycles	5
1.2.3.4	Cell protection	6
1.2.3.5	Communication and interactions between cells	8
1.2.3.6	Food and micronutrient accumulation source4	0
1.2.3.7	Sedimentary EPS cycling 4	0
1.2.3.8	EPS-mineral interactions molecular mechanisms 4	1
1.3 Su	ummary and research questions addressed in this thesis	3
Referenc	es	7

1.1 The Gironde estuary

1.1.1 Definition of estuaries

Estuaries are transition zones between rivers and oceans, characterized by dynamic and high-energy environments (Dyer, 1997). Estuaries are characterized by salinity gradients: they are made up a transition zone between a fresh water river and the marine environment with a salinity ranging from 0.1‰ to 30-35‰ (Pritchard, 1967). Estuaries develop during relative sea level rise and generally occupy the seaward parts of submerged valleys that subsequently fil with a mixture of river and marine sediments (Martinius & Van den Berg, 2011). Two types of estuaries can be distinguished: (i) macrotidal estuaries are tide-dominated; (ii) microtidal or mesotidal estuaries are wave-dominated (Hayes, 1980). Tidally dominated estuaries show a predominance of elongated tidal bars and tidal channel systems and the absence (or limited development) of wave-generated barriers and/or beaches parallel to the coast (Dalrymple & Choi, 2007). In microtidal and mesotidal estuaries, waves are dominant at the mouth, producing littoral transport and creating a barrier that partially closes the mouth (Hayes, 1980). There typically is a permanent competition between tidal energy and river flow in the estuaries. The impact of river flow increases progressively as the tidal energy dissipates by friction on the estuarine banks.

1.1.2 Physiography and hydrology of the Gironde estuary

The Gironde estuary is located in south-west of France, at the confluence of the Garonne and the Dordogne rivers, draining the water from the watershed of which includes the Pyrenees and the Massif Central (Benaouda, 2008). It is one of the largest estuaries in Europe, with a length of 75 km and a surface area of 630 km², from the junction of the Garonne and the Dordogne (Bec d'Ambès) to the estuarine mouth (Soulac-sur-Mer) at the Bay of Biscay (Figure 1.1). The maximum width of the estuary is of 12 km near the mouth, and the maximum depth is 20 m. The tidal range comprises 1.5 meters during neap tides at the estuarine mouth and 6.3 meters during spring tides in Bordeaux (Castaing et Allen, 1981; Jalon-Rojas et al., 2018). The average annual river flow entering the estuary is about 900 m³.s⁻¹, with two distinct seasons: (i) the period of high river flow extends from December to June with a mean flow that can reach 3 000 m³.s⁻¹. Major river flow extends from July to November (e.g., 1930, 1981, 1988, 2010); (ii) The period of low river flow extends from July to November

with a mean flow of less than 500 m³.s⁻¹ (Coynel et al., 2004). During low river flow, tidal current reversal can occur up to 160 km upstream from the estuarine mouth (Chaumillon et al., 2013).

1.1.3 Physicochemical conditions in the Gironde estuary

The Gironde estuary has a well-developed turbidity maximum zone (TMZ). The TMZ is characterized by very turbid water with a high concentration of particles in suspension. In the Gironde, the TMZ extends over a distance of 70 to 80 km along the estuary axis. The concentration of suspended particulate matter (SPM) varies is of 1 to 10 g.L⁻¹ in surface water and reaches several hundreds of g.L⁻¹ near the bottom of the estuary (i.e., "crème de vase"; Castaing & Allen, 1981; Sottolichio et al. 2011). The position of the TMZ in the estuary varies seasonally with changes in river flow: during periods of high river flow, the TMZ is restricted to the estuary funnel, whereas during periods of low flow it is essentially located in the estuarine channels (Sottolichio et al., 2011; Jalon-Rojas et al., 2015; Figure 1.1). The TMZ expands and the SPM concentration increases during spring tides, river floods or storms. The total transport of SPM during these periods is estimated between 2.5×10^6 and 4×10^6 tons through the estuary (Migniot, 1968). Relative positions of the TMZ during the year have been summarized on a figure by Virolle et al. 2021 (Figure 1.1).

Two hydrodynamic mechanisms explain the formation of the TMZ (Dyer, 1988; Allen et al., 1980):

- (i) In the Gironde estuary, the tidal flood current is stronger than the ebb current, with the difference increasing landward. The results in a "tidal pumping mechanism", which is determined by this tidal wave asymmetry. This mechanism is active all year long and results in the erosion of bottom mud and upstream transport of suspended particles. The settling of fine particles and the accumulation of mud at the bottom of the channel occurs at the tide nodal point, i.e., the zone where the river current equals the tidal current (amphidromic point).
- (ii) During the high river stage, a density gradient forms with a seaward-oriented current at surface and a landward-oriented bottom current. The density nodal point is the zone where fresh surface water and salty bottom water meet. It is an effective trap for fine sediments during the winter, when the estuary is well-stratified.

The Gironde TMZ is made of fine-grained material with a size between 0.45 and 150 µm. It comprises detrital minerals (e.g., organic colloids, clay minerals, micas, quartz) and a biogenic fraction (e.g., phytoplankton, such as diatoms, and zooplankton; Verney et al., 2006). In the TMZ, mineral particles form complexes with reactive organic matter (including expolymeric substances) eventually producing flocs (Eisma, 1986). This complexation process is enhanced by salinity. In the Gironde estuary, Gibbs et al. (1989) documented the formation of flocs at salinity higher than 0.2 g.L⁻¹. The water characteristics (dissolved oxygen, salinity and temperature) have been monitored continuously since 2005 in the City of Bordeaux, near our main investigation area, the Bordeaux North Point Bar (Schmidt et al., 2016; Figure 1.1). Monthly trends have been calculated for the period 2005-2014. Dissolved oxygen concentrations ranged between 9.5 and 12.5 mg.L⁻¹ in winter when low water temperatures favor oxygen solubility. With the gradual increase in temperature from spring to summer, there is a gradual decrease in dissolved oxygen concentration, with values between 3.1 and 8.2 mg.L⁻¹. The oxygen saturation, which is independent of the temperature, is between 39 and 104%. Brief episodes of phytoplankton bloom in the spring produced dissolved oxygen and account for the slight oversaturation (Schmidt et al., 2016). The salinity varied from 0.09 to 2.0 g.L⁻¹, with the highest values observed during the summer. The temperature ranged from an average annual low of 7°C in February to an average high of 24°C in July. The TMZ is present during five to eight months per year in Bordeaux, which explains the strong variations of turbidity between 100 and 5000 NTU (nephelometric turbidity unit).



Figure 1.1: Map of the depositional environments of the Gironde estuary. Red dot localizes the Bordeaux North Point Bar. Double arrows indicate the seasonal position of the turbidity maximum zone (TMZ). The insert at the bottom right shows the location of the Gironde estuary in France. Map from Virolle et al. (2021).

1.1.4 Sedimentology of the Gironde estuary

The Gironde estuary can be classified as a wave- and tide-dominated estuary (Allen & Posamentier, 1993) that can be subdivided into three sedimentary and geomorphological zones, as recently described by Virolle et al. (2021): (i) the fluvio-estuarine channels, (ii) the inner estuary funnel and (iii) the outer estuary funnel (Figure 1.2).

- (i) The fluvio-estuarine channels extend over an 85 km long area from the Bec d'Ambès to the upstream limit of tidal action, at 160 km from the mouth, near La Réole. This area is constituted by the hydrographic network of the Garonne and Dordogne rivers, influenced by tides and fluvial currents, and composed of meandering channels (up to 10 meters deep) and heterolithic point bars. Our main investigation area, the Bordeaux North Point Bar, is located in this zone (Figure 1.1).
- (ii) The inner estuary funnel is tide-dominated channel extends from 75 to 35 km from the mouth of the estuary and includes the bayhead delta of the Gironde. The bay

head delta is composed of elongated and lobate (e.g., U-shaped) tidal sand bars separated by 6 to 10 meters deep muddy channels.

(iii) The outer estuary funnel is tide-dominated. This is where the estuary reaches its maximal width. The outer estuary funnel extends from the Gironde mouth to 35 km upstream. It is composed of the muddy central basin (20 km long) and of the sandrich tidal inlet channel fill (15 km long). The latter includes a few elongated tidal sand bars (e.g., Banc de Richard).

These three zones are surrounded by an extensive mud-dominated intertidal zone (Figure 1.1), which sediments are covered by diatom biofilms.



Figure 1.2: The various sedimentary facies present along the Gironde estuary. Adapted from Allen (1991).

There are three types of sand bars in the Gironde estuary: fluvial point bars, estuarine point bars and estuarine tidal bars (Alen, 1991):

- (i) The fluvial point bars are located upstream of the bay line and consist of conglomerates at the base, fining up to cross-bedded coarse sand without clay lamination.
- (ii) After the bay line, we find heterolithic point bars. These bars are composed of upward thinning medium grained sand with sigmoidal cross-beds and ripples. There are a vast number of clay laminae within the coarse-sand matrix more abundant towards the top. These heterolithic bars are generally between 1-2 km long and 100-

500 m wide and located in the estuarine channel (75-130 km from the mouth of the Gironde estuary; Figure 1.1).

(iii) Finally, the tidal bars are made up of vertical fining upward medium-grained sand displaying ripple and sigmoidal cross-beds. Bars displays also abundant clay laminae. The tidal bars are between 3-5 km long and 300m-1.5 km wide and predominantly located in an area between 35 and 75 km from the mouth of the Gironde estuary (Figure 1.1).

1.1.5 Bordeaux North Point Bar

The Bordeaux North Point Bar is our main investigation area. It is located north of the City of Bordeaux, approximately 94 km upstream from the estuary mouth. The point bar is 1 100 m long (north-south axis) and 200 m wide (east-west axis; Figure 1.3). The western channel bank is composed of a 3-m thick section of muddy estuarine coastal-plain sediments that were deposited up to the high-tide limit (Figure 1.4). Three sedimentary environments can be distinguished at the point bar: (i) an intertidal mudflat, (ii) a muddy chute channel located between the crest of the point bar and the channel bank flat and (iii) a network of tidal dunes, some observable at low tide (Figure 1.3). The size of the point bars can increase during very high tidal amplitude. Diatom biofilms proliferate on the intertidal mudflats and the chute channels but less abundantly on the tidal dunes due to high hydrodynamic regime.



Figure 1.3: (A) Map of the Gironde estuary showing the location of the Bordeaux North Point Bar, and a satellite image of the point bar at low tide (Google Earth Pro). Three sedimentary domains are visible in the intertidal zone of the point bar: the mud flat, the muddy chute channel and the crest of the point bar covered with sand dunes. Section shown in Figure 1.4 is highlighted in red. (B) Picture of the mudflat covered with diatoms. (C) Picture of the sand dunes area at low tide. Biofilms are not visible in this high energy area.

This point bar is heterolithic, as it is composed of fluvial-sourced sand reworked by tidal currents that alternate with decimeter to meter thick mud-dominated layers (Musial et al., 2012; Virolle et al., 2021). Such alternation of sand and mud are organized as meter-thick lateral accretion unit, prograding towards the channel (Figure 1.4).



Figure 1.4: Schematic cross section of the Bordeaux North Point Bar. Accretion of the sand-mud alternances is heading towards the channel. High and low tide levels are represented by dashed blue lines. The bar is 500 m wide and around 10 m depth.

Tidal cycles and currents are primary controls on the short term sediment deposition. The semidiurnal tidal cycle (12.5 hours) comprises flood and ebb currents. On the point bar, dunes migrate with the flood and ebb, when the current velocity is above 50 cm.s⁻¹ resulting in the formation of tidal bundles (Figure 1.5). High and low tide slack waters are recorded in the form of cm-thick clay drapes (Fenies et al., 1999; Figure 1.5). Dunes cover the lower part of the intertidal zone, and the entire subtidal zone, up to the canal thalweg (Figure 1.4). On the intertidal mudflat, where the current is slower, sand ripples alternate with cm-thick clay drapes, forming lenticular or wavy beddings. The spring-neap cycle (14.7 days) also modulates the thickness of tidal bundles (Allen et al., 1977), as well as that of ripples and clay drapes in the intertidal zone (Musial et al., 2012). Four facies were identified on the point bar:

- (i) *Fluvial flood facies* consisted of coarse-grained sand mixed with detrital gravels intercalated with floating rounded to sub-angular soft mud clasts.
- (ii) *Tidal sand dunes facies* composed of fine-to-medium sands with trough crossbedding and reactivation surfaces. Dune foresets were locally draped by muds forming mud couplets.
- (iii) Heterolithic fine sands and laminated mud facies made up of thick laminated heterolithic intervals composed of grey to brown laminated clay and fine-to-medium micaceous sands.
- (iv) Fluid and laminated mud facies consisted of fluid mud layers alternating with very fine-grained sands to silty lenses and slack-water clay drapes.

A detailed description of the facies on the Bordeaux North Point Bar can also be found in chapter 3 of this thesis, and details of the point bar stratigraphy are described in Virolle et al. (2021).



Figure 1.5: Cross section of a trench dug within the surface of point bar, showing a small sand dune with clay drapes and trough cross-bedding. Clay couplets (green circle) and reactivation surface (red dotted line) are visible and highlighted in this trench. Adapted from Virolle et al., 2019a.

The average mineral composition of the point bar sediment is 37 % quartz, 17 % clay and silt sized particles, 14 % lithics (wood debris, mud clasts), 11 % feldspars, 5% micas and 3 % carbonates (Virolle et al., 2021). Based on XRD analysis, Virolle and coworkers (2021) determined four main clay minerals: chlorite, illite, kaolinite, and smectite. The clay fraction consists on average of illite (45%), smectite (35%), chlorite (10%) and kaolinite (10%; Figure 1.6). The average suspended assemblage of clay minerals in the water column is composed of illite (49%), smectite (22%), kaolinite (14%) and chlorite (15%; Jouanneau & Latouche, 1981).



Figure 1.6: X-ray diffractograms of the $\leq 2 \mu m$ fraction showing dominant clay minerals in the BORNW-11 sample at 2.81m depth: illite (45%), smectite (35%), chlorite (10%) and kaolinite (10%) from Virolle et al., 2021. The facies of this sample is heterolitic medium-grained sand dunes, equivalent to facies F2 that can be found in this study.

1.1.6 Mineralogy of clay minerals

Clay minerals are defined by a layer structure with one dimension in the nanometer range (1:1 TO layer or 2:1 TOT layer), anisotropy of the layers, the existence of several types of surfaces (e.g. external or basal surface), plasticity and hardening on drying or firing (Bergaya et al, 2013). This should not to be confused with clay sized-particles, which is a strictly granulometric term that groups sedimentary particles with a diameter $< 3.9 \,\mu\text{m}$ (Wentworth, 1922). Gironde clay minerals are mainly classified as phyllosilicates, i.e., alumino-silicates with a layered structure (Bergaya et al., 2013). At the atomic scale, clay minerals are composed of tetrahedra or octahedra organized into planar layers. Tetrahedra and octahedra are linked together by sharing oxygen atoms connected by a covalent bond (Brigatti et al., 2013). A tetrahedron is formed by a cation (generally Si⁴⁺, more rarely Al³⁺) coordinated to four oxygen anions O²⁻. This tetrahedron shares three oxygen atoms with the neighboring tetrahedra (Figure 1.7). Clay mineral layers consist of repetitive tetrahedral and/or octahedral sheets. The oxygen atoms are organized into hexagonal networks, forming the base of the tetrahedral layer. The octahedral layer is formed by octahedra duh. An octahedron consists in one Al³⁺, Mg²⁺ or Fe²⁺ coordinated to six anions, mostly oxygens but also hydroxyls (OH⁻). Octahedra share common

anions and are organized in planar layers. The connections between the sheets are made when the apical oxygens of the tetrahedron become the vertices of the octahedron.

Depending on the combination of layers, three main structural types are distinguished (Brigatti et al., 2013; Figure 1.7: (i) a 1:1-layer structure consists of the repetition of one tetrahedral and one octahedral layer (e.g., kaolinite, halloysite). In contrast, (ii) a 2:1-layer structure consists of repetitions of one octahedral sheet between two tetrahedral sheets (e.g., illite, smectite); (iii) 2:1:1 minerals include an octahedral layer composed of cations and hydroxyls inside the inter-layer space (e.g., chlorite). In 1:1-clay minerals, the tetrahedral surface is composed of oxygen atoms and the octahedral surface is mostly composed of O-H groups (Figure 1.7). In kaolinite, the layers are piled up without any inter-layer molecules. In 2:1-clay minerals, both surfaces consist of tetrahedral basal oxygen atoms, while the hydroxyl groups of the octahedral layer are replaced by oxygen atoms from the tetrahedral sheets (Brigatti et al., 2013). The interlayer space includes weakly-bound cations (e.g., K⁺, Mg²⁺) or water molecules (Figure 1.7).



Figure 1.7: Organization of clays, from the atomic to the mineral scale. (A) The two different atomic organizations arrangements: (A.1) a tetrahedron composed of one central cation (generally Si^{4+}) connected to four oxygen anions or (B.2) an octahedron consisting of one Al^{3+} or Mg^{2+} bound to six anions, mostly oxygen but also hydroxyl groups. (B) The two different sheet organizations: the tetrahedral sheet repetition of tetrahedron linked together (or, dioctehedral sheet), repetition of octahedra linked together. (C) The two different organization of layers: a 1:2 TO layer and a 1:1 TOT layer. (D) The resulting clay mineral particles. Smectite is a type-1:2 clay with the unique feature of hydrated interfoliar cations (unlike illite or chlorite). Kaolinite is a 1:1-clay with layers that are stacked without any interlayer molecules. Reproduced from Tournassat et al., 2015.

1.1.7 Clay minerals reactivity

The surface properties of clay minerals depend on many properties: the chemical composition, the surface type, the nature of the interacting atoms, the type of charge and the type of exchangeable cations (Schoonheydt & Johnston, 2013). Three zones of interaction can be distinguished in clay minerals: (i) the basal surface, (ii) the edge of the layer and (iii) the interlayer space (only for certain clay species, e.g., smectite; Figure 1.8). The electronegativity of the oxygen atoms in the clay mineral structure determines the hydrophilic or hydrophobic character of the surface (Schoonheydt & Johnston, 2013). The clay mineral basal surface is composed of oxygen atoms in Si–O bonds, and referred to as the siloxane surface. The siloxane

surface has a considerable covalent character and is hydrophobic (Brigatti et al., 2013). While the siloxane surface is neutral, a permanent charge can be introduced through isomorphic substitution, which is the replacement of an atom of the sheet by a less electronegative one. For example, the replacement of Al by a Si cation in the tetrahedral sheet, or of Mg by Al in the octahedral sheet, leads to a local distortion (Schoonheydt & Johnston, 2013). As a consequence, the charge on the neighboring oxygen atoms is expected to increase, which can modify the reactivity of the siloxane surface. The negative charge that results from isomorphous substitution is balanced by the presence of exchangeable cations of which Ca²⁺, Mg²⁺, K⁺, and Na⁺ ions are the most common (Schoonheydt & Johnston, 2013). Changes in surface properties combined with exchangeable cations can polarize the surface oxygen atoms and make the properties more hydrophilic (Nulens et al., 1998). This result in three types of surfaces in clay minerals: (i) neutral siloxane surfaces, (ii) charged surfaces and (iii) hydroxylated surfaces (Figure 1.8):

- (i) Neutral siloxane surfaces are nonpolar and interact weakly with water molecules, because of the absence of hydrogen bond formation (Croteau et al., 2009). Indeed, the external oxygen atoms on the siloxane surface are relatively weak electron donors and nonpolar (Schoonheydt & Johnston, 2013). The neutral siloxane surfaces are favorable adsorption domains for non-polar and semi-polar molecules such as hydrophobic parts of proteins (Poorni & Natarajan, 2013).
- (ii) Charged siloxane surfaces are produced through isomorphous substitutions. This process results in the development of a negative charge on the siloxane surface (Schoonheydt & Johnston, 2013). The negative charge is balanced by presence of exchangeable cations (e.g., Ca²⁺ or Mg²⁺), which can interact with water molecules by forming hydrogen bonds, making the surface partially or fully hydrophilic. The charged siloxane surfaces are adsorption domains for polar molecules such as hydrophilic parts of proteins (Poorni & Natarajan, 2013).
- (iii) Hydroxylated surfaces are pH dependent and interact strongly with water molecules. Hydroxyl groups present a positive or negative charge depending on the adsorbed metal ion and on the pH of the ambient aqueous solution (Schoonheydt & Johnston, 2013). Depending on pH, the hydroxylated surfaces are adsorption domains for polar molecules such as hydrophilic parts of polysaccharides (Poorni & Natarajan, 2013).

The reactivity of clay minerals can be measured through the **cation-exchange capacity (CEC)**, the **specific surface area** and the **anion exchange capacity (AEC)**. The CEC is defined as the sum of the exchangeable cations that a mineral can adsorb at a given pH in meq.100 g⁻¹. In other words, it is a measurement of the negative charges carried by the mineral (Bain & Smith, 1994). These negative charges are created by isomorphous substitutions (i.e., permanent charges), by broken bonds at layer edges and external surfaces, or by dissociation of accessible hydroxyl groups. The specific surface area of clay mineral is the total area accessible for sorption processes. The anion-exchange capacity (AEC), i.e. the sum of exchangeable anions that a clay mineral can adsorb, has been shown to be proportional to the surface area (Bain & Smith, 1994).



Figure 1.8: Surface properties of the clay minerals found in the Gironde estuary. (A) The different surfaces between 1:1- and 2:1- clay type. The 1:1 clay type has a hydrophobic neutral siloxane surface and a hydrophilic hydroxyl surface. The 2:1 clay type has a hydrophobic siloxane surface and a hydrophilic charged siloxane surface which can interact with exchangeable cations. (B) The main areas of interaction in the clay mineral particles found in the Gironde estuary. Smectite has the most interaction zones with basal surface edges and interlayer space. Illite, chlorite and kaolinite cannot interact at the interlayer space but can do so on edges and basal surfaces.

1.1.8 Properties of the clay mineral species found in the Gironde estuary

Four clay minerals have been found in the Gironde estuary using FT-IR spectroscopy and XRD analyses (Figures 1.6 and 1.10): kaolinite, chlorite, smectite and illite. In Gironde, these minerals have a detrital origin, derived by erosion of the Massif-Central, drained by the Dordogne, and from the Pyrenees drained by the Garonne, which constitute the drainage area constituting the watershed of the Gironde estuary.
- (i) **Kaolinite:** The kaolin group presents a 1:1-layer structure with an average chemical formula of Al₂Si₂O₅(OH)₄ (Figure 1.7D). The kaolinite layer is essentially neutral and has a thickness of 7.2 Å. Two contiguous layers are linked through hydrogen bonding. The O-H groups of the hydroxyl surface present different properties depending on their position and orientation within the layer. For example, outer O-H groups form strong hydrogen bonds if they are oriented nearly perpendicular to the tetrahedral layer (Brigatti et al., 2013). The kaolinite standard material used in this study for clay EPS mixing (ref. KGa-1; Clay Minerals Society) has a chemical composition of 44.2% SiO₂, 39.7% Al₂O₃, 1.39% TiO₂, 0.13% Fe₂O₃, 0.08% FeO and MnO with some trace elements (e.g., K₂O, MgO). The FT-IR spectrum of kaolinite (Madejova et al., 2017) is characterized by wavenumber peaks at 3694 cm⁻¹ (O-H bands), 3669 cm⁻¹ (O-H bands), 3652 cm⁻¹ (O-H bands), 3620 cm⁻¹ (O-H bands), 1102 cm⁻¹ (Si-O bands), 1033 cm⁻¹ (Si-O-Si bands), 1011 cm⁻¹ (Si-O bands), 938 cm⁻¹ (O-H bands from Al₂OH) and 915 cm⁻¹ (O-H bands from Al₂OH; Figure 1.9). Kaolinite is a clay mineral with a low reactivity, especially towards xanthan, as demonstrated in a study by Dontsova and Bigham (2005). This low reactivity can be explained by low CEC, comprised between (3-15 meq.100g⁻¹), AEC (7-20 meq.100g⁻¹). The surface area of kaolinite is intermediate (44 m².g⁻¹; Van Olphen & Fripiat, 1981; Bain & Smith, 1994).
- (ii) **Illite:** The illite group presents a 2:1-layer structure. The illite sheet is formed of three layers: one octahedral layer sandwiched between two opposing tetrahedral layers (Fig. 3D) with a thickness of 100 Å. The average chemical formula of illite is (K,H₃O)(Al,Mg,Fe)₂(Si,Al)4O₁₀[(OH)₂,(H₂O)]. The interlayer space is occupied by potassium cations (K^+) , explaining why illite does not have a swelling property. The illite standard material used in this study (ref. IMt-2; Clay Minerals Society) has a chemical composition of 49.3% SiO₂, 24.25% Al₂O₃, 7.83% K₂O, 7.32%, 2.56% MgO, Fe₂O₃, 0.55% TiO₂ and FeO, 0.43% CaO with some trace elements (e.g., P_2O_5). The FT-IR spectrum of illite shows typical peaks at 3623 cm⁻¹ (O-H bands), 1100 cm⁻¹ (Si-O bands), 1030 cm⁻¹ (Si-O bands) and 916 cm⁻¹ (O-H bands from Al₂OH; Madejova et al., 2017; Figure 1.9). Illite is more reactive than kaolinite, with a CEC comprised between 10-40 meq.100 g⁻¹, an AEC between 4-17 meq.100 g⁻¹ and a specific surface area of *ca*. 183 m².g⁻¹ (Van Olphen & Fripiat, 1981; Bain & Smith, 1994). These properties may explain the high reactivity of illite towards xanthan, as documented by Dontsova and Bigham (2005).

- (iii) Chlorite: The chlorite group presents a 2:1:1-layer structure with an average chemical formula (Si₃Al)(MgFeAl)₆O₁₀(OH)₈. The interlayer space is generally occupied by magnesium and iron oxyhydroxides (Mg-Fe)₃OH₆) and the layer has a thickness of thick 14–14.5 Å (Brigatti et al., 2013; Figure 1.7D). Ripidolite used in this study is a classical chlorite mineral enriched in Mg and Fe (so called Mg-Fe-Chlorite). The standard (ref. CCa-2; Clay Minerals Society) has a chemical composition of 26.6% Fe₂O₃, 26% SiO₂, 20.8% FeO, 20% Al₂O₃, 17.2% MgO, 0.5% TiO₂, 0.25% CaO with some trace elements (e.g., K₂O). Ripidolite has a CEC comprised between 10 and 40 meq.100 g⁻¹, an AEC comprised between 5 and 20 meq.100 g⁻¹ and a surface area of 36 m².g⁻¹ (Van Olphen & Fripiat, 1981; Bayley, 1988). The FT-IR spectrum of ripidolite is characterized by peaks at 3671 cm⁻¹ (O-H bands), 3570 cm⁻¹ (O-H bands), 3429 cm⁻¹ (O-H bands), 989 cm⁻¹ (Si-O bands) and 822 cm⁻¹ (Si-O bands; Madejova et al., 2017; Figure 1.9).
- Smectite: The smectite group presents a 2:1-layer structure. Smectites have a total (iv) negative layer charge comprised between 0.2 and 0.6 e per unit cell (deviation from neutrality due to isomorphic substitution). Montmorillonite, a smectite type clay used in this study, has a dioctahedral character and an average chemical formula of (Na,Ca)_{0.3} (Al,Mg)₂ Si₄O₁₀(OH)₂ (Figure 1.7D). The standard (ref. SAz-2; Clay Minerals Society) has a chemical composition of 60.4% SiO₂, 17.6% Al₂O₃, 6.46% MgO, 2.82% CaO, 1.42% Fe₂O₃, 0.24% TiO₂, 0.2% K₂O, 0.1% MnO with some trace elements (e.g., Na₂O). Montmorillonite has a CEC comprised between 100 and 150 meq.100 g⁻¹, a specific surface area of 97.42 m².g⁻¹ and an AEC comprised between 20 and 30 meg.100 g⁻¹ (Van Olphen & Fripiat, 1981; Bain & Smith, 1994). The FT-IR spectrum of montmorillonite is characterized by typical peaks at 3621 cm⁻¹ (O-H bands), 3253 cm⁻¹ (H₂O vibration), 1635 cm⁻¹ (H₂O vibration), 1030 cm⁻¹ (Si-O bands) and 924 cm⁻¹ (O-H bands from Al₂OH; Johnston & Premachandra, 2001; Madejova et al., 2017; Figure 1.9). Various exchangeable cations can occupy interlayer positions in smectite, e.g., Na⁺, K⁺, Ca²⁺ or Mg²⁺. Interlayer cations are commonly hydrated and exchangeable (Brigatti et al., 2013). The swelling properties of smectites is controlled by the type of the specific exchangeable cations in the interlayer space, the layer charge, and the Brownian swelling that occurs between two different smectite particles (Ferrage et al., 2010). Different molecules can insert themselves into the interfoliar space, for example water, which will make

Chapter 1

the particles swell. Other molecules can interact with the interfoliar space as well, for example EPS molecules produced by bacteria. (Alimova et al., 2009).



Figure 1.9: Fourier transform infrared (FT-IR) spectra of clay mineral standards used in this study: kaolinite, illite, chlorite (ripidolite type) and smectite (montmorillonite type). The most common peaks are O-H bands (around 3670, 3565 and 3621 cm⁻¹), Si-O bands (around 1100, 1030 and 990 cm⁻¹) and O-H bands from Al₂OH (around 920 cm⁻¹).

1.1.9 Summary of the results of the ClayCoat Project

This thesis follows the ClayCoat Project, which aimed to understand the mechanisms responsible for the formation and distribution of detrital clay coatings by studying a modern analogue of estuarine reservoirs the Gironde estuary. This project was funded by Engie Exploration Production service (2015-2017), Neptune Energy (2018-2022). The formation of a clay coating around the quartz grains delays or inhibits the development of quartz overgrowth during sandstone diagenesis. This allows to strongly limit the decrease of the petrophysical properties of the reservoir rocks. Petrophysical properties are impacted by mechanical and chemical compaction processes and the overgrowth of quartz during the diagenetic processes. Among the clays that can constitute coatings, authigenic chlorites, and in particular iron-rich chlorites, are the most frequently studied. The link between inhibition of quartz overgrowths by the formation of authigenic chlorite crystals in deeply buried sandstone reservoirs has been demonstrated (Ehrenberg, 1993). The process of chloritization (formation of chlorite crystals) is carried out via the transformation of precursor clay minerals, such as berthierine, during diagenesis (Beaufort et al., 2015). Virolle's work in a modern estuarine environment (i.e., the Gironde estuary) has allowed to advance on the understanding of the mechanisms responsible for the formation and distribution of clay precursors, potentially responsible for the setting of clay coatings during diagenesis. Virolle's investigation analyzed and compared tidal bars and point bars in the Gironde estuary with ancient estuarine reservoir bodies of the Cape Hay Formation (Australia, Permian), the Sego Formation (Colorado, USA, Cretaceous) or the McMurray Formation (Alberta, Canada, Cretaceous; Musial et al, 2012; Saïag et al, 2016; Aschoff et al, 2018; Virolle et al., 2019a). His work revealed for the first time the presence of clay coats (composed of detrital clay minerals) around the grains in all the sampled sand bars (Virolle et al., 2019b). Moreover, the primary elements that may be involved in the chloritization process (Al, Fe, Si, Mg) are already present at the time of deposition and are

preserved several meters deep (Virolle et al., 2022). Berthierine is hypothesized to be the clay mineral precursor to chlorite coatings and is formed during the eogenesis with shallow burial (<1000m) via the transformation of detrital minerals such as kaolinite, smectite or micas (Virolle et al., 2021). The presence of reactive iron is essential for this transformation (Beaufort et al., 2015). The work of the current thesis is connected to the ClayCoat Project through the characterization of the potential role of diatom biofilms in the formation of detrital clay coats around sand grains in a highly energetic hydrodynamic regime (Topic 3). The preservation of diatom biofilm exopolymers at depth was also studied to determine their impact on the diagenesis of primary clay minerals. The following section will present the state of the art of diatom biofilms in the estuarine domain.

1.2 Benthic biofilm and Exopolymeric Substances in estuaries: state of the art

1.2.1 Generalities on diatoms

The surface of the estuarine intertidal zone is often covered with a microphytobenthic biofilm (Admiraal, 1976; Underwood & Paterson, 1993; Underwood & Kromkamp, 1999; Decho, 2000). Diatoms are the most common group of microorganisms inhabiting intertidal soft sediments (sand and/or clay; Smith & Underwood, 1998). These benthic eukaryotes are unicellular photosynthetic algae with a characteristic cell wall impregnated with silica (SiO₂, nH₂O; Qin et al., 2008). This cell wall, or frustule, is composed of two large units, referred to as valves, which are linked together by several thinner structures, termed girdle elements (Hoek et al., 1995; Figure 1.10). Depending on the taxon, the size of the cells, and consequently that of the frustule, varies from a few micrometers to more than 500 micrometers (Lavoie & Hamilton, 2008). The exchange of material across the frustule takes place through pores and slits. The two valves of the frustule are called epivalve and hypovalve and the girdle elements are referred to as epicingulum and hypocingulum, respectively (Round et al., 1990; Figure 1.10). The epivalve and the epicingulum grouped together are called epithecae, while the hypovalve and the hypocingulum form a structure called the hypotheca (Figure 1.10). Because the valves and girdle elements are quite rigid, cell growth can occur in one direction as the epitheca and the hypotheca move apart (Hoek et al., 1995). During cell division by vegetative reproduction, each frustule of the daughter cell has one valve from the mother cell, and one formed just after the cell division. New parts of the wall are formed in the protoplasts and added by exocytosis. After each cellular division, the new valve and girdle are smaller than the old one, and so is the cell. Cell size is restored through sexual reproduction (triggered by cell size and environmental factors), which results in the formation of a special cell called an auxospore that grows and expands in a very controlled way before producing a frustule (Lavoie & Hamilton, 2008).



Figure 1.10: Cryo-scanning electron microscope images of diatom frustules from the Gironde estuary. (A) and (B) *Navicula* sp., frontal (A) and side (B) view with names of the different parts of the frustule. (C) Fractured frustule which allows to see the internal organization of the different parts and their organization. The interior of the cell was probably removed during cryofracture. (D) Internal view of a valve that shows the internal organization of the latter.

The cell organelles and cytoplasm are protected by this silica wall. Diatom cells comprise generally plastids, mitochondria, dictyosomes and other organelles packed into a thin peripheral layer of cytoplasm, compressed between cell walls by one or two large central vacuoles (Figure 1.11). The nucleus is generally easy to see, suspended at the center of the cell or lied to one side of the cell. Lipid vesicles or other reserve organelles are often visible within the cytoplasm (Figure 1.11). Due to the carotenoid pigments (β -carotene, diatoxanthin, diadinoxanthin and fucoxanthin) masking the color of the chlorophylls (a and c), the chloroplasts have a pale yellow to brown color, which has determined the classification of diatoms in the "brown algae" group (Lavoie & Hamilton, 2008; Figure 1.11).



Figure 1.11: (A) Polarizing microscope image of a diatom (*Nitzchia* sp.) from the Gironde estuary surface biofilm. Details of the cell and the silica frustule are visible. (B) Polarizing microscope image of a slide stained with Triphenyltetrazolium (TTC). The reduction of TTC into formazan stains gives organelles with a functional an active electron transport system a pink appearance, here most likely the mitochondria (diatom: *Nitzchia* sp.). (C) Epifluorescence microscope image of a fluorescein di-acetate (FDA) stained diatom (*Pleurosigma* sp.; BH2-RFCA microscope; 490 nm). The hydrolyze of FDA by non-specific enzymes results in green fluorescence in the nucleus and the lipid vesicles. Chloroplasts show a red autofluorescence due to photosynthetic pigments.

Diatoms are divided into two main groups: (i) centric diatoms, with an axial symmetry and a radial or concentric ornamentation, and (ii) pennate diatoms, with a bilateral symmetry, which can present a conjunctive line interrupting the striae. When this line is discontinuous (pseudo-raphe) or continuous (raphe; Lange-Bertalot et al., 2017). The raphe plays a major role in diatom motility through the excretion of exopolymeric substances (EPS). The classification of diatoms is based on morphological characteristics of the frustule in valvular front view, in connective view (side) and on the appearance of the valves (Paulmier, 1997; Lavoie & Hamilton, 2008; Lange-Bertalot et al., 2017). The number of extant identified species is estimated between 20,000 and 30,000, but Mann and Droop (1996) suggest that there could be as many as 200,000 species. Diatoms have two contrasting lifestyles: (i) planktonic forms thrive in the water column, while (ii) benthic forms are attached to sediment surface (Lavoie & Hamilton, 2008). The oldest fossil diatoms are dated as Lower Jurassic (*ca.* 190 Ma), and the oldest pennate benthic diatoms as Late Cretaceous (*ca.* 75 Ma). Diatoms are thought to have progressively

colonized inland brackish and freshwater environments between the end of the Cretaceous and the Oligocene, mainly during periods of elevated sea level. The adaptation to dynamic sedimentary environments, such as river and estuaries, appears strongly linked with the evolution of the raphe (Sims et al., 2006). Two modes of life can be distinguished in benthic diatoms: epipsammic (sessile forms attached to the particles of sandy sediments) or epipelic (free-living forms that move over muddy sediment surfaces; Round, 1984; MacIntyre et al., 1996; Consalvey et al., 2004). Diatoms make up the most abundant taxa in the mudflat environments, but are part of a large diversity of eukaryotic and prokaryotic organisms grouped under the term microphytobenthic organisms (e.g., including prokaryotes such as cyanobacteria or filamentous sulfur bacteria; Hubas et al., 2018). In benthic habitats, microalgae and bacteria often secrete EPS into the surrounding environment to form a stabilizing and protective microenvironment and to allow for attachment to the sediment (Decho, 2000; Underwood & Paterson, 1995). The association of microorganisms and EPS matrix is referred to as a biofilm. In diatoms, EPS are synthesized in the Golgi apparatus, then externally secreted via specialized vesicles through the raphe or perforations along the frustule (Underwood and Paterson 2003; Crawford et al., 2001; Wotton, 2004). The following part will present the general characteristics of the EPS, then the unique characteristics of the diatom EPS. Finally, a section will review the roles of the EPS for the biofilm in general in estuarine environment.

1.2.2 Microphytobenthic biofilms and exopolymeric substances

1.2.2.1 Background on EPS

EPS is a complex mix of molecules of different sizes and molecular weights (e.g., polysaccharides, proteins, nucleic acids, etc.) ranging from mucilages in various stages of hydration (gels, slimes) to dissolved components soluble in aqueous media (Decho, 1990: Marvasi et al., 2010). The matrix also includes a wide variety of small molecules and ions sorbed to the EPS (e.g., Na, Mg, Ca; Decho & Gutierrez, 2017). Thus, EPS are generally separated into two different fractions, depending on their function and location (Underwood et al., 1995): (i) bound EPS (also termed bulk EPS) and (ii) colloidal EPS (also called soluble EPS; Nielsen and Jahn, 1999). There is also a third, less studied fraction, the capsular EPS which are closely associated with the cell wall (Hubas et al., 2018), which is not subject of this thesis. Bound EPS (e.g., condensed gels) are found further from the cell, often as a densely packed network, while colloidal EPS (e.g., soluble macromolecules) are mainly dissolved and

interact more with sediments and/or water (Underwood & Paterson, 2003). While colloidal EPS are easily extracted with water from sediments, bound EPS are more tightly absorbed to sediment particles or cells (Underwood et al., 1995; Yallop et al., 2000). Bound and colloidal carbohydrates have been shown to differ in their vertical distribution with sediment depth (Taylor & Paterson 1998). These authors report that colloidal carbohydrates are concentrated at the sediment surface and that their concentration decreases with depth, but that bound carbohydrates increase in concentration with sediment depth. The results of this study are, however, to be taken in context because the extraction of bound EPS was not performed, as the measurements were done on sediment samples. Using a more efficient extraction method to obtain the bound EPS fraction would be a good way to verify this assumption. Carbohydrates typically represent between 60 and 80% of the weight of total EPS, which also includes uronic acids, proteins and lipids (Underwood and Paterson, 2003). Extraction of EPS from benthic diatom cultures of Cylindrotheca closterium and Navicula salinarum reveals a different contribution of the bound and colloidal monosaccharide fractions. The monosaccharide (carbohydrate) fraction of bound EPS contained glucose (82-85%; Staats et al., 1999) mannose (0.5-7.6%), xylose (0.9-3.9%), rhamnose (1-9.1%), galactose (2.2-4.3%) and arabinose (0.6-2.8%).

A major role of bound EPS for the biofilm community is support of vertical migration (e.g., of diatoms), sorption of nutrients, water retention, adhesion and aggregation of cells, food and nutrient sources and cohesion and biostabilization of the sediment (Decho, 2000; Yallop et al., 2000; Decho & Gutierrez 2017; Hubas et al., 2018; Figure 1.12). Colloidal EPS present a more balanced distribution of the various monosaccharides: 22.9-41.6% of glucose, 5.6-14.7% of rhamnose, 12.2-19.1% of galactose, 20.2-46.1% of xylose, 4.1-13.8% of mannose and no arabinose (Staats et al., 1999). Taylor et al. (1999) have measured a mean in situ concentration of 1.8 mg glucose eq.g⁻¹ dry sediment for colloidal EPS on mudflats surface of two estuaries (Humber and Eden, UK) over emersion period. Their roles in the environment include exoenzymatic activity, export of cell products and wastes, sorption and transport of nutrients, communication, and promotion of polysaccharide gel formation (Decho, 2000; Decho & Gutierrez 2017; Hubas et al., 2018; Figure 1.12). The secretion of different types of EPS is varies and depends on the response of the organisms within the biofilm to environmental consitions. For example, De Brouwer et al. (2002) demonstrated, using a diatom culture, that colloidal EPS is continuously released into the medium. In contrast, bound EPS is produced exclusively in the light. Extraction of EPS fractions is described in detail in the material and method chapter 2.



Figure 1.12: Different fractions of EPS in biofilms and their various roles and functions. Adapted from Hubas et al. 2018.

1.2.2.2 Diatom EPS specificities

The biochemical composition of the EPS of approximately 30 genera of diatoms has been reviewed by Hoagland et al. (1993). However, only a few benthic species are included in that study and no EPS fractions were separated. For example, *Nitszchia frustulum perpusilla*, a benthic diatom, has EPS composed of 74% of carbohydrate (34% mannose, 24% rhamnose, 8% galactose and 34% unknown monosaccharides) and 9% sulfated sugars. Another benthic diatom, *Navicula incerta* has EPS with a monosaccharide composition of 33% rhamnose, 20% fucose, 10% mamnose, 9% xylose, 8% galactose and 20% of unknown sugars. It is demonstrated that the bound EPS presents more glucose contrary to the colloidal EPS, which more balanced distribution of monosaccharide (Staats et al., 1999). In addition to anionic polysaccharides, benthic diatom EPS can be made up of a substantial portions of acidic polysaccharides, proteins, sulphate esters, methylated sugars, uronic acids and some lipids (Hoagland et al., 1993; Staats et al., 1999; McConville et al., 1999). Depending on the diatom genus, carbohydrates contribute between 60 and 80% of the total EPS weight (Staats et al., 1999). EPS secretion requires an energy investment from the microorganisms and studies have shown a positively correlation between rate of primary production and carbohydrate production (Staats et al. 2000; Perkins et al. 2010). The secretion of EPS by benthic diatoms will furthermore depend on the environment conditions. Colloidal EPS are produced both with and without light (Perkins et al. 2006), in contrast to bound EPS, which is only produced only in the presence of light (Orvain et al., 2003). EPS from diatom cultures is enriched in glucose when produce during light period. The glucose contribution to bound EPS fraction increase by 10-15%, but while no changes in the colloidal EPS are observed (De Brouwer & Stal, 2001; Figure 1.13). In certain cases, tidal emersion can also impact the EPS composition, with glucose increases in >100 kDa and <10kDa fractions (De Brouwer & Stal, 2001; Figure 1.13). Furthermore, a higher production rate is observed at the end of low tide (Van Duyl et al. 1999). Passarelli et al. 2015 link the increased production of EPS in the summer with a higher photosynthetic rate. Other factors that can impact the production of EPS include nutrient limitation (Stal, 2003) or seasonal and daily cycles (Orvain et al., 2014; Passarelli et al., 2015). The relative contribution of protein in EPS varies from species to species (between 5.4-16.9% of the total EPS weight; Staats et al., 1999). The amino acid composition in the protein fraction of EPS also differe between diatom species (Staats et al., 1999). Detailed studies of the protein composition of EPS have been performed for some benthic diatoms, but only for epipsammic species. For example, Amphora longipes EPS has an amino acid composition consisting of alanine (17%), glycine (13%), valine (13%), serine (13%), and aspartate (13%; Xiao & Zheng, 2016). Some extracellular proteins are specific to diatoms: for example frustulin, a glycoproteins most likely covering the cell, but this was shown in only few investigations (Kröger et al., 1994). Frustulin has domains enriched with acidic cysteine residues that are stabilized by Ca²⁺ bridges on the cell wall surface (Chiovitti et al., 2003).



Figure 1.13: Conceptual model of different benthic diatom EPS fractions and features of their production patterns from Underwood and Paterson, 2003. For specifics of the EPS, please refer to the manuscript.

1.2.3 Benthic biofilms biological functions

Diatoms are merely the top of the biofilm iceberg, since microphytobenthos (MPB) comprise a large diversity of eukaryotic and prokaryotic organisms, which interact in multiple ways and contribute to the functioning of the whole biofilm community (Hubas et al., 2018). The biological functions of EPS in estuarine environments for will be discussed with an emphasis on diatoms. The different roles of EPS in diatom biofilms are summarized in table 1.1 and detailed in the following section.

1.2.3.1 Adhesion to the sediment and substratum stabilization

The first step in microbial colonization of sediment surfaces is the establishment of the biofilm through EPS secretion (Decho, 2000). By creating a biofilm, organisms establish and maintain an association between individual cells and/or between cells and their immediate environment. Although the majority of EPS is produced by photoautotrophs, such as diatoms, heterotrophic bacteria also contribute to the EPS pool (Braissant et al., 2007; Taylor et al.,

2013). EPS can bind sediment particles together by forming adhesive bridges, forming a network that traps and protects the cells (De Winder et al, 1999; Yallop et al., 2000; Sutherland, 2001). EPS can also coat sediment particles, increasing the cohesiveness (Underwood & Paterson, 1993; Tolhurst et al., 2002; Lundkvist et al., 2007; Grabowski et al., 2011) and reducing resuspension and erosion (Paterson, 1995; Decho, 2000; Morelle et al., 2020). EPS can also reduce sediment roughness by filling the space between particles and smoothing the surface of particles (De Winder et al., 1999). All these processes limit sediment erosion and promote sediment stabilization, and consequently, the development of biofilms significantly increases the stability of intertidal mudflats (Paterson, 1989; Sutherland et al., 1998). A number of studies report on the positive effects of diatom biofilms on sediment stability, which is often related to positive correlation between EPS concentrations in the sediment and various properties of sediment stability (Paterson, 1989; Underwood and Paterson 1993; Sutherland et al., 1998; Paterson and Hagerthey 2001; Stal, 2010). Malarkey et al. (2015) experimentally demonstrate that low concentrations of xanthan mixed with the sediment (1%) significantly reduce particle movement and increase the stability of small-scale bedform (e.g., current ripples) development, because EPS inhibit the grains from moving independently. In riverine sediments, Gerbersdorf et al. (2008) demonstrate for both colloidal and bound EPS moieties a strong positive correlation with the critical shear stress for erosion. EPS can influence the sediment properties by binding fine-grained sediment, changing the water content and enhancing the organic composition. Zhou et al. (1998) further show that ratio of sugar monomers may be important in EPS interaction with sediments. These authors suggest that the cohesive properties of the EPS and the ability to form aggregates is predominantly related to rhamnose and fucose monomers. Comparison of EPS stabilizing properties from diatom and bacteria was tested in laboratory flumes (Lundkvist et al. 2007). The diatom EPS increases the biostabilisation by 80%, and bacterial EPS enhances this approximately by 12%. The increase in erosion threshold induces by EPS from benthic diatoms is 120%, and by EPS from bacteria 20%. Diatoms and bacteria combined increase the erosion threshold by 150%, showing the significant potential of interactions within the MPB. By impacting sediment properties through EPS secretion, MPB and in particular the diatom component, may be consider as ecosystem engineers (Paterson & Daborn, 1991; Gerbersdorf et al., 2009).

1.2.3.2 Frustule stabilization

The frustule is coated by thick mucilaginous EPS material approximately 10 nm thick, interrupted only near the raphe (Crawford et al., 2001). The interruption of this particular part can be related to the secretion of EPS by the raphe for the gliding mobility. This EPS coat that encases each cell, composed of bound EPS, is non-adhesive, soft, and compressible and contains a degree of crosslinking that makes it cohesive (Crawford et al., 2001). Bound EPS may have a protective function for the cell and their composition may vary considerably between diatom taxa (Hoagland et al. 1993). EPS has also a role of stabilization and sealing of the different parts of the frustule. Without a thin EPS coat, the frustule easily breaks up into separate parts (Round et al., 1990).

1.2.3.3 Cell motility in response to light and tidal cycles

Diatoms adjust their position by gliding at the sediment surface in response to a range of stimuli, including tidal fluctuation, diurnal rhythm, nutrient availability, and UV-B irradiance (Consalvey et al., 2004; Molino et al., 2016). They migrate towards the surface of the sediment at low tide in order to perform photosynthesis. At high tide, they move down, inside the sediment at a depth ranging from one millimeter to possibly a few centimeters (Consalvey et al., 2004; Hubas et al., 2018; Round & Palmer, 1966). The rate of diatom movement has been measured and theorical distance covered during one hour has been calculated for several benthic diatom species and varies between 0.17-0.28 µm.s⁻¹ (theorical distance: 0.6-1 mm.h⁻¹) vertically and 2.5-9µm.s⁻¹ horizontally (theorical distance: 32-9 mm.h⁻¹; Consalvey et al., 2004). This movement occurs through the secretion of multi-stranded adhesive mucilage tethers composed of specific EPS at the raphe, where, as discuss above, an opening provides the secretion (Higgins et al., 2003). Smith and Underwood (1998) show that diatom secretion of EPS is closely related to their motility and occurred predominantly during the dark period. Gliding is enable through an adhesive complex composed of transmembrane linker molecules between adhesive EPS in contact with the substrate and intracellular motor proteins (e.g., actin filaments; Edgar and Pickett-Heaps, 1984; Molino et al., 2016; Figure 1.14). The gliding force of diatoms is generated by myosin in actin filaments (Poulsen et al., 1999). EPS secretion is continuous as diatoms slide across the surface or into the sediment. The mucilaginous strands break away from the cell and are left behind as "trails" that eventually accumulate as components of the biofilms (Higgins et al., 2003). Diatoms are also able to increase EPS secretion and to migrate vertically in the sediment when UV irradiance or temperature are excessive, in order to avoid potential cell damage (Perkins et al., 2010; Wolfstein & Stal 2002; Fig 4E). UV exposure results in modifications of EPS properties, e.g., a decrease in average molecular size (Decho, 1990).



Figure 1.14: Schematic diagram of a benthic diatom cell with detail of the components of the adhesion complex secreted through the raphe (from Molino et al. 2016). Diatom gliding movement is then generated by myosin actin interactions.

1.2.3.4 Cell protection

Diatoms are particularly sensitive to changes in nutrient concentrations (primarily phosphorus and nitrogen), pH and to organic loads from agricultural and industrial activities (Lavoie & Hamilton, 2008). Tidal flats are variable in chemical composition and physical condition (salinity, temperature etc.) and EPS can act as a buffer maintaining stable conditions (e.g., pH, salinity) in the immediate cell environment (Decho, 1990; Decho 2000). EPS contain high amounts of hydrophilic molecules (e.g., hydroxyl functional groups of polysaccharides), preserving the biofilm hydration and protecting the microorganisms against desiccation, particularly during low tide (Decho, 1990; Potts, 1994). Sulfate functional groups present in EPS have the capacity to retain water molecules that play an important role in imparting a gellike consistency to the EPS (Wingender et al., 1999). Elevated concentrations of some metal species (e.g., cadmium or zinc) are toxic for diatom cells (Morin et al., 2007), resulting in a decrease of their abundance within the biofilm. In the absence of EPS, frustule deformation is

more common when cells are exposed to heavy metals (Gold et al., 2003; Morin et al., 2007; Morin et al., 2012). Mature laboratory biofilms of diatoms increase the EPS production in presence of Cd contamination (Duong et al., 2010). Organic pollutions (e.g., chlordecone) can also induce changes both in the biofilm community and in EPS properties, e.g., sorption and viscoelasticity (Hubas et al., 2022). EPS are rich in negatively charged functional groups (e.g., carboxyls, sulfates; Braissant et al., 2009; Figure 1.15). These functional groups have distinct pKa and deprotonate at various pH (Figure 1.15). As a consequence, the EPS matrix acts as a cation sponge, binding metals (Decho & Gutierrez, 2017). This binding mostly occurs through cation exchange processes, with metal cations substituting for water on EPS. Polysaccharide moieties, including abundant carboxyl and hydroxyl groups, are particularly efficient in metal binding (Decho, 1990). For example, EPS extracted from a marine bacterium culture have the ability to bind metals due to abundant carboxyl moieties (Gutierrez et al., 2012). As a result, molecules absorbed onto EPS do not reach the cells, which is why biofilms can grow in contaminated environments when other organisms often cannot (Sutherland, 2001; Morin et al., 2007). Other factors can result in a change in EPS secretion in order to protect organisms: for example, increased EPS production is shown in the presence of a grazing nematode (Hubas et al., 2010).



Figure 1.15: Diagram showing the functional groups commonly associated with exopolymeric substances and their possible interaction with metal (M^+) from Braissant et al. 2007. pKa ranges for each functional group are indicated in the upper bar and for phosphoryl groups both pK1 and pK2 are considered.

1.2.3.5 Communication and interactions between cells

Quorum sensing (QS) consists of communication and coordination between dense populations of microorganisms in the biofilm through the production, detection and response by cells to signaling molecules (Decho et al., 2010; Decho & Gutierrez, 2017). QS relies on the production and release of signaling molecules, called autoinducers (Camilli & Bassler 2006). The development of biofilms on the sediment surface is thought to be mediated by QS, especially through enhanced EPS production (Decho, 2000; Solano et al., 2014). However, there is a lack of field studies in mudflats to confirm such a hypothesis. Diffusible signaling molecules allow the self-regulation of the microbial community when cells density is high (Decho, 2000). The cells within a biofilm can move and reorient themselves in relation to one another and escape from invasion by other cells, owing to the diffusion of molecular messages (e.g. fatty acid amides) in the EPS (Decho & Gutierrez, 2017). QS can also impact the development of biofilm architecture by intercellular communication (Decho, 2000). Diatoms secrete signaling molecules, such as pheromones, inducing reproduction and transition from mitosis to meiosis (Moeys et al. 2016; Figure 1.16). However, as highlighted by Hubas et al. (2018), the signaling molecules produced by diatoms are poorly known, especially under natural conditions. For example, the synchronized migration of benthic diatoms during diurnal and tidal cycles can be coordinated through cell-to-cell communication in the biofilm matrix. But It is not known how fluctuating conditions (e.g., pH, dessication, high temperature) in natural environments influence chemical signaling and coordination of microbial activities (Decho & Gutierrez, 2017). Inter-microbial species competition for nutrients and space also takes place in the biofilm. For example, the EPS of the benthic diatom *Navicula phyllepta* inhibits the development of some bacterial species (e.g., *Flavobacterium sp.*), or promoting that of others (e.g., *Roseobacter, Shewanella* sp.; Doghri et al., 2017). Diatoms can secrete allelopathic halogenated compounds in the EPS matrix against competing species, as well as antibacterial compounds such as free fatty acids and esters (Hubas et al., 2018). Molecules that may act as signals for some bacteria, may act as antibiotics against other bacteria (Schertzer et al., 2009; Johnson et al., 2016).



Figure 1.16: The life cycle of *Seminavis robusta* linked to sex-inducing pheromone from Moeys et al. (2016). During the vegetative phase, average cell size decreases. Once below a certain cell size, called the sexual size threshold, diatoms become capable of sexual reproduction. When passing this, both homothallic (MT⁻) and heterothallic (MT⁺) reproduction type cells start the production of sex-inducing pheromones, SIP⁺ and SIP⁻ respectively. Under the influence of these pheromones, MT⁻ secretes the attraction pheromone diproline and MT⁺ probably expresses a diproline receptor. Diproline signalling then leads to mate finding and pair formation, after which gametes and zygotes are formed. The resulting auxospore cells, elongate then to initial cell size.

1.2.3.6 Food and micronutrient accumulation source

Due to their sorptive properties, EPS can accumulate metals (e.g., Ca, Si, Fe) and organic micronutrients (e.g., low-molecular weight (LMW) compounds, such as simple sugars or amino acids) that can be used by the biofilm community (Decho, 1990; Gutierrez et al., 2012; Decho & Gutierrez, 2017). Bound EPS contain exo-enzymes responsible for the mobilization and transformation of organic matter, for example, the hydrolysis of large macromolecules (e.g., polysaccharides) into smaller ones (e.g., LMW organic compounds; Decho, 1990). In the biofilm, EPS can be used as a carbon source by bacteria (Underwood & Paterson, 2003). Low molecular EPS components are more rapidly degraded by bacteria than high molecular weight polymers (Smith & Underwood, 1998). Bacteria are the main EPS degraders in the biofilm, with a rapid transfer of carbon fixed by diatoms to the heterotrophic community (Bellinger et al., 2009; Taylor et al., 2013). Up to 60% of photosynthetically fixed carbon can be transferred to heterotrophic bacteria within 36 h (Oakes et al., 2010). *Pseudomonas* sp. and possibly some other EPS making up the biofilm. Similar to bacteria, some diatoms may also assimilate the EPS, or EPS-breakdown products, from other diatom species (Taylor et al., 2013).

1.2.3.7 Sedimentary EPS cycling

In estuaries, EPS is lost from sediments by various mechanisms, e.g., solubilization due to currents, removal by overlying water and bacterial degradation (Underwood & Paterson, 2003). Typically abundant at the sediment surface, the quantity of EPS decreases with depth (Paterson et al., 2000; De Brouwer & Stal, 2001). The main cause of this decrease is likely the degradation of EPS by heterotrophic bacteria. Van Duyl et al. (1999) find a positive correlation between heterotrophic microbial activity and the amount of colloidal EPS secreted by diatoms. Degradation of EPS consists of a multistep process, by sequential removal of different components, ranging from highly labile to relatively refractory parts (Nankai et al., 1999). Although the EPS consumption of heterotrophic bacteria can be important, some prokaryotes also secret a significant amount of their own EPS (e.g., sulfate-reducing bacteria; Braissant et al., 2007). Several studies suggest that the bound EPS fractions represent a refractory pool left behind following the biodegradation of labile, colloidal EPS components (De Winder et al., 1999; De Brouwer & Stal, 2001). This can result in accumulation of refractory, biodegraded EPS deeper in the sediment (Gerbersdorf et al., 2009; Oakes et al., 2010). Aggregates of EPS

and EPS fixed to mineral surfaces may prevent microbial degradation of these polymers (Kleber et al., 2015). A small amount of EPS can be present at depth in the sediment, but the properties seems to differ from the EPS produced at the surface (e.g. reactivity modifications). The "deep" EPS differs from the surface EPS due to a combination of heterotrophic microbial degradation and new production by heterotrophic microorganisms. For example, Braissant et al. (2007) shown that the EPS of sulfate-reducing bacteria have an unusually high content of sulfur, which had not been reported for EPS before. The elevated sulfur content seemed to correlate with increased sulfide concentrations in the sediment.

1.2.3.8 EPS-mineral interactions molecular mechanisms

EPS links to mineral surfaces through an ionic interaction between carboxylate groups (negatively charged) and the edges of the clay platelets (Henao & Mazeau, 2009). Xanthan, frequently used as a model for microbial EPS in laboratory studies, is known to adsorb onto clay depending on the surface properties (e.g., the EPS adsorption is greater on montmorillonite than kaolinite surfaces; Dontsova & Bigham, 2005). The charge of the tetrahedra in clay minerals is positively correlated with xanthan sorption. This indicates the importance of cation bridging in EPS-clay interactions (Dontsova & Bigham, 2005). For example, negatively charged groups in EPS (e.g., carboxylic and sulfate acidic groups) can form bidentate bridges with minerals (e.g., clay minerals, oxides) in the presence of divalent cations (Kleber et al., 2007; Braissant et al. 2009; Ghashoghchi et al., 2017; Fig. 4D). The biofilm community can adapt the production of EPS to alter the interaction with the environment by secreting a larger amount of EPS depending on the type of mineral (Poorni & Natarajan, 2013). For example, a hematite mineral surface increases the amount of proteinaceous compounds in EPS in compared to kaolinite. EPS are therefore very reactive molecules responsible for many vital roles in biofilms. By performing these roles, EPS impact the surrounding environment, especially the physical processes related to sediment deposition. This thesis aims to understand in more detail impacts of EPS secretion in estuaries, especially in deep sediments.

Function of EPS in diatom biofilm	Environmental impact for the microbial community	EPS components involved	References
Sorption of organic compounds	Accumulation of organic nutrients from the environment (e.g., Fe or LMW compounds) and protection against environmental pollutants (e.g., chlordecone).	Polysaccharides and proteins	Decho 1990; Decho, 2000; Gutierrez et al., 2012; Decho & Gutierrez, 2017; Hubas et al., 2022
Sorption of inorganic compounds	Enhance trace element bioavailability from the environment (e.g., iron), protection against environmental pollution (e.g., cadmium) and promotion of polysaccharide gel formation.	Polysaccharides and proteins	Decho, 1990; Decho, 2000; Sutherland, 2001; Morin et al., 2007; Duong et al., 2010
Adhesion, cohesion of biofilm and cell aggregation	Attachement to sediment surface. Spatial organization of the community.	Polysaccharides and proteins	Decho, 1990; De Winder et al., 1999; Decho, 2000; Sutherland, 2001
Water retention	Maintenance of highly hydrated microenvironment around organisms especially during low tide.	Polysaccharides, sulfate esters and proteins	Decho, 1990; Wingender et al., 1999
Micro-environment stabilization	Maintaining stable conditions in the immediate surroundings of cells, protecting against quick environmental changes.	Polysaccharides and proteins	Decho, 1990; Potts, 1994; Decho, 2000; Decho & Gutierrez, 2017
Enzymatic activity	Retention and stabilization of enzymes Enables the digestion of macromolecules for nutrient acquisition.	Proteins and polysaccharides	Decho 1990; Decho, 2000; Decho & Gutierrez, 2017; Hubas et al., 2018
Nutrient sources	Organic carbon source for heterotrophic bacteria within the biofilm.	Potentially all EPS but specifically the LMW colloidal parts	Decho 1990; Van Duyl et al., 1999; Braissant et al., 2009; Underwood & Paterson, 2003; Bellinger et al., 2009; Taylor et al. 2013
Sediment stabilization	Biofilm stabilization, community settlement and protection against erosion.	Proteins and polysaccharides	Paterson, 1995; De Winder et al., 1999; Decho, 2000; Sutherland, 2001; Tolhurst et al., 2002; Lundkvist et al., 2007; Gerbersdorf et al., 2008; Grabowski et al., 2011; Malarkey et al., 2015; Morelle et al., 2020
Motility on the sediment	Protection on the sediment according to environmental stress (temperature, UV, etc.). Adaptation to light condition.	Proteins (actin/myosin) and polysaccharides	Edgar & Pickett-Heaps, 1984; Smith & Underwood, 1998; Poulsen et al., 1999; Higgins et al., 2003; Consalvey et al., 2004; Molino et al., 2016
Communication between cells	Localization of cells, cooperation and/or competition and reproduction within the biofilm.	The whole matrix that transports chemical messages	Decho, 2000; Solano et al., 2014; Moeys et al., 2016; Decho & Gutierrez, 2017; Doghri et al., 2017; Hubas et al., 2018

Table 1.1: Main functions extracellular polymeric substances of MPB biofilm on mudflats (adapted from Flemming & Wingender 2010).

1.3 Summary and research questions addressed in this thesis

This thesis studies (i) the physico-chemical properties of exopolymeric substances in the Gironde estuary, (ii) their preservation with depth in the sedimentary column, and (iii) their potential roles during early diagenesis (e.g., interaction with sedimentary particles and metals). To this aim, we will also investigate the modifications resulting from microbial metabolisms in the sediment, the properties of sediments and the interaction between EPS and sediments and the geochemistry of porewaters. This study aims to serve as a basis for future studies on the role of EPS in estuarine sedimentary systems (e.g., sediment trapping, interaction with metals), to extrapolate to other sedimentary environments (e.g., rivers, deltas), and to apply to early diagenesis and biogeochemical models, and to preservation of biofilm and EPS biosignatures.

Topic 1. Preservation of EPS, evolution of EPS properties and EPS-sediment interactions with depth (Chapter 3).

At the surface of estuarine mudflats, EPS are mainly produced by photoautotrophic microorganisms (e.g., benthic diatoms and cyanobacteria; Taylor et al., 2002). The amount of EPS typically decreases exponentially in the first millimeters to centimeters of the sediment (Taylor et al., 1999; Paterson et al., 2000; Braissant et al. 2009). This decrease may be linked to consumption by heterotrophic bacteria (Taylor et al., 2013; Bohorquez et al. 2017). Studies of EPS preservation are generally limited to a depth of 30 cm because it is assumed that all polymers are totally consumed at depth. Kleber et al. (2015) proposed that EPS can be protected from degradation by inherent recalcitrance of certain EPS parts against consumption or protection through the interactions with minerals. In the sediment, heterotrophic communities (e.g., sulfate-reducing bacteria) are also able to produce their own EPS, albeit at lower production rates than that of photosynthetic organisms (Braissant et al., 2007). The first objective of this thesis will therefore be to study the presence and preservation of EPS in sediment cores taken from the Gironde estuary through the EPS extraction and physicochemical characterization. The study of EPS-mineral interactions will be conducted for the first time to a depth of 6m. The chapter 3 of this thesis aims at answering the following questions:

Q1: Are the EPS preserved at depth in the sediment? If so, how do EPS physicochemical properties evolve?

Q2: Do EPS still interact with sediments at depth? If so, are there modifications of EPS-mineral complexes between the surface and deeper sediments?

Q3: Can we link the preservation of EPS and the sedimentary dynamics in an estuary? In other words, does the preservation of EPS depend on depositional conditions?

Topic 2. Geochemical gradient, microbial activity, EPS and related impacts in the sediment early diagenesis (Chapter 4).

The preservation of EPS in the sediment (Topic 1) could have a major impact on the fate of metals and on biogeochemical cycles. Indeed, EPS possess reactive functional groups (e.g., carboxyls, sulfates) that are able to bind large quantities of cations (e.g., Pace et al., 2018), and can be considered as "metal sponges". Heterotrophs preferentially consume labile forms of EPS, notably low molecular weight compounds (Smith & Underwood, 1998), which can result in the release of adsorbed compounds (e.g., Braissant et al., 2007). As a result, the consumption of EPS leads to changes in porewater chemistry equilibria that could impact the precipitation of mineral phases in sediments. It has also been shown that, in estuarine conditions, the sorption of metals by EPS depends on external parameters such as salinity or pH (Schlekat et al., 1998). Porewater geochemistry allows to monitor the evolution of dissolved chemical elements during sediment early diagenesis and to calculate if mineral phases have a potential to precipitate or to dissolve. This also highlights the presence of gradient changes related to microbial metabolism at depth (O'Sullivan et al., 2013; Hong et al., 2019). Various microbial activities change the chemical environment and impact the fate of metal species in the sediment (e.g., solubility of cadmium or iron). The cycles of some key metal species such as manganese and iron are tightly coupled with the sulfur cycle, and are mediated by microbial activity (Burdige, 1993; Kappler et al. 2021). For example, the sulfide produced by sulfate reducing bacteria (SRB) can precipitate as iron sulfides (Jorgensen & Nelson, 2004; Kraal et al., 2013), leading to a sequestration of sulfur and iron in the sediment. Through their metabolism, SRB also consume EPS, potentially liberating bound cations. Beyond serving as a potential source of energy for heterotrophic microorganisms, EPS can impact the chemistry of the sediment either as nucleus or as inhibitors of mineral precipitation (e.g., Dupraz et al., 2009). For example, bacterial and algal EPS may increase iron solubility, accelerate Fe(II) oxidation (Norman et al., 2015) or bind heavy metals (Hao et al., 2016). The second objective is to better understand the potential interactions between microbial metabolisms, the Gironde sedimentary EPS and the subsurface metal cycles. To this aim, we monitored the porewater geochemical gradients along three estuary cores from an estuarine point bar. The variations of pH, dissolved O₂,

and the concentrations of sulfate, nitrate, sulfide and metals (Ca, Mg, Fe, Mn) with depth allows to the establish the stratification of microbial metabolisms in the sediment. For the first time these hypotheses can be corroborated with measurements microbial activity (colorimetric assays and calorimetry). A conceptual model linking microbial activity, geochemical gradients and potential precipitation/dissolution of mineral phases is proposed in <u>chapter 4</u> of this thesis to answer the main research questions raised in this section:

Q4: How do microorganisms impact sediment geochemistry in the Gironde estuary? Can we link metal concentrations to a stratification of microbial metabolisms at depth? What is the impact of microbial metabolisms on the precipitation/dissolution of mineral phases in the sediments?

Q5: Is there a role for EPS in the precipitation/dissolution of metals in estuarine sediments?

Topic 3. Role of EPS in the aggregation of clay and sand: an experimental approach (Chapter 5).

Many studies have investigated the impact of EPS in sedimentary environments. In estuaries, the presence of EPS in the sediment promotes the cohesiveness and stability of sediments (Tolhurst et al., 2002), impacting bedform morphology (De Winder et al., 1999; Malarkey et al., 2015). The physicochemical properties of minerals, especially clay minerals, are strongly modified by their interactions with EPS (Chenu, 1993). These mechanisms have led to laboratory studies showing that electrostatic force and chemical interactions are the main mechanisms leading to the formation of EPS-mineral complexes (Fang et al., 2012; Lin et al., 2016). For example, xanthan can form bidentate bridges with clay minerals in the presence of divalent cations (Ghashoghchi et al., 2017), and EPS secreted by bacteria can interact with various parts of clay minerals, including the interlayer space (Alimova et al., 2009). In diatom EPS, charged functional groups and neutral oxygen groups form the basis for EPS sorptive and adhesive properties, which allow the development of the biofilm in dynamic intertidal zones (Decho, 2000). Wooldridge et al. (2017) proposed a pivotal role for EPS in the aggregation of clay- and sandy sediments by correlating the distribution of photopigments to the proportion of detrital clay coats in estuaries. However, to date no experimental evidence supports this hypothesis. The third objective of this thesis is therefore to experimentally demonstrate a role of EPS in the aggregation of sedimentary particles in estuaries. Additional analyses of EPS properties will be conducted to understand their physicochemical properties and

roles in the aggregation detrital sedimentary particles and in the formation of clay coats. The main research questions addressed in chapter 5 of this thesis are:

Q6: Do EPS produced in diatom biofilms interact with sedimentary particles (e.g., sand and clay) in the Gironde estuary? If so, what are the products of these interactions?

Q7: What are the possible mechanisms of EPS-mineral interactions in estuarine sediments?

References

- Admiraal, W., 1977, Influence of light and temperature on the growth rate of estuarine benthic diatoms in culture: Marine Biology, v. 39, p. 1–9.
- Alimova, A., Katz, A., Steiner, N., Rudolph, E., Wei, H., Steiner, J.C., and Gottlieb, P., 2009, Bacteria-clay interaction: Structural changes in smectite induced during biofilm formation: Clays and Clay Minerals, v. 57, p. 205–212, doi:10.1346/CCMN.2009.0570207.
- Allen, G.P., 1991, Sedimentary Processes and Facies in the Gironde Estuary: A Recent Model for Macrotidal Estuarine Systems, p. 29–39.
- Allen, G.P., and Posamentier, H.W., 1993a, Sequence stratigraphy and facies model of an incised valley fill; the Gironde Estuary, France: Journal of Sedimentary Research, v. 63, p. 378–391, doi:10.1306/D4267B09-2B26-11D7-8648000102C1865D.
- Allen, G.P., and Posamentier, H.W., 1993b, Sequence stratigraphy and facies model of an incised valley fill; the Gironde Estuary, France: Journal of Sedimentary Research, v. 63, p. 378–391.
- Allen, G.P., Salomon, J.C., Bassoullet, P., Du Penhoat, Y., and de Grandpré, C., 1980, Effects of tides on mixing and suspended sediment transport in macrotidal estuaries: Sedimentary Geology, v. 26, p. 69– 90, doi:10.1016/0037-0738(80)90006-8.
- Allen, G.P., Sauzay, G., and Castaing, P., 1977, Transport and deposition of suspended sediment in the Gironde estuary, France, in Wiley, M. ed., Estuarine Processes, Academic Press, p. 63–81, doi:10.1016/B978-0-12-751802-2.50013-8.
- Aschoff, J.L., Olariu, C., and Steel, R.J., 2018, Recognition and significance of bayhead delta deposits in the rock record: A comparison of modern and ancient systems: Sedimentology, v. 65, p. 62–95.
- Bain, D.C., and Smith, B.F.L., 1994, Chemical analysis, in Wilson, M.J. ed., Clay Mineralogy: Spectroscopic and Chemical Determinative Methods, Dordrecht, Springer Netherlands, p. 300–332, doi:10.1007/978-94-011-0727-3_8.
- Benaouda, A., 2008, Dynamique saisonnière des sédiments en suspension dans l'estuaire de la Gironde: modélisation opérationnelle de la réponse aux forçages hydrodynamiques [These de doctorat]: Bordeaux 1, https://www.theses.fr/2008BOR13618 (accessed April 2022).
- Bergaya, F., Theng, B.K.G., and Lagaly, G., 2013, Handbook of Clay Science: Elsevier.
- Bohórquez, J., McGenity, T.J., Papaspyrou, S., García-Robledo, E., Corzo, A., and Underwood, G.J.C., 2017, Different Types of Diatom-Derived Extracellular Polymeric Substances Drive Changes in Heterotrophic Bacterial Communities from Intertidal Sediments: Frontiers in Microbiology, v. 8, doi:10.3389/fmicb.2017.00245.
- Braissant, O., Decho, A.W., Dupraz, C., Glunk, C., Przekop, K.M., and Visscher, P.T., 2007, Exopolymeric substances of sulfate-reducing bacteria: Interactions with calcium at alkaline pH and implication for formation of carbonate minerals: Geobiology, v. 5, p. 401–411, doi:10.1111/j.1472-4669.2007.00117.x.
- Braissant, O., Decho, A.W., Przekop, K.M., Gallagher, K.L., Glunk, C., Dupraz, C., and Visscher, P.T., 2009, Characteristics and turnover of exopolymeric substances in a hypersaline microbial mat: FEMS Microbiology Ecology, v. 67, p. 293–307, doi: 10.1111/j.1574-6941.2008.00614.x.
- Brigatti, M.F., Galán, E., and Theng, B.K.G., 2013, Chapter 2 Structure and Mineralogy of Clay Minerals, in Bergaya, F. and Lagaly, G. eds., Developments in Clay Science, Elsevier, Handbook of Clay Science, v. 5, p. 21–81, doi:10.1016/B978-0-08-098258-8.00002-X.
- de Brouwer, J., and Stal, L., 2001, Short-term dynamics in microphytobenthos distribution and associated extracellular carbohydrates in surface sediments of an intertidal mudflat: Marine Ecology Progress Series, v. 218, p. 33–44, doi:10.3354/meps218033.
- Brouwer, J.F.C.D., Wolfstein, K., and Stal, L.J., 2002, Physical characterization and diel dynamics of different fractions of extracellular polysaccharides in an axenic culture of a benthic diatom: European Journal of Phycology, v. 37, p. 37–44, doi:10.1017/S0967026201003419.
- Burdige, D.J., 1993, The biogeochemistry of manganese and iron reduction in marine sediments: Earth-Science Reviews, v. 35, p. 249–284, doi:10.1016/0012-8252(93)90040-E.
- Camilli, A., and Bassler, B.L., 2006, Bacterial Small-Molecule Signaling Pathways: Science (New York, N.Y.), v. 311, p. 1113–1116, doi:10.1126/science.1121357.

- Castaing, P., and Allen, G.P., 1981, Mechanisms controlling seaward escape of suspended sediment from the Gironde: A macrotidal estuary in France: Marine Geology, v. 40, p. 101–118, doi:10.1016/0025-3227(81)90045-1.
- Chaumillon, E., Féniès, H., Billy, J., Breilh, J.-F., and Richetti, H., 2013, Tidal and fluvial controls on the internal architecture and sedimentary facies of a lobate estuarine tidal bar (The Plassac Tidal Bar in the Gironde Estuary, France): Marine Geology, v. 346, p. 58–72.
- Chenu, C., 1993, Clay— or sand—polysaccharide associations as models for the interface between microorganisms and soil: water related properties and microstructure, in Brussaard, L. and Kooistra, M.J. eds., Soil Structure/Soil Biota Interrelationships, Amsterdam, Elsevier, p. 143–156, doi:10.1016/B978-0-444-81490-6.50016-9.
- Chiovitti, A., Higgins, M.J., Harper, R.E., Wetherbee, R., and Bacic, A., 2003, The Complex Polysaccharides of the Raphid Diatom Pinnularia Viridis (bacillariophyceae)1: Journal of Phycology, v. 39, p. 543–554, doi:10.1046/j.1529-8817.2003.02162.x.
- Coynel, A., Schäfer, J., Hurtrez, J.-E., Dumas, J., Etcheber, H., and Blanc, G., 2004, Sampling frequency and accuracy of SPM flux estimates in two contrasted drainage basins: Science of The Total Environment, v. 330, p. 233–247, doi: 10.1016/j.scitotenv.2004.04.003.
- Crawford, S.A., Higgins, M.J., Mulvaney, P., and Wetherbee, R., 2001, Nanostructure of the diatom frustule as revealed by atomic force and scanning electron microscopy: Journal of Phycology, v. 37, p. 543–554.
- Croteau, T., Bertram, A.K., and Patey, G.N., 2009, Simulation of Water Adsorption on Kaolinite under Atmospheric Conditions: The Journal of Physical Chemistry A, v. 113, p. 7826–7833, doi:10.1021/jp902453f.
- Dalrymple, R.W., and Choi, K., 2007, Morphologic and facies trends through the fluvial-marine transition in tide-dominated depositional systems: A schematic framework for environmental and sequencestratigraphic interpretation: Earth-Science Reviews, v. 81, p. 135–174, doi: 10.1016/j.earscirev.2006.10.002.
- De Winder, B., Staats, N., Stal, L.J., and Paterson, D.M., 1999, Carbohydrate secretion by phototrophic communities in tidal sediments: Journal of Sea Research, v. 42, p. 131–146.
- Decho, A.W., 2000, Microbial biofilms in intertidal systems: an overview: Continental shelf research, v. 20, p. 1257–1273.
- Decho, A., 1990, Microbial exopolymer secretions in ocean environments: their role (s) in food webs and marine processes: Oceanogr. Mar. Biol. Annu. Rev., v. 28, p. 73–153.
- Decho, A.W., and Gutierrez, T., 2017, Microbial extracellular polymeric substances (EPSs) in ocean systems: Frontiers in microbiology, v. 8, p. 922.
- Decho, A.W., Norman, R.S., and Visscher, P.T., 2010, Quorum sensing in natural environments: emerging views from microbial mats: Trends in Microbiology, v. 18, p. 73–80, doi: 10.1016/j.tim.2009.12.008.
- Doghri, I., Lavaud, J., Dufour, A., Bazire, A., Lanneluc, I., and Sablé, S., 2017, Cell-bound exopolysaccharides from an axenic culture of the intertidal mudflat Navicula phyllepta diatom affect biofilm formation by benthic bacteria: Journal of Applied Phycology, v. 29, p. 165–177, doi:10.1007/s10811-016-0943-z.
- Dontsova, K.M., and Bigham, J.M., 2005, Anionic Polysaccharide Sorption by Clay Minerals: Soil Science Society of America Journal, v. 69, p. 1026–1035, doi:10.2136/sssaj2004.0203.
- Duong, T.T., Morin, S., Coste, M., Herlory, O., Feurtet-Mazel, A., and Boudou, A., 2010, Experimental toxicity and bioaccumulation of cadmium in freshwater periphytic diatoms in relation with biofilm maturity: Science of The Total Environment, v. 408, p. 552–562, doi: 10.1016/j.scitotenv.2009.10.015.
- Dupraz, C., Reid, R.P., Braissant, O., Decho, A.W., Norman, R.S., and Visscher, P.T., 2009, Processes of carbonate precipitation in modern microbial mats: Earth-Science Reviews, v. 96, p. 141–162, doi: 10.1016/j.earscirev.2008.10.005.
- Dyer, K.R., 1988, Fine Sediment Particle Transport in Estuaries, in Dronkers, J. and van Leussen, W. eds., Physical Processes in Estuaries, Berlin, Heidelberg, Springer, p. 295–310, doi:10.1007/978-3-642-73691-9_16.
- Dyer, K.R., 1997, Partially mixed and well-mixed estuaries: Estuaries—a physical introduction, 2nd edition, eds. John Wiley and Sons, p. 136–164.
- Ehrenberg, S.N., 1993, Preservation of anomalously high porosity in deeply buried sandstones by graincoating chlorite: examples from the Norwegian continental shelf: AAPG Bulletin, v. 77, p. 1260–1286.

- Fang, L., Cao, Y., Huang, Q., Walker, S.L., and Cai, P., 2012, Reactions between bacterial exopolymers and goethite: A combined macroscopic and spectroscopic investigation: Water Research, v. 46, p. 5613– 5620, doi: 10.1016/j.watres.2012.07.046.
- Fenies, H., Resseguier, A.D., and Tastet, J.-P., 1999, Intertidal clay-drape couplets (Gironde estuary, France): Sedimentology, v. 46, p. 1–15, doi:10.1046/j.1365-3091.1999.00196.x.
- Ferrage, E., Lanson, B., Michot, L.J., and Robert, J.-L., 2010, Hydration Properties and Interlayer Organization of Water and Ions in Synthetic Na-Smectite with Tetrahedral Layer Charge. Part 1. Results from X-ray Diffraction Profile Modeling: The Journal of Physical Chemistry C, v. 114, p. 4515–4526, doi:10.1021/jp909860p.
- Flemming, H.-C., and Wingender, J., 2010, The biofilm matrix: Nature reviews microbiology, v. 8, p. 623-633.
- Ghashoghchi, R.A., Hosseini, M.R., and Ahmadi, A., 2017, Effects of microbial cells and their associated extracellular polymeric substances on the bio-flocculation of kaolin and quartz: Applied Clay Science, v. 138, p. 81–88, doi: 10.1016/j.clay.2017.01.002.
- Gold, C., Feurtet-Mazel, A., Coste, M., and Boudou, A., 2003, Effects of cadmium stress on periphytic diatom communities in indoor artificial streams: Freshwater Biology, v. 48, p. 316–328, doi:10.1046/j.1365-2427.2003.00980.x.
- Grabowski, R.C., Droppo, I.G., and Wharton, G., 2011, Erodibility of cohesive sediment: The importance of sediment properties: Earth-Science Reviews, v. 105, p. 101–120.
- Gutierrez, T., Biller, D.V., Shimmield, T., and Green, D.H., 2012, Metal binding properties of the EPS produced by Halomonas sp. TG39 and its potential in enhancing trace element bioavailability to eukaryotic phytoplankton: BioMetals, v. 25, p. 1185–1194, doi:10.1007/s10534-012-9581-3.
- Hao, L. et al., 2016, Binding of heavy metal ions in aggregates of microbial cells, EPS and biogenic iron minerals measured in-situ using metal- and glycoconjugates-specific fluorophores: Geochimica et Cosmochimica Acta, v. 180, p. 66–96, doi: 10.1016/j.gca.2016.02.016.
- Hayes, M.O., 1980, General morphology and sediment patterns in tidal inlets: Sedimentary Geology, v. 26, p. 139–156, doi:10.1016/0037-0738(80)90009-3.
- Henao, L.J., and Mazeau, K., 2009, Molecular modelling studies of clay–exopolysaccharide complexes: Soil aggregation and water retention phenomena: Materials Science and Engineering: C, v. 29, p. 2326– 2332, doi: 10.1016/j.msec.2009.06.001.
- Higgins, M.J., Molino, P., Mulvaney, P., and Wetherbee, R., 2003, The Structure and Nanomechanical Properties of the Adhesive Mucilage That Mediates Diatom-Substratum Adhesion and Motility1: Journal of Phycology, v. 39, p. 1181–1193, doi:10.1111/j.0022-3646.2003.03-027.x.
- Hoagland, K.D., Rosowski, J.R., Gretz, M.R., and Roemer, S.C., 1993, Diatom Extracellular Polymeric Substances: Function, Fine Structure, Chemistry, and Physiology: Journal of Phycology, v. 29, p. 537– 566, doi:10.1111/j.0022-3646.1993.00537.x.
- Hoek, C., Mann, D., Jahns, H.M., and Jahns, M., 1995, Algae: an introduction to phycology: Cambridge university press.
- Hong, Y., Wu, J., Wilson, S., and Song, B., 2019, Vertical Stratification of Sediment Microbial Communities Along Geochemical Gradients of a Subterranean Estuary Located at the Gloucester Beach of Virginia, United States: Frontiers in Microbiology, v. 9, https://www.frontiersin.org/article/10.3389/fmicb.2018.03343 (accessed May 2022).
- Hubas, C. et al., 2022, Chlordecone-contaminated epilithic biofilms show increased adsorption capacities: Science of The Total Environment, v. 825, p. 153942, doi: 10.1016/j.scitotenv.2022.153942.
- Hubas, C., Passarelli, C., and Paterson, D.M., 2018, Microphytobenthic biofilms: composition and interactions, in Mudflat ecology, Springer, p. 63–90.
- Hubas, C., Sachidhanandam, C., Rybarczyk, H., Lubarsky, H., Rigaux, A., Moens, T., and Paterson, D., 2010, Bacterivorous nematodes stimulate microbial growth and exopolymer production in marine sediment microcosms: Marine Ecology Progress Series, v. 419, p. 85–94, doi:10.3354/meps08851.
- Jalón-Rojas, I., Schmidt, S., and Sottolichio, A., 2015, Turbidity in the fluvial Gironde Estuary (southwest France) based on 10-year continuous monitoring: sensitivity to hydrological conditions: Hydrology and Earth System Sciences, v. 19, p. 2805–2819, doi: 10.5194/hess-19-2805-2015.
- Jalón-Rojas, I., Sottolichio, A., Hanquiez, V., Fort, A., and Schmidt, S., 2018, To What Extent Multidecadal Changes in Morphology and Fluvial Discharge Impact Tide in a Convergent (Turbid) Tidal River: Journal of Geophysical Research: Oceans, v. 123, p. 3241–3258, doi:10.1002/2017JC013466.

- Johnston, C.T., and Premachandra, G.S., 2001, Polarized ATR-FTIR Study of Smectite in Aqueous Suspension: Langmuir, v. 17, p. 3712–3718, doi:10.1021/la010184a.
- Jørgensen, B.B., and Nelson, D.C., 2004, Sulfide oxidation in marine sediments: Geochemistry meets microbiology:, doi:10.1130/0-8137-2379-5.63.
- Kappler, A., Bryce, C., Mansor, M., Lueder, U., Byrne, J.M., and Swanner, E.D., 2021, An evolving view on biogeochemical cycling of iron: Nature Reviews Microbiology, v. 19, p. 360–374, doi:10.1038/s41579-020-00502-7.
- Kleber, M., Eusterhues, K., Keiluweit, M., Mikutta, C., Mikutta, R., and Nico, P.S., 2015, Chapter One -Mineral–Organic Associations: Formation, Properties, and Relevance in Soil Environments, in Sparks, D.L. ed., Advances in Agronomy, Academic Press, v. 130, p. 1–140, doi: 10.1016/bs.agron.2014.10.005.
- Kleber, M., Sollins, P., and Sutton, R., 2007, A conceptual model of organo-mineral interactions in soils: selfassembly of organic molecular fragments into zonal structures on mineral surfaces: Biogeochemistry, v. 85, p. 9–24, doi:10.1007/s10533-007-9103-5.
- Kraal, P., Burton, E.D., and Bush, R.T., 2013, Iron monosulfide accumulation and pyrite formation in eutrophic estuarine sediments: Geochimica et Cosmochimica Acta, v. 122, p. 75–88, doi: 10.1016/j.gca.2013.08.013.
- Kröger, N., Bergsdorf, C., and Sumper, M., 1994, A new calcium binding glycoprotein family constitutes a major diatom cell wall component.: The EMBO Journal, v. 13, p. 4676–4683, doi:10.1002/j.1460-2075.1994.tb06791.x.
- Lange-Bertalot, H., Hofmann, G., Werum, M., Cantonati, M., and Kelly, M.G., 2017, Freshwater benthic diatoms of Central Europe: over 800 common species used in ecological assessment: Koeltz Botanical Books Schmitten-Oberreifenberg, v. 942.
- Lavoie, I., and Hamilton, P.B., 2008, Guide d'identification des diatomées des rivières de l'Est du Canada: PUQ.
- Lin, D., Ma, W., Jin, Z., Wang, Y., Huang, Q., and Cai, P., 2016, Interactions of EPS with soil minerals: A combination study by ITC and CLSM: Colloids and Surfaces B: Biointerfaces, v. 138, p. 10–16, doi: 10.1016/j.colsurfb.2015.11.026.
- Lundkvist, M., Grue, M., Friend, P.L., and Flindt, M.R., 2007, The relative contributions of physical and microbiological factors to cohesive sediment stability: Continental Shelf Research, v. 27, p. 1143–1152, doi: 10.1016/j.csr.2006.01.021.
- MacIntyre, H.L., Geider, R.J., and Miller, D.C., 1996, Microphytobenthos: the ecological role of the "secret garden" of unvegetated, shallow-water marine habitats. I. Distribution, abundance and primary production: Estuaries, v. 19, p. 186–201.
- Madejová, J., Gates, W.P., and Petit, S., 2017, Chapter 5 IR Spectra of Clay Minerals, in Gates, W.P., Kloprogge, J.T., Madejová, J., and Bergaya, F. eds., Developments in Clay Science, Elsevier, Infrared and Raman Spectroscopies of Clay Minerals, v. 8, p. 107–149, doi:10.1016/B978-0-08-100355-8.00005-9.
- Malarkey, J. et al., 2015, The pervasive role of biological cohesion in bedform development: Nature Communications, v. 6, p. 6257, doi:10.1038/ncomms7257.
- Mann, D.G., and Droop, S.J.M., 1996, Biodiversity, biogeography and conservation of diatoms, in Biogeography of freshwater algae, Springer, p. 19–32.
- Martinius, A.W., and Van den Berg, J.H., 2011, Atlas of sedimentary structures in estuarine and tidallyinfluenced river deposits of the Rhine-Meuse-Scheldt system: their application to the interpretation of analogus outcrop and subsurface depositional systems: Houten, EAGE publications.
- Marvasi, M., Visscher, P.T., Perito, B., Mastromei, G., and Casillas-Martínez, L., 2010, Physiological requirements for carbonate precipitation during biofilm development of Bacillus subtilis etfA mutant: FEMS Microbiology Ecology, v. 71, p. 341–350, doi:10.1111/j.1574-6941.2009.00805.x.
- Migniot, C., 1968, Étude des propriétés physiques de différents sédiments très fins et de leur comportement sous des actions hydrodynamiques: La Houille Blanche, p. 591–620, doi: 10.1051/lhb/1968041.
- Moeys, S. et al., 2016, A sex-inducing pheromone triggers cell cycle arrest and mate attraction in the diatom Seminavis robusta: Scientific Reports, v. 6, p. 19252, doi:10.1038/srep19252.
- Molino, P.J., Chiovitti, A., Higgins, M.J., Dugdale, T.M., and Wetherbee, R., 2016, Diatom Adhesives: Molecular and Mechanical Properties, in Smith, A.M. ed., Biological Adhesives, Cham, Springer International Publishing, p. 57–86, doi:10.1007/978-3-319-46082-6_3.

- Morelle, J., Claquin, P., and Orvain, F., 2020, Evidence for better microphytobenthos dynamics in mixed sand/mud zones than in pure sand or mud intertidal flats (Seine estuary, Normandy, France): PLOS ONE, v. 15, p. e0237211, doi: 10.1371/journal.pone.0237211.
- Morin, S. et al., 2012, Consistency in Diatom Response to Metal-Contaminated Environments, in Guasch, H., Ginebreda, A., and Geiszinger, A. eds., Emerging and Priority Pollutants in Rivers, Berlin, Heidelberg, Springer Berlin Heidelberg, The Handbook of Environmental Chemistry, v. 19, p. 117–146, doi:10.1007/978-3-642-25722-3 5.
- Morin, S., Vivas-Nogues, M., Duong, T.T., Boudou, A., Coste, M., and Delmas, F. ois, 2007, Dynamics of benthic diatom colonization in a cadmium/zinc-polluted river (Riou-Mort, France): Fundamental and Applied Limnology, p. 179–187, doi:10.1127/1863-9135/2007/0168-0179.
- Musial, G., Reynaud, J.-Y., Gingras, M.K., Féniès, H., Labourdette, R., and Parize, O., 2012, Subsurface and outcrop characterization of large tidally influenced point bars of the Cretaceous McMurray Formation (Alberta, Canada): Sedimentary Geology, v. 279, p. 156–172.
- Nankai, H., Hashimoto, W., Miki, H., Kawai, S., and Murata, K., 1999, Microbial System for Polysaccharide Depolymerization: Enzymatic Route for Xanthan Depolymerization by Bacillus sp. Strain GL1: Applied and Environmental Microbiology, v. 65, p. 2520–2526.
- Nielsen, P.H., and Jahn, A., 1999, Extraction of EPS, in Microbial extracellular polymeric substances, Springer, p. 49–72.
- Norman, L., Worms, I.A.M., Angles, E., Bowie, A.R., Nichols, C.M., Ninh Pham, A., Slaveykova, V.I., Townsend, A.T., David Waite, T., and Hassler, C.S., 2015, The role of bacterial and algal exopolymeric substances in iron chemistry: Marine Chemistry, v. 173, p. 148–161, doi: 10.1016/j.marchem.2015.03.015.
- Nulens, K., Toufar, H., Janssens, G.O.A., and Schoonheydt, R., 1998, Clay Minerals and Clay Mineral-water Interaction: A Combined EEM-Monte Carlo Study: The Latest Frontiers of the Clay Chemistry, Proceedings on the Chemistry of Clays and Clay Minerals, https://lirias.kuleuven.be/1754199 (accessed April 2022).
- O'Sullivan, L.A., Sass, A.M., Webster, G., Fry, J.C., Parkes, R.J., and Weightman, A.J., 2013, Contrasting relationships between biogeochemistry and prokaryotic diversity depth profiles along an estuarine sediment gradient: FEMS Microbiology Ecology, v. 85, p. 143–157, doi:10.1111/1574-6941.12106.
- Pace, A., Bourillot, R., Bouton, A., Vennin, E., Braissant, O., Dupraz, C., Duteil, T., Bundeleva, I., Patrier, P., and Galaup, S., 2018, Formation of stromatolite lamina at the interface of oxygenic–anoxygenic photosynthesis: Geobiology, v. 16, p. 378–398.
- Passarelli, C., Meziane, T., Thiney, N., Boeuf, D., Jesus, B., Ruivo, M., Jeanthon, C., and Hubas, C., 2015, Seasonal variations of the composition of microbial biofilms in sandy tidal flats: Focus of fatty acids, pigments and exopolymers: Estuarine, Coastal and Shelf Science, v. 153, p. 29–37, doi: 10.1016/j.ecss.2014.11.013.
- Paterson, D., and Daborn, G., 1991, Sediment Stabilisation by Biological Action, in Peregrine, D.H. and Loveless, J.H. eds., Developments in Coastal Engineering, University of Bristol, p. 111–120.
- Paterson, D.M., Tolhurst, T.J., Kelly, J.A., Honeywill, C., De Deckere, E., Huet, V., Shayler, S.A., Black, K.S., De Brouwer, J., and Davidson, I., 2000, Variations in sediment properties, Skeffling mudflat, Humber Estuary, UK: Continental Shelf Research, v. 20, p. 1373–1396.
- Paulmier, G., 1997, Atlas des Diatomophycées des côtes françaises et des aires marines adjacentes:
- Perkins, R.G., Lavaud, J., Serôdio, J., Mouget, J.L., Cartaxana, P., Rosa, P., Barille, L., Brotas, V., and Jesus, B.M., 2010, Vertical cell movement is a primary response of intertidal benthic biofilms to increasing light dose: Marine Ecology Progress Series, v. 416, p. 93–103, doi:10.3354/meps08787.
- Perkins, R.G., Mouget, J.-L., Lefebvre, S., and Lavaud, J., 2006, Light response curve methodology and possible implications in the application of chlorophyll fluorescence to benthic diatoms: Marine Biology, v. 149, p. 703–712, doi:10.1007/s00227-005-0222-z.
- Poorni, S., and Natarajan, K.A., 2013, Microbially induced selective flocculation of hematite from kaolinite: International Journal of Mineral Processing, v. 125, p. 92–100, doi: 10.1016/j.minpro.2013.10.002.
- Potts, M., 1994, Desiccation tolerance of prokaryotes: Microbiological Reviews, v. 58, p. 755-805, doi:10.1128/mr.58.4.755-805.1994.
- Poulsen, N.C., Spector, I., Spurck, T.P., Schultz, T.F., and Wetherbee, R., 1999, Diatom gliding is the result of an actin-myosin motility system: Cell Motility, v. 44, p. 23–33, doi: 10.1002/(SICI)1097-0169(199909)44:1<23::AID-CM2>3.0.CO;2-D.

- Pritchard, D.W., 1967, What is an estuary: Physical Viewpoint: https://tamug-ir.tdl.org/handle/1969.3/24383 (accessed April 2022).
- Qin, T., Gutu, T., Jiao, J., Chang, C.-H., and Rorrer, G.L., 2008, Photoluminescence of silica nanostructures from bioreactor culture of marine diatom Nitzschia frustulum: Journal of nanoscience and nanotechnology, v. 8, p. 2392–2398.
- Round, F.E., 1984, The ecology of algae: CUP Archive.
- Round, F.E., Crawford, R.M., and Mann, D.G., 1990, Diatoms: biology and morphology of the genera: Cambridge university press.
- Round, F.E., and Palmer, J.D., 1966, Persistent, vertical-migration rhythms in benthic microflora.: II. Field and Laboratory Studies On Diatoms From The Banks Of The River Avon: Journal of the Marine Biological Association of the United Kingdom, v. 46, p. 191–214, doi:10.1017/S0025315400017641.
- Saïag, J., Brigaud, B., Portier, É., Desaubliaux, G., Bucherie, A., Miska, S., and Pagel, M., 2016, Sedimentological control on the diagenesis and reservoir quality of tidal sandstones of the Upper Cape Hay Formation (Permian, Bonaparte Basin, Australia): Marine and Petroleum Geology, v. 77, p. 597– 624.
- Schlekat, C.E., Decho, A.W., and Chandler, G.T., 1998, Sorption of cadmium to bacterial extracellular polymeric sediment coatings under estuarine conditions: Environmental Toxicology and Chemistry, v. 17, p. 1867–1874, doi:10.1002/etc.5620170930.
- Schmidt, S., Etcheber, H., Sottolichio, A., and Castaing, P., 2016, Le réseau MAGEST: bilan de 10 ans de suivi haute-fréquence de la qualité des eaux de l'estuaire de la Gironde: Mesures haute résolution dans l'environnement marin côtier. Presses du CNRS.
- Schoonheydt, R.A., and Johnston, C.T., 2013, Chapter 5 Surface and Interface Chemistry of Clay Minerals, in Bergaya, F. and Lagaly, G. eds., Developments in Clay Science, Elsevier, Handbook of Clay Science, v. 5, p. 139–172, doi:10.1016/B978-0-08-098258-8.00005-5.
- Sims, P.A., Mann, D.G., and Medlin, L.K., 2006, Evolution of the diatoms: insights from fossil, biological and molecular data: Phycologia, v. 45, p. 361–402.
- Smith, D.J., and Underwood, G.J., 1998, Exopolymer production by intertidal epipelic diatoms: Limnology and Oceanography, v. 43, p. 1578–1591.
- Smith, D.J., and Underwood, G.J.C., 2000, The production of extracellular carbohydrates by estuarine benthic diatoms: the effects of growth phase and light and dark treatment: Journal of Phycology, v. 36, p. 321–333, doi:10.1046/j.1529-8817.2000.99148.x.
- Sottolichio, A., Hurther, D., Gratiot, N., and Bretel, P., 2011, Acoustic turbulence measurements of near-bed suspended sediment dynamics in highly turbid waters of a macrotidal estuary: Continental Shelf Research, v. 31, p. S36–S49, doi: 10.1016/j.csr.2011.03.016.
- Staats, N., Stal, L.J., and Mur, L.R., 2000, Exopolysaccharide production by the epipelic diatom Cylindrotheca closterium: effects of nutrient conditions: Journal of Experimental Marine Biology and Ecology, v. 249, p. 13–27, doi:10.1016/S0022-0981(00)00166-0.
- Staats, N., Winder, B.D., Stal, L.J., and Mur, L.R., 1999, Isolation and characterization of extracellular polysaccharides from the epipelic diatoms Cylindrotheca closterium and Navicula salinarum: European Journal of Phycology, v. 34, p. 161–169, doi:10.1080/09670269910001736212.
- Sutherland, I.W., 2001, Exopolysaccharides in biofilms, flocs and related structures: Water Science and Technology, v. 43, p. 77-86.
- Sutherland, T.F., Grant, J., and Amos, C.L., 1998, The effect of carbohydrate production by the diatom Nitzschia curvilineata on the erodibility of sediment: Limnology and Oceanography, v. 43, p. 65–72, doi:10.4319/lo.1998.43.1.0065.
- Taylor, J.D., McKew, B.A., Kuhl, A., McGenity, T.J., and Underwood, G.J.C., 2013, Microphytobenthic extracellular polymeric substances (EPS) in intertidal sediments fuel both generalist and specialist EPSdegrading bacteria: Limnology and Oceanography, v. 58, p. 1463–1480, doi:10.4319/lo.2013.58.4.1463.
- Taylor, I.S., and Paterson, D.M., 1998, Microspatial Variation in Carbohydrate Concentrations with Depth in the Upper Millimetres of Intertidal Cohesive Sediments: Estuarine, Coastal and Shelf Science, v. 46, p. 359–370, doi:10.1006/ecss.1997.0288.
- Taylor, I.S., Paterson, D.M., and Mehlert, A., 1999, The quantitative variability and monosaccharide composition of sediment carbohydrates associated with intertidal diatom assemblages: Biogeochemistry, v. 45, p. 303–327, doi:10.1007/BF00993005.

- Tolhurst, T.J., Gust, G., and Paterson, D.M., 2002, The influence of an extracellular polymeric substance (EPS) on cohesive sediment stability, in Proceedings in marine science, Elsevier, v. 5, p. 409–425.
- Underwood, G., and Kromkamp, J., 1999, Primary Production by Phytoplankton and Microphytobenthos in Estuaries. Advances in Ecological# 12 in G/WResearch: Elsevier.
- Underwood, G.J., and Paterson, D.M., 1993, Seasonal changes in diatom biomass, sediment stability and biogenic stabilization in the Severn Estuary: Journal of the Marine Biological Association of the United Kingdom, v. 73, p. 871–887.
- Underwood, G.J.C., and Paterson, D.M., 2003, The importance of extracellular carbohydrate productionby marine epipelic diatoms, in Advances in Botanical Research, Academic Press, v. 40, p. 183–240, doi:10.1016/S0065-2296(05)40005-1.
- Underwood, G.J.C., Paterson, D.M., and Parkes, R.J., 1995, The measurement of microbial carbohydrate exopolymers from intertidal sediments: Limnology and Oceanography, v. 40, p. 1243–1253.
- Van Olphen, H., and Fripiar, J.J., 1981, Data Handbook for Clay Materials and Other Non-Metallic Minerals: Soil Science, v. 131, p. 62.
- Verney, R., Brun-Cottan, J.-C., Lafite, R., Deloffre, J., and Taylor, J.A., 2006, Tidally-induced shear stress variability above intertidal mudflats in the macrotidal seine estuary: Estuaries and Coasts, v. 29, p. 653–664, doi:10.1007/BF02784290.
- Virolle, M., Brigaud, B., Beaufort, D., Patrier, P., Abdelrahman, E., Thomas, H., Portier, E., Samson, Y., Bourillot, R., and Féniès, H., 2022, Authigenic berthierine and incipient chloritization in shallowly buried sandstone reservoirs: Key role of the source-to-sink context: GSA Bulletin, v. 134, p. 739–761, doi:10.1130/B35865.1.
- Virolle, M., Brigaud, B., Bourillot, R., Féniès, H., Portier, E., Duteil, T., Nouet, J., Patrier, P., and Beaufort, D., 2019a, Detrital clay grain coats in estuarine clastic deposits: Origin and spatial distribution within a modern sedimentary system, the Gironde Estuary (south-west France): Sedimentology, v. 66, p. 859– 894.
- Virolle, M., Brigaud, B., Féniès, H., Bourillot, R., Portier, E., Patrier, P., Derriennic, H., and Beaufort, D., 2021, Preservation and distribution of detrital clay coats in a modern estuarine heterolithic point bar in the Gironde estuary (Bordeaux, France): Journal of Sedimentary Research, v. 91, p. 812–832, doi:10.2110/jsr.2020.146.
- Virolle, M., Brigaud, B., Luby, S., Portier, É., Féniès, H., Bourillot, R., Patrier, P., and Beaufort, D., 2019b, Influence of sedimentation and detrital clay grain coats on chloritized sandstone reservoir qualities: Insights from comparisons between ancient tidal heterolithic sandstones and a modern estuarine system: Marine and Petroleum Geology, v. 107, p. 163–184.
- Wentworth, C.K., 1922, A scale of grade and class terms for clastic sediments: The journal of geology, v. 30, p. 377–392.
- Wooldridge, L.J., Worden, R.H., Griffiths, J., Thompson, A., and Chung, P., 2017, Biofilm origin of claycoated sand grains: Geology, v. 45, p. 875–878, doi:10.1130/G39161.1.
- Wotton, R.S., 2004, The utiquity and many roles of exopolymers (EPS) in aquatic systems: Scientia marina, v. 68, p. 13–21.
- Yallop, M.L., Paterson, D.M., and Wellsbury, P., 2000, Interrelationships between Rates of Microbial Production, Exopolymer Production, Microbial Biomass, and Sediment Stability in Biofilms of Intertidal Sediments: Microbial Ecology, v. 39, p. 116–127, doi:10.1007/s002489900186.



Chapter 2: Material and methods


Table of contents

Chapter 2: Material and methods55					
2.1	Methodology and its specificities relative to the Gironde estuary	58			
2.2	Comparison of EPS extraction procedures and quality of the purified material	62			
2.3	Sediment sampling and measurements	67			
2.3.1	Sedimentary core extraction	67			
2.3.2	Reference mineral material	68			
2.3.3	Surface sediment and core samples	68			
2.3.4	FTIR spectroscopy (EPS and clay minerals)	69			
2.3.5	Grain size analyzes	70			
2.3.6	Measurements of microbial activity in the sediment	70			
2.4	Water chemistry	71			
2.4.1	Porewater sampling	71			
2.4.2	Porewater chemical analyzes	72			
2.4.3	Porewater geochemical modeling	73			
2.5	EPS extraction and characterization	74			
2.5.1	EPS extraction	74			
2.5.2	EPS physico-chemistry: colorimetric assays	74			
2.5.3	FTIR spectroscopy on EPS	75			
2.5.4	EPS acid-base titration	76			
2.5.5	EPS cation binding capacity	77			
2.6	Microscopy	77			
2.6.1	Cryo-scanning electronic microscopy	77			
2.6.2	Optical microscopy: petrographic thin section and microbiological glass slides	78			
2.6.3	Atomic force microscopy	79			
Referen	nces	80			
Annexe	28	83			
Ann	ex 1: EPS extraction protocol	83			
Ann	ex 2: Alcian Blue assay protocol	84			
Annex 3: Phenol sulfuric assay protocol85					
Annex 4 EPS acid-base titration protocol					
Annex 5 Fluorescein Diacetate (FDA) assay protocol					
Annex 6 Triphenyltetrazolium (TTC) assay protocol					
Annex 7 Sediments preparation for analysis and experiment					
Annex 8 Sediments preparation for Granulometry					
Annex 9 Diatom F/2 medium					

2.1 Methodology and its specificities relative to the Gironde estuary

This section aims to give an overview of the protocols used in this thesis, even if specific methodological sections are detailed in other sections and associated scientific papers. We will also explain the adaptations that have been made to adapt to the conditions in the Gironde estuary. This work benefitted from collaborations with several laboratories and institutes. Table 2.1 summarizes the methods, the instruments, the laboratories where the analyses were done and the people involved. My first experience with EPS consisted in the purification and physicochemical characterization of EPS from hypersaline microbial mats from Great Salt Lake and Cuba during my master thesis (parts of the results were published in Pace et al., 2018). In this thesis, the protocol for EPS extraction had to be modified because of the high proportions of detrital sediments in the samples, specifically silts and clay. In order to obtain higher EPS yields we added additional steps (e.g., ultrasonication) to our initial EPS extraction protocol.

Analyzes	Studied objects and objectives	Method	Instrument	Locality	People involved
Biofilm sampling	Surface diatom biofilm for community characterization and EPS extraction	Surface scraping to sample the biofilm and reduce the quantity of sediment	Scraper	Bordeaux North point bar	R. Bourillot T. Duteil
EPS extraction and purification	Extract purified exopolymeric substances (EPS)	Centrifugation, ultrasonication and ethanol precipitation	Centrifuge, ultrasonic bath, dialysis bags	EPOC, Bordeaux	P.T. VisscherO. BraissantP. DésveronnièresE. DurandT. Duteil
EPS physico-chemical characterization	Determine the quantity, reactivity and physico-chemical properties of EPS	Fourier Transform InfraRed spectroscopy (FTIR)	Nicolet IS50 (ThermoScientific) spectrometer in attenuated total reflection (ATR) mode	IC2MP, Poitiers	B. Grégoire T. Duteil
		Colorimetric assays: Alcian Blue (acidic sites) Phenol sulfuric (neutral sugars) and Lowry (proteins)	Reagents, spectrophotometer, standards (xanthan, albumin)	EPOC, Bordeaux	P.T. Visscher O. Braissant R. Bourillot T. Duteil
		Isothermal Calorimetry	TAM III ITC (Waters / TA instruments, Delaware, USA)	Center for Biomechanics and Biocalorimetry, Basel	O. Braissant T. Duteil
Sediment core sampling	Sample sedimentary cores (up to 6 meters long) for subsequent	Piston-coring, box-coring. Access to the field site by boat	Portable vibro-corer, tubes, box core	Bordeaux North point bar EPOC, Bordeaux	H. Derriennic M. Virolle E. Portier H. Féniès R. Bourillot T. Duteil
Core opening and photographs	Open the cores for subsequent sediment and EPS analyzes, take high resolution photographs		Core-opening saw, photography bench	EPOC, Bordeaux	I. Billy O. Ther
Sedimentological description	Sedimentological description and interpretation of cores	Observation		EPOC, Bordeaux	E. Portier H. Fenies

					T. Duteil
Core analyzes	Semi-quantification of major and minor elements (Al, Si, S, K, Ca, Mn, Fe, Rb, Zn, Pb)	X-ray fluorescence spectrometry (XRF)	XRF Avaatech bench	EPOC, Bordeaux	I. Billy O. Ther
Sediments properties	Characterization of sediment properties: grain size, total organic carbon	Laser granulometry	Mastersizer 2000 (Malvern, UK)	EPOC, Bordeaux	M. Perello C. Larvor T. Duteil
		Non Purgeable Organic Carbon method (NPOC)	TOC-LCSH analyzer (Shimadzu, Japan)	IC2MP, Poitiers	M. Leloup T. Duteil
		spectroscopy (FTIR)	(ThermoScientific) spectrometer in attenuated total reflection (ATR) mode		T. Duteil
Preparation of reference minerals for experiments	Preparation of clay mineral standards (Clay Minerals Society, USA)	Grinding and washing	Grinder, centrifuge, reagents	EPOC, Bordeaux	T. Duteil
Petrographic thin sections	Preparation of thin sections for petrographic and mineral analyzes	Sampling of sediment minicores (2 inches in diameter), impregnation in epoxy and preparation of thin section	Equipment for thin section preparation	EPOC, Bordeaux	L. Pigot
Microscopic analyzes	Microscopic characterization of biofilms, sediments or EPS: sediment composition, EPS- sediment interactions, biofilm state and communities	Polarizing and epifluorescence microscopy	-Nikon Eclipse polarizing microscope -Olympus BH-2 UV epifluorescence microscope (BH- RFCA) -Cameras	EPOC, Bordeaux	R. Bourillot I. Betelu J. Caumartin A. Henry T. Duteil
		Environmental scanning electronic microscopy (E- SEM) on experimental samples	Phenom X Pro SEM (Phenom-World B.V., Netherlands)	University of Paris-Saclay, GEOPS, Orsay	J. Nouet M. Virolle T. Duteil
		Cryo-scanning electronic microscopy (cryo-SEM) on	Gemini SEM 300 (Zeiss, Germany)	Bordeaux Imaging Center, Bordeaux	E. Gontier I. Svahn T. Duteil

		natural and experimental samples			
		Atomic force microscopy	Dimension3100 AF microscope (Bruker Corporation)	University of Paris-Saclay, GEOPS, Orsay	J. Nouet R. Bourillot T. Duteil
Microbial activity	Measurements of metabolic activity in the sediment	Metabolic assays: FDA (enzymatic activity), TTC (metabolic activity)	Spectrophotometer, reagents	EPOC, Bordeaux	O. Braissant T. Duteil
		Metabolic heat measurement and modified Gompertz model	aTAM air (TA Instruments, New Castle, DE)	Center for Biomechanics and Biocalorimetry, Basel	O. Braissant T. Duteil
Porewater extraction	Extraction of pore water from sedimentary cores	Drilling of holes in the core tube and rhizon sampling	Rhizons	EPOC, Bordeaux	T. Duteil
Geochemical analyses Biochemical activity	Analyses of sediment porewater to characterize vertical geochemical gradients. Microelectrode profiles (in situ) IC and ICP-OES (ex situ).	Ion Chromatography	Dionex equipped with CS12A analysis column and an IONPAC CG12A protection column	EPOC, Bordeaux	M. Franceschi T. Duteil
	Modelling of mineral saturation indices.	Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES)	ICP-AES (iCAP 6000 Series; ThermoFisher)	EPOC and Eurofins laboratory, Bordeaux	T. Duteil, MJ. Olivier
		Geochemical modeling	Phreeqc software	EPOC, Bordeaux	M. Franceschi T. Duteil
		Specific polarographic microelectrodes to measure O ₂ , HS ⁻ and pH	Polarographic microelectrodes coupled with a picoammeter	EPOC, Bordeaux Bordeaux North point bar	P.T. Visscher

Table 2.1: Synthesis of the methods used in this thesis with the principal objectives, the type of instruments used, the laboratory collaborations and the persons involved in analyzes.

2.2 Comparison of EPS extraction procedures and quality of the purified material

There is no standard method for EPS extraction. The choice of an extraction method depends on the studied material (biofilm, culture, sediment) and on the information needed to address the research questions. Knowing that our samples had low EPS concentration, we privileged a protocol allowing to extract the EPS without selecting a specific fraction (e.g. colloidal, bound EPS). In order to compare studies, it is critical to take into account the protocol of EPS extractions. The various procedures can show important differences in EPS yields, and some protocols induce contamination with intracellular material. Two groups of extraction approaches are used: (i) physical extraction e.g., centrifugation, ultrasonication; and (ii) chemical extraction e.g. formaldehyde, EDTA (Lui and Fand, 2002; Comte et al., 2006; Takahashi et al., 2009). Here is a non-exhaustive list of extraction methods with their pros and cons:

- **Centrifugation**, is generally efficient for the extraction of colloidal carbohydrates and proteins (Comte et al., 2006). This is also a method that minimizes cell lysis and contamination by intracellular material (Klock et al., 2007). Klock et al. (2007) observed that this method slightly increases yields for diatom EPS extraction.
- Ultrasonication helps to break bonds between EPS and minerals (Dignac et al., 1998).
 It is often combined with other methods and allows to improve the extraction yield, as demonstrated experimentally when combined with dowex resin extraction (Dignac et al., 1998; Comte et al., 2006).
- Cold alcohol extraction (<0°C) is particularly effective for extracting polysaccharides and proteins with limited structural modification of these molecules (D'Abzac et al., 2010). The principle is that EPS compete with ethanol for water molecules. When there are not enough remaining water molecules, the EPS precipitate. A concern with this type of extraction is that it preferentially selects polymers larger than 100 kD (De Brouwer & Stal, 2001). On the other hand, this precipitation isolates extracted EPS from low molecular weight organic carbon (LMWOC), and consequently from intracellular elements (e.g., intracellular carbohydrates such as chrysolaminarine) produced during cell lysis (De Brouwer and Stal, 2004). This method does not allow the selection of one

EPS fraction (e.g. colloidal or bound EPS) or another during precipitation, segregation must be done beforehand (D'Abzac et al., 2010). Successive precipitations can also improve the extraction of EPS, the organic carbon content and the percentage of structurally identified EPS fractions (Klock et al., 2007).

- **Cation Exchange Resins (e.g., Dowex)** remove cations from the organo-mineral aggregates, leading to a breakup of the links and to subsequent release of EPS (Frolund et al., 1996). The destabilization of the matrix by the resin and the mechanical shear forces applied during the stirring allow the release of the bound EPS (D'Abzac et al., 2010). This method is very efficient in terms of yield and minimal disruption of the exopolymers (Frolund et al., 1996; Takahashi et al., 2009). It also favors protein extraction because proteins have a higher affinity for multivalent cations than polysaccharides (Frolund et al., 1996; Dignac et al., 1998).
- Water extraction with DI or saline water allows to extract an important quantity of EPS without selection of a molecule size. This method has also the lowest impact on the structure of the EPS (Comte et al., 2006) although Takahashi et al. (2009) observed a contamination by intracellular diatom compounds through cell lysis when using DI water to extract EPS of a marine diatom species.
- Formaldehyde or glutaraldehyde allows to extract large amount of EPS but induces contaminations. Comte et al. (2006) demonstrated that the biochemical composition of EPS was largely impacted by this technique. Formaldehyde or glutaraldehyde react with amine groups of proteins or amino sugars, thus modifying the structure and properties of the proteins in EPS. These molecules crosslink the amines of EPS, which makes EPS less reactive because the amine functions are unavailable. There is a possibility to partially remove glutaraldehyde by dialyzing the extracts, but most of the crosslinking is irreversible.
- EDTA is a chelating agent. EDTA removes divalent ions from EPS and reduces the strength of the EPS matrix. It also helps to release EPS from cell or mineral surfaces, when EPS are stabilized by ionic interactions via divalent ions (Comte et al. 2006). However, the formation of complexes between EDTA and EPS leads to changes in EPS chemical properties, inducing for example shifts in FTIR peaks (Liu & Fang, 2002; Comte et al., 2006; D'Abzac et al., 2010). Dialysis is a solution to remove the EDTA after extraction.

Two or more extraction methods can be coupled to increase EPS yields, e.g. dowex resin plus sonication (Nielsen and Jahn, 1999; Comte et al., 2006). The extraction efficiency also depends on the type of organism or material. The most efficient extraction methods for diatom cultures (without alcohol precipitation) were respectively: freshwater, dowex resin, saline water (15psu) and glutaraldehyde (Takahashi et al., 2009; Figure 2.1), though in that case freshwater induced contamination. For cyanobacterial mats, the most efficient extraction methods (with alcohol precipitation) were respectively: artificial seawater (ASW) + EDTA, ASW, 10% NaCl + EDTA and NaCL (Klock et al., 2007). Contrastingly, the most efficient extraction technique for activated sludge (without alcohol precipitation) are respectively: formaldehyde, glutaraldehyde, EDTA, heating, sonication+ dowex resin and dowex resin only (Comte et al., 2006). Two fractions are generally extracted in mudflat or estuarine biofilm or sediment EPS (Underwood et al., 1995). Colloidal EPS are extracted in water or artificial sea water (depending on the salinity of the studied site) and then centrifuged to recover the supernatant containing the colloidal fraction. In a second step, the bound EPS are extracted from the residual sediments with the use of EDTA or dowex resins (Underwood et al., 1995; De Winder et al., 1999; Pierre et al., 2012; Orvain et al., 2014). In this study, we focused on the colloidal fraction of EPS (section 2.5.1).



Figure 2.1: Comparison of EPS extraction methods applied to diatom cultures (*Navicula jeffreyi*). (A) Amount of carbohydrate pg.cell⁻¹ measured against glucose standards and (B) Quantity of protein in pg.cell⁻¹ measured against BSA (Takahashi et al., 2009).

Once extracted, the EPS physico-chemical properties are characterized by different methods. The most common is the phenol sulfuric assay for carbohydrate sugars concentration (colloidal or bound; Dubois et al. 1956). For protein concentrations, the most common is the Lowry assay (Lowry et al. 1951) but there is also the Bradford assay (Bradford, 1976). It is possible to characterize in more detail the different fractions of monosaccharides in EPS using GC-MS (Taylor et al., 1999; Bellinger et al., 2005; Figure 2.2) or by ultrafiltration followed by high performance liquid chromatography (HPLC) analysis (De Brouwer and Stal, 2001). FTIR can also be used to determine, in addition to the composition, the chemical functions in EPS (Braissant et al., 2007; D'Abzac et al., 2010).



Figure 2.2: Variation in monosaccharide composition of EPS extracted from diatom biofilm using GC-MS (Taylor et al., 1999).

2.3 Sediment sampling and measurements

2.3.1 Sedimentary core extraction

The portable vibro-corer developed by EPOC ("Amaury" corer; Service Terrain of the laboratory) is adapted to extract long cores from soft sandy and muddy sediment. The Amaury corer is stationary and avoids the effects of fluid overpressure that could deform the sediments. It consists of an aluminum tube ca. 7.6 cm (3 inches) in diameter. Inside this tube, a mobile piston is attached to a wire, and a fixed "orange peel" keeps the sediment inside the tube during the coring process. During coring, the casing is left in place to allow the installation of the aluminum tube and to continue the coring session which can go to depths of 8 m (by using a 2m PVC tube extension). The tube is pushed in the sediment and raised very slowly, so as not to compact or deform the sediment or prevent the fluid from rising. After extraction, in the field, the vibracores are (i) oriented seaward so as to distinguish between ebb and flood dune stratifications, and (ii) are cut into one meter long sections to facilitate their transport. To avoid water mixing, the sections were kept straight until the pore water was sampled. Following microelectrode profiling and pore water extraction (see 2.4.1), cores are opened photographed PAACS platform of the EPOC laboratory in Bordeaux. X-radiographies and XRF are then performed on the open cores (see Virolle, 2019 for details about the procedures). One part was used for sampling and another was left undisturbed for sediment descriptions.

Short cores (0.7 m) are sampled manually. To do so, a plexiglass tube with sharpened ends was gently pushed into the sediment (Figure 2.3B). The core was then gently removed to avoid any modification of the sedimentary structures, and kept straight during transport. Finally, sediment boxes are extracted using steel sampling boxes (length: 28cm, width: 17.5cm, depth: 6.2cm; Figure 2.3A).

In this thesis most of the analyses were performed on 3 cores sampled in june 2019: BXN-LC (long vibracore, 6.08 meters deep), BXN-SC (short hand cores; 0.7 meter deep) and BXN-B (boxcore; 0.28 m deep).



Figure 2.3: (A) Sampling box on the field before push it to sample 30 cm of sediment. (B) The box and the short core on the field pushed in sediment juste before sampling. (C) Details of the method used to sample diatom biofilm for EPS extraction.

2.3.2 Reference mineral material

Reference mineral materials are used in experiments (chapter 5). Four clay reference minerals are selected for the experiments (Clay Minerals Society; Chantilly, USA): kaolinite (KGa-1b), illite (Imt-2), montmorillonite (Saz-2) and chlorite (CCa-2) (their properties are detailed in section 1.1.8.). Claystone samples are mechanically crushed using a RETSCH PM 400 grinder in order to separate the micrometer sized clay crystals. Clay solutions are prepared according to annex 7 of this chapter.

Fontainebleau quartz sand (Sibelco, France) was used as reference for quartz (96.5% of the grains size $<120\mu$ m). The sand is cleaned and rinsed before use to remove organic matter and impurities (annex 7).

2.3.3 Surface sediment and core samples

For surface biofilm sampling, the sediment surface is scraped using a large plastic spatula to collect as much biofilm as possible and reduce the quantity of sediment (Figure 2.3C).

In cores, EPS extraction started just after core opening. For this, 20 cm long sediment half core sections are sampled in the long core (10 cm above and below hole made for water extraction). For grain size measurements, ca. 2 cm³ of sediment are sampled (Figure 2.4). For thin sections, plastic plugs (diameter 1.5 inch) are inserted into the wet sediment. Plugs are subsequently

dried (40°C; 24 hours) and impregnated with blue epoxy resin under vacuum to make thin sections. Each sediment sample is weighed before and after drying.



Figure 2.4: Example of sampling for grain size analysis in a vibracore.

2.3.4 FTIR spectroscopy (EPS and clay minerals)

Fourier Transform Infrared Spectroscopy (FT-IR) is performed on a Nicolet IS50 (ThermoScientific) Spectrometer in Attenuated Total Reflection (ATR) mode at the IC2MP laboratory (Poitiers, France). For this, one drop of EPS and/or clay solution is placed on the diamond ATR element and evaporated (Figure 2.8). Spectra are acquired between 650 cm⁻¹ and 4000 cm⁻¹, with a 4 cm⁻¹ spectral resolution and 100 scans are co-added for each spectrum.

2.3.5 Grain size analyzes

Approximately 1 gram of sediment (16 samples) is first sieved to remove large particles (>1200 μ m). Sediment grain size is then measured with a Mastersizer 2000 laser granulometer (Malvern, United Kingdom; Figure 2.14), with an obscuration rate of 2–25% at the EPOC laboratory (Bordeaux, France). Each sample is measured in triplicate. The median grain size (D₅₀, in μ m) and the proportion of sand (63-1200 μ m), silt- (<63 μ m), and clay-sized (<4 μ m) particles are calculated for each sample. A detailed protocol is available in annex 8.

2.3.6 Measurements of microbial activity in the sediment

The FDA (fluorescein diacetate) assay measures the enzymatic activity, more particularly that of non-specific hydrolase (lipase, esterase, and protease). The principle is the hydrolysis of the acetate moieties of the FDA molecule (Braissant et al., 2020). The released fluorescein is then extracted, and its concentration is calculated using the absorbance at 490nm measured with a spectrophotometer. The TTC (triphenyltetrazolium chloride) assay is a marker of metabolically active cells. The principle is the reduction of TTC to formazan by the electron transport chain during cell metabolism (either enzymatically or through direct reaction with NADH or NADPH; Braissant et al., 2020). The formazan has a rose-red colour. The absorbance is measured at 480 nm using a spectrophotometer in order to calculate the concentration of formazan.

In order to degrade enzymes and potential spores, a double sterilization process is applied to prepare blank samples: (i) autoclave (autoclaved 20 minutes at 121°C) and (ii) glutaraldehyde addition (final concentration 1.5%). Detailed protocols are given in annex 2.5 for FDA and annex 2.6 for TTC.

Microbial activity was also measured with isothermal microcalorimetry. Analyzes are performed with a TAM air calorimeter (TA Instruments, New Castle, DE). Two grams of sediments are put inside the calorimeter with 200 μ L of R2A culture medium (Reasoner & Geldreich, 1985; Figure 2.5). Blanks are prepared using sterilized samples autoclaved 20 minutes at 121°C. For each depth, the measurements are done in triplicate. The heat release is measured during 24h to quantify the microbial activity. The growth rate (μ), the lag phase (λ) and the heat produced (Q) are calculated by fitting the heat over a time curve with the modified

Gompertz model (Gil et al., 2006). The maximum heatflow is used to estimate the number of active cells assuming a heat production rate of 3pW.cell⁻¹. For aerobic microbial plate counts, 1g of sample is dissolved in 10ml of PBS and then subjected to 10 folds dilution series. The appropriate dilutions are then deposited in petri dishes filled with agarized R2A medium (Reasoner & Geldreich, 1985). Bacterial communities are counted after 1, 7 and 30 days (CFU1, 7 and 30) and aerobic microbial counts were calculated using the sample dilutions.



Figure 2.5: (A) Isothermal calorimetry thermometric TAM air. (B) Detail of the sediment sample with the culture medium before putting it in the calorimeter.

2.4 Water chemistry

2.4.1 Porewater sampling

Following extraction, cores are sealed and kept vertical to avoid water mixing. Rhizons (Rhizosphere Research Products; Wageningen) are used for pore water sampling. Rhizons are made of a hydrophilic porous polymer tube (pore diameter of $0.1 \mu m$; outer diameter 2.4 mm; filter section 5cm) extended with a polyvinyl chloride tube. Rhizons present several advantages, for example they cause low disturbance to the sediment, they have a small area of influence, and the pore diameter implies no further filtering before analysis (Seeberg-Elverfeldt et al.,

2005). To improve water recovery, Rhizon samplers were saturated with deionized water for 30 minutes in a beaker before use. For the long core (BXN-LC), 16 holes were drilled in the core tube, and Rhizons were inserted horizontally in the sediment to extract the water in situ (Figure 2.6B). 25 mL syringe were connected with Luer-Lock system to the Rhizons with wooden retainer to create a vacuum (Figure 2.6A). Water extraction was performed during 24h, then syringes were closed with Luer-Lock plug and stored in the fridge (4°C). Same operations were made for the box and short core.



Figure 2.6: (A) Detail of the water extraction made with the rhizon with the different parts. (B) Portions of the core during the water extraction with the rhizon placed at the targeted depths.

2.4.2 Porewater chemical analyzes

In situ measurements of pH, oxygen and sulfide are performed using polarographic microelectrodes combined with a picoammeter (Unisense, Aarhus, Denmark; Visscher et al., 2000). Shallow O₂ profiles (max. 2 cm deep) are done directly in the field (Figure 2.7A) using

a micromanipulator, with a vertical resolution of 250 μ m. pH, HS⁻ and O₂ profiles are measured in the cores by inserting the microelectrodes in the holes used for porewater sampling (Figure 2.7B).

The concentrations of major cation (Ca²⁺, Mg²⁺, Mn²⁺, Na⁺, K⁺) and anions (Cl⁻, NO₃⁻, SO₄²⁻ and PO₄³⁻) concentrations are measured in porewater samples (ex situ) by ion chromatography (Dionex ICS-1500, with an analytical precision of 0.2 mg.L⁻¹ ±5%) at the EPOC laboratory. Metal concentrations (Cd²⁺, Ca²⁺, Mg²⁺, Mn²⁺, total dissolved Fe, Pb²⁺, Zn²⁺) are measured by ICP-OES at Eurofins laboratory. Concentrations of Fe²⁺ and Fe³⁺ cations are measured using the iron (II/III) LCK 320 colometric test (Hach Lange) with a Helios Epsilon spectrophotometer (Thermo scientific).



Figure 2.7: (A) Microelectrode measurements (dissolve O_2) in the field. The holder is equipped with a micromanipulator that allows a 250 μ m vertical resolution. (B) Microelectrode measurements in the core.

2.4.3 Porewater geochemical modeling

Porewater compositions are entered in the PhreeQC software in order to calculate the saturation indices of mineral phases, e.g., oxides, sulfides or carbonates. PhreeQC calculates thermodynamic equilibria based on the Pitzer model (Pitzer, 1979).

2.5 EPS extraction and characterization

2.5.1 EPS extraction

The goals of the EPS extraction was to have as much material as possible to carry out the subsequent physico-chemical characterizations without altering the EPS structure. We followed the classical method for extracting EPS from intertidal sediments developed by Underwood & Paterson (1995). It consists in DI water extraction followed by cold alcohol precipitation (here we used cold propanol). This simple and reproducible method allows for the characterization of colloidal EPS of microbial assemblages in natural sediments.

Water extraction followed by ethanol precipitation ensures a good extraction efficiency without changing the physical properties of proteins or polysaccharides (Comte et al., 2005; Takahashi et al., 2009). In order to increase the EPS yield, we have added an ultrasonication step to break bonds between EPS and particles (Dignac et al. 1998). Finally, we purify EPS by dialysis (>10 kDa) in addition to the classical extraction methods. The detailed protocol can be found in Annex 1.

Extraction methods with potentially higher yields (e.g., EDTA) were not used because of potential contamination of EPS during chemical extractions. The subsequent extraction of bound EPS using e.g., Dowex resin could have been an interesting complement, but was not envisaged at the beginning of this thesis.

2.5.2 EPS physico-chemistry: colorimetric assays

We used three complementary assays to characterize EPS. All these assays are based on the Beer-lambert law for the calculation of the concentration of colored complexes or molecules formed by the reaction. Detailed protocols are available in annex 2 (Alcian Blue assay), annex 3 (phenol sulfuric assay) and on Thermos scientific web site for the Pierce modified Lowry protein assay (https://www.thermofisher.com/order/catalog/product/23240).

1/the <u>Alcian Blue assay</u> is an adaptation from the method of Passow & Alldredge (1995). This method is a proxy for the density of reactive sites in EPS (mainly carboxylic acids and sulfates) through the fixation of Alcian Blue these sites. It is a negative test, i.e. the lower the absorbance the more reactive the EPS is. Xanthan is used as a laboratory equivalent of EPS for the standard

samples. We chose xanthan rather than the widely used glucose, because xanthan is probably closer in size and properties to the natural EPS.

2/the <u>phenol sulfuric assay</u> is adapted from the method of Dubois et al. (1956). This method is used to measure the concentration sugar polymers in EPS. Pure sulfuric acid hydrolyzes the sugar polymers and phenol reacts with the formed monomers, resulting in a dark brown colored compound. This is a positive test, i.e. the higher the absorbance is, the more polysaccharides there are in EPS. Xanthan is used as a laboratory equivalent of EPS for the standard samples. A caution point is to use pure fuming sulfuric acid.

3/<u>the Pierce modified Lowry assay</u> is adapted from a method by Lowry et al. (1951) commonly used for protein quantification. The procedure consists in the reaction of proteins with cupric sulfate and tartrate in alkaline solution, resulting in the formation of tetradentate chelated copper complexes. This complex produces a blue color measurable at 750 nm. Again, this is a positive test, i.e. the higher the absorbance is, the more protein there is in EPS. Bovin serum albumin (BSA) was used as a protein standard.

2.5.3 FTIR spectroscopy on EPS

Fourier Transform Infrared Spectroscopy (FT-IR) analyses of the samples were performed on a Nicolet IS50 (ThermoScientific) Spectrometer in Attenuated Total Reflection (ATR) mode at the IC2MP laboratory (Poitiers, France). One drop of EPS solution was placed on the diamond ATR element and evaporated (Figure 2.8). Spectra were acquired between 650 cm⁻¹ and 4000 cm⁻¹, with a 4 cm⁻¹ spectral resolution and 100 scans were co-added for each spectrum.



Figure 2.8: (A) Nicolet IS50 (ThermoScientific) Spectrometer. (B) Zoom on the drop of EPS solution on the diamond ATR element before evaporation.

2.5.4 EPS acid-base titration

Acid-base titrations have been performed on two EPS samples (surface water EPS and at 6.08 m in BXN-LC) to determine the potential types and densities of the functional groups present in the EPS. For this, 3 mL of EPS are acidified with 100 μ L of HCL (1 M) to lower the pH, and 100 μ L of KCl (1 M) are added to provide sufficient ionic concentration to stabilize the pH electrode. Once acidified, the solution is left under constant N₂ flow for 20 minutes to avoid potential CO₂ dissolution and carbonate or bicarbonate formation during the titration. The solution is titrated with NaOH (0.1 M) added stepwise in 10 μ L increments and 5 μ L increments when the pH rise is fast. The pH is recorded up to 11 with a pH microelectrode (Sentix 60, A005106A030) connected to a Consort C561 meter (De Bruyne, Belgium). Control titrations are effected with DI water under the same experimental conditions. The experimental set-up has been adapted to work with small volumes of EPS (Figure 2.13). The titration curves were analyzed using the PROTOFIT 2.1 software, assuming three main biding sites (Turner & Fein, 2006). A detailed protocol can be found in annex 4.

2.5.5 EPS cation binding capacity

The binding of Ca^{2+} and Mg^{2+} to EPS is measured by isothermal titration calorimetry (ITC) at the University of Basel. For each measurement a 4mL hasteloy ampoule is filled with 2.7 mL of freshly dialyzed and degassed EPS solution. TRIS is adjusted at a final concentration of 20mM and the ITC syringe is filled with 300 µL of divalent cation solution (CaCl₂ or MgCl₂) dissolved in the same buffer. The ampoule with the EPS and the syringe with calcium or magnesium ions is placed in the TAM III ITC (Waters / TA instruments, Delaware, USA). The titration is achieved through 17 injections of 15 µL of the cation solution to EPS samples, dextran was used as a commercially available polysaccharide as a reference compound. Binding parameters and affinity were calculated using TAM assistant. The cation binding capacity is currently being calculated, so the data are not shown in this thesis.

2.6 Microscopy

2.6.1 Cryo-scanning electronic microscopy

We chose cryo-SEM to document EPS and their interactions with sediments, because conventional SEM causes drying and retraction of the highly hydrated organic matrix. Small fractions of sediments are placed in gold or silver cups (0.9-1 mm in diameter; 0.3 mm in depth; Figure 2.9A). Then, a cryo-fixation is performed by high-pressure freezing (HPF; pressure: 2,050 bars) with an EM HPM 100 device (Leica Microsystems) at the Bordeaux Imaging Center (Bordeaux). HPF reduces ice crystals formation and ensures a very fast freezing process (in a few milliseconds). Cryo-fixation prevents the collapse of EPS, allowing optimal observations of the three dimensional organo-mineral architecture and ensures a simultaneous immobilization of all the EPS macromolecular components. Samples are stored in a cryo-bank (liquid nitrogen; -196°C) until processing. Cryofracture, sublimation, platinum coating and transfer of the sample to the SEM chamber are done with a cryo-transfer system PP3010T (Quorum Technologies; Figure 2.9A). Due to the highly hydrated nature of our samples, it was necessary to adapt the duration of sublimation. A classic sublimation lasts between 2 and 5min but it was not enough to defrost our sample for observations. After a few tests and some samples

lost, we fixed the best sublimation duration at 40min even if it does not allow a 100% defrost each time. Samples are subsequently coated with Platinum during 40 seconds (Figure 2.9B), and observed under cryo-SEM with a Gemini SEM 300 (Zeiss; Germany; Figure 2.9A) at the BIC. In this manuscript, a mineralogical characterization of the sedimentary particles was performed with an EDX probe at an energy of 12keV (EDX system: XFlash 6/60; BRUKER, Germany).



Figure 2.9: (A) Gemini SEM 300 SEM (Bordeaux Imaging Center). The insert on the bottom left shows gold cups filled with sediment just before HPF cryo-fixation. (B) Cryo-transfer unit PP3010T photographed during the sample metallization.

2.6.2 Optical microscopy: petrographic thin section and microbiological glass slides

Petrographic thin sections have been observed and photographed with a Nikon Eclipse polarizing microscope equipped with a camera. This allows the determination of the microfacies and of the nature of sedimentary particles. Thin section are subsequently scanned in high-resolution (4800 ppp) using an Epson V750 Pro scanner. The relative surface area of each category of grains (quartz, coated sands, etc.) is calculated using random point counting with the *JMicrovision* software (https://jmicrovision.github.io). For each thin section, 12 representative areas of 5x5 mm are selected for point counting, for a total of about 800 random points counts per thin section.

Microbiological preparations have also been observed under the same microscope in order to determine diatoms (genus level) and to check for the presence of other organisms (e.g., green algae, Euglenids). For this, a drop of the biofilm is placed on a glass slide and covered with a lamella.

Similar preparations have been made to observe the location of reactions of three assays: FDA, TTC and Alcian Blue (see above). For this we mixed a drop of biofilm with a drop of reagent. Alcian Blue and TTC assays can be observed under a polarizing microscope. The FDA assay necessitates an incubation time of 30 minutes in the dark. The observation of fluoresceine is done under an epifluorescence microscope Olympus BH-2 equipped with a UV BH-RFCA filter cube.

2.6.3 Atomic force microscopy

For AFM (Atomic Force Microscopy), a drop of the clay-sand-EPS mix is deposited on a glass plate and air-dried. Observations are carried out on a Dimension 3100 AF microscope (Bruker) at University of Paris-Saclay (GEOPS, Orsay).

References

- Bellinger, B., Abdullahi, A., Gretz, M., and Underwood, G., 2005, Biofilm polymers: relationship between carbohydrate biopolymers from estuarine mudflats and unialgal cultures of benthic diatoms: Aquatic Microbial Ecology, v. 38, p. 169–180, doi:10.3354/ame038169.
- Bradford, M.M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. p. 7.
- Braissant, O., Astasov-Frauenhoffer, M., Waltimo, T., and Bonkat, G., 2020, A Review of Methods to Determine Viability, Vitality, and Metabolic Rates in Microbiology: Frontiers in Microbiology, v. 11, p. 547458, doi:10.3389/fmicb.2020.547458.
- Braissant, O., Decho, A.W., Dupraz, C., Glunk, C., Przekop, K.M., and Visscher, P.T., 2007,
 Exopolymeric substances of sulfate-reducing bacteria: Interactions with calcium at alkaline pH and implication for formation of carbonate minerals: Geobiology, v. 5, p. 401–411, doi:10.1111/j.1472-4669.2007.00117.x.
- De Brouwer, J., and Stal, L., 2001, Short-term dynamics in microphytobenthos distribution and associated extracellular carbohydrates in surface sediments of an intertidal mudflat: Marine Ecology Progress Series, v. 218, p. 33–44, doi:10.3354/meps218033.
- Brouwer, J.F.C.D., Wolfstein, K., and Stal, L.J., 2002, Physical characterization and diel dynamics of different fractions of extracellular polysaccharides in an axenic culture of a benthic diatom: European Journal of Phycology, v. 37, p. 37–44, doi:10.1017/S0967026201003419.
- Comte, S., Guibaud, G., and Baudu, M., 2006, Relations between extraction protocols for activated sludge extracellular polymeric substances (EPS) and EPS complexation properties Part I. Comparison of the efficiency of eight EPS extraction methods: Enzyme and Microbial Technology, p. 9.
- D'Abzac, P., Bordas, F., Van Hullebusch, E., Lens, P.N., and Guibaud, G., 2010, Extraction of extracellular polymeric substances (EPS) from anaerobic granular sludges: comparison of chemical and physical extraction protocols: Applied microbiology and biotechnology, v. 85, p. 1589–1599.
- De Brouwer, J., and Stal, L., 2004, Does warm-water extraction of benthic diatoms yield extracellular polymeric substances or does it extract intracellular chrysolaminaran? European Journal of Phycology, v. 39, p. 129–131, doi:10.1080/09670260410001703815.
- Dignac, M.-F., Urbain, V., Rybacki, D., Bruchet, A., Snidaro, D., and Scribe, P., 1998, Chemical description of extracellular polymers: implication on activated sludge floc structure: Water Science and Technology, v. 38, p. 45–53.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P. t, and Smith, F., 1956, Colorimetric method for

determination of sugars and related substances: Analytical chemistry, v. 28, p. 350-356.

- Frølund, B., Palmgren, R., Keiding, K., and Nielsen, P.H., 1996, Extraction of extracellular polymers from activated sludge using a cation exchange resin: Water Research, v. 30, p. 1749–1758, doi:10.1016/0043-1354(95)00323-1.
- Gil, M.M., Brandão, T.R.S., and Silva, C.L.M., 2006, A modified Gompertz model to predict microbial inactivation under time-varying temperature conditions: Journal of Food Engineering, v. 76, p. 89–94, doi: 10.1016/j.jfoodeng.2005.05.017.
- Klock, J.-H., Wieland, A., Seifert, R., and Michaelis, W., 2007, Extracellular polymeric substances (EPS) from cyanobacterial mats: characterisation and isolation method optimisation: Marine Biology, v. 152, p. 1077–1085, doi:10.1007/s00227-007-0754-5.
- Liu, H., and Fang, H.H.P., 2002, Extraction of extracellular polymeric substances (EPS) of sludges: Journal of Biotechnology, v. 95, p. 249–256, doi:10.1016/S0168-1656(02)00025-1.
- Lowry, O., Rosebrough, N., Farr, A., and Randall, R., 1951, Protein measurement with the Folin phenol reagent: J biol Chem, v. 193, p. 265–275.
- Nielsen, P.H., and Jahn, A., 1999, Extraction of EPS, in Wingender, J., Neu, T.R., and Flemming, H.
 C. eds., Microbial Extracellular Polymeric Substances, Berlin, Heidelberg, Springer Berlin Heidelberg, p. 49–72, doi:10.1007/978-3-642-60147-7 3.
- Orvain, F., De Crignis, M., Guizien, K., Lefebvre, S., Mallet, C., Takahashi, E., and Dupuy, C., 2014,
 Tidal and seasonal effects on the short-term temporal patterns of bacteria, microphytobenthos and exopolymers in natural intertidal biofilms (Brouage, France): Journal of Sea Research, v. 92, p. 6–18, doi: 10.1016/j.seares.2014.02.018.
- Pace, A., Bourillot, R., Bouton, A., Vennin, E., Braissant, O., Dupraz, C., Duteil, T., Bundeleva, I., Patrier, P., and Galaup, S., 2018, Formation of stromatolite lamina at the interface of oxygenic– anoxygenic photosynthesis: Geobiology, v. 16, p. 378–398.
- Passow, U., and Alldredge, A.L., 1995, A dye-binding assay for the spectrophotometric measurement of transparent exopolymer particles (TEP): Limnology and Oceanography, v. 40, p. 1326–1335.
- Pierre, G., Zhao, J.-M., Orvain, F., Dupuy, C., Klein, G.L., Graber, M., and Maugard, T., 2014, Seasonal dynamics of extracellular polymeric substances (EPS) in surface sediments of a diatomdominated intertidal mudflat (Marennes–Oléron, France): Journal of Sea Research, v. 92, p. 26– 35, doi: 10.1016/j.seares.2013.07.018.
- Pitzer, K.S., 1979, Relativistic effects on chemical properties: Accounts of Chemical Research, v. 12, p. 271–276.
- Reasoner, D.J., and Geldreich, E.E., 1985, A new medium for the enumeration and subculture of bacteria from potable water: Applied and Environmental Microbiology, v. 49, p. 1–7, doi:10.1128/aem.49.1.1-7.1985.

- Seeberg-Elverfeldt, J., Schlüter, M., Feseker, T., and Kölling, M., 2005, Rhizon sampling of porewaters near the sediment-water interface of aquatic systems: Limnology and oceanography: Methods, v. 3, p. 361–371.
- Takahashi, E., Ledauphin, J., Goux, D., and Orvain, F., 2009, Optimising extraction of extracellular polymeric substances (EPS) from benthic diatoms: comparison of the efficiency of six EPS extraction methods: Marine and Freshwater Research, v. 60, p. 1201, doi:10.1071/MF08258.
- Taylor, I.S., Paterson, D.M., and Mehlert, A., 1999, The quantitative variability and monosaccharide composition of sediment carbohydrates associated with intertidal diatom assemblages: Biogeochemistry, v. 45, p. 303–327, doi:10.1007/BF00993005.
- Turner, B.F., and Fein, J.B., 2006, Protofit: A program for determining surface protonation constants from titration data: Computers & Geosciences, v. 32, p. 1344–1356, doi: 10.1016/j.cageo.2005.12.005.
- Underwood, G.J.C., Paterson, D.M., and Parkes, R.J., 1995, The measurement of microbial carbohydrate exopolymers from intertidal sediments: Limnology and Oceanography, v. 40, p. 1243–1253.
- Virolle, M., Brigaud, B., Luby, S., Portier, É., Féniès, H., Bourillot, R., Patrier, P., and Beaufort, D., 2019, Influence of sedimentation and detrital clay grain coats on chloritized sandstone reservoir qualities: Insights from comparisons between ancient tidal heterolithic sandstones and a modern estuarine system: Marine and Petroleum Geology, v. 107, p. 163–184.
- Visscher, P.T., Reid, R.P., and Bebout, B.M., 2000, Microscale observations of sulfate reduction: correlation of microbial activity with lithified micritic laminae in modern marine stromatolites: Geology, v. 28, p. 919–922.
- de Winder, B., Staats, N., Stal, L.J., and Paterson, D.M., 1999, Carbohydrate secretion by phototrophic communities in tidal sediments: Journal of Sea Research, v. 42, p. 131–146, doi:10.1016/S1385-1101(99)00021-0.

Annexes

Annex 1: EPS extraction protocol

Use latex gloves to prevent contamination of EPS or destruction of dialysis bags.

- <u>Dialysis bags preparation:</u> 1. Cook dialysis tubing in 2% Na₂CO₃ (20g), 1mM EDTA (0.372g) in 1L of DI water for
- 1. Cook dialysis tubing in 2% Na₂CO₃ (20g), ImM EDTA (0.3/2g) in 1L of DI water for 30min. Add the EDTA first, and then when it is dissolved, add the Na₂CO₃.
- 2. Rinse the dialysis bags and put them in distilled water. They are ready for use, or you can put them in the fridge for a future utilization. Method:
- 1. Weigh the mass of the sample (wet weight) then dilute it in 2 times its volume of DI water. Put the solution formed in ultrasonic bath for 10 min then gently stir during 4h with a magnetic stirrer. Note: keep an aliquot of the sample to measure the dry mass (dry weight).
- 2. Centrifuge the obtained solution for 20min (2800 rpm), recover the supernatant and discard the sediment stuck to the bottom of the tube (sand and clay) and cells. If the supernatant is to clayey, repeat this step.
- 3. Filter the supernatant through 0.22 μm polycarbonate filters (to avoid contamination of EPS). If the supernatant is to clayey, repeat filtration.
- 4. Precipitate the filtrate using three volumes of cold propanol (<0°C) per volume of filtrate for a day. EPS should form a white/grey aggregate in the bottom of the container.
- 5. Centrifuge the solution to recover the precipitate (2800xg, 20min). For each centrifugation step, throw the propanol and centrifuge again to increase the EPS pellet.
- 6. At the end of the centrifugation, recover the EPS pellet with a metal spatula and place them in placed in dialysis bags (10kDa) taking care not to puncture the bag. Make sure the bag is well closed with a knot plus a plier at both ends.
- Dialyze three days in a buffer solution (2L of DI water and 0,744g EDTA) and three other days against 2L of DI water (>18 MΩ) at 4°C. Keep Liquid EPS solutions in the fridge at 4°C before use.
- 8. Cut the dialysis bag just after the node and recover the EPS in a 15 or 50 mL centrifuge tube (falcon-type).



Figure 2.101: (A) Result of the precipitation of the EPS at stage 4. (B) EPS pellet collected in the bottom of the centrifuge tube after the step 5. (C) Dialysis bag with the final EPS solution at the end of the manipulation.

Annex 2: Alcian Blue assay protocol

Always wear gloves while handling acid and samples. Ensure all glassware used is clean; ideally acid washed with 1% HCl and rinsed at least 3 times with distilled water. Prepare pH meter by soaking electrode in 0.1N HCl for at least 2 hours before calibrating with pH standards. Allow the spectrophotometer to warm up for at least 15 minutes prior to taking measurements.

Alcian Blue 8GX solution

- 1. Prepare a solution of acetic acid 5% and adjust the pH between 2.6 and 3.0. pH is very important to have a good reaction.
- 2. Take the volume needed and dissolve in it a small quantity of Alcian Blue to obtain a saturated solution. Estimate the absorbance at 614 nm, and calculate the dilution needed to obtain a solution having an absorbance of almost 1.5.
- 3. Filter the final solution (0.5 μ m), and measure its absorbance. It should be comprised around 1.0.

Method:

- 1. Dissolve 0.12g of Xanthan in 50 mL of DI water (0.25% Xanthan solution). The dissolution of the Xanthan is hard so it's better to prepare your Xanthan solution one day before and put it on fridge. Before using it, vortex it.
- 2. Prepare your standard curve with Xanthan: 0, 20, 40, 60, 80 and 100 μ L of Xanthan 0.25% in triplicate.
- 3. Prepare your sample by adding 100µL of purified EPS in triplicate.
- 4. Add 2mL of Alcian Blue and vortex it for 10s. Let react during 30 min.
- 5. Centrifuge (2800xg, 5min) the tube to drop the formed Alcian Blue pellets.
- 6. Measure the absorbance of the supernatant at 614nm. Be careful not to touch or resuspend the Alcian Blue pellets, which are very sticky. It is a negative test, the less absorbance there is, and the more reactive the EPS is.
- 7. Plot the data for the standard samples to obtain a curve. Get the regression equation to calculate the amount present in other samples. An example of equation measured for experiments in this study was: x=(1.17-y)/3.57 (x= amount of EPS and y= measured absorbance). Final solutions are in mg eq. Xanthan.mL EPS sample⁻¹.



Figure 2.112: (A) Example of standard samples where we can see that the Alcian Blue assay is a negative test. (B) Resulted standard curve.

Annex 3: Phenol sulfuric assay protocol

Always wear lab coat, glasses and gloves because of the risks with phenol and concentrated acid and under extractor hood. Use glass tubes, not plastic tubes to avoid the risk of dissolution. Ensure all glassware used is clean; ideally acid washed with 1% HCl and rinsed at least 3 times with distilled water. Prepare pH meter by soaking electrode in 0.1N HCl for at least 2 hours before calibrating with pH standards. Allow the spectrophotometer to warm up for at least 15 minutes prior to taking measurements.

Method:

- 1. Dissolve 0.12g of Xanthan in 50 mL of DI water (0.25% Xanthan solution). The dissolution of the Xanthan is hard so it's better to prepare your Xanthan solution one day before and put it on fridge. Before using it, vortex it.
- 2. Prepare your standard curve with Xanthan: 0, 20, 40, 60, 80 and 100 μ L of Xanthan 0.25% in triplicate.
- 3. Prepare your sample by adding 50µL of purified EPS in triplicate.
- 4. Add 500μ L of DI water.
- 5. Add 25μ l of phenol (heat the phenol ($50-60^{\circ}$ C) if it is solid at room temperature).
- 6. Mix this solution by vortexing for 10 second. The solution may turn slightly cloudy due to the phenol droplets formed.
- 7. Put the tube on sand and 2mL of concentrated sulfuric acid (>95%) as fast as possible (cut the end of the plastic pipette to facilitate the adding of acid before the experiment). Close the tube with a glass marble.
- 8. Wait 30 minutes to 1 hour to allow the reaction to complete and allows the tube cools down.
- 9. Measure the absorbance of the solution at 490nm.
- 10. Plot the data for the standard samples to obtain a curve. Get the regression equation to calculate the amount present in other samples. An example of equation measured for experiments in this study was: x=(y-0.036)/9.16 (x= amount of EPS and y= measured absorbance). Concentrations are expressed in mg eq.xanthan.mL EPS sample⁻¹.



Figure 2.123: (A) Example of standard samples where we can see that the phenol sulfuric assay is a positive test. (B) Resulted standard curve.

Annex 4 EPS acid-base titration protocol

Always wear glasses and gloves. Do the titration under N_2 atmosphere. Prepare your titration sheet and pH meter by soaking electrode in 0.1N HCl for at least 2 hours before calibrating with pH standards. Method:

- 1. In a 5 ml beaker, add a known amount of pure EPS with a stirrer. If EPS is highly concentrated, dilute it in DI water.
- 2. Add 100 ul of KCl 1M for the ionic balance.
- 3. Transfer into the titration setup, close the beaker with parafilm. Ensure nitrogen flow through the setup to avoid CO₂ dissolution at high pH. Place pH electrode in the system away from the stirrer.
- 4. Add 100ul of 1M HCl to the system. Stir the beaker at least 20 minutes to degas the CO₂ and for a stable pH around 2. If the pH remains above 2.5, add more HCl.
- 5. Add 10 μ l of 0.1M NaOH. Record the pH when the pH reading is stable.
- 6. Repeat until you reach pH jump. Decrease the volume at 5µL during six step to be more precise near the pH jump.
- 7. Repeat the 10μ L adding until you reach pH 11
- 8. Use the PROTOFIT 2.1 software (Turner and Fein, 2006) to estimate the reactive functions in titrated EPS.



Figure 2.13: Example of titration setup used in this study.

Annex 5 Fluorescein Diacetate (FDA) assay protocol

Allow the spectrophotometer to warm up for at least 15 minutes prior to taking measurements.

FDA solution

Dissolve 50mg of FDA in 50ml of acetone.

Method:

- 1. Take 2 grams of sample ideally in glass tubes for later extraction with organic solvents. Make triplicate in order to have good data.
- 2. Add 4 mL of solution per gram of sample and homogenize. Adapt your solution from the natural environment of your sample (for example "Instant Ocean", saline solution, or sterile filtered site water).
- 3. Prepare in triplicate blank by heat inactivation of the bacteria in the sample. Autoclave the sample two-time to ensure killing all the spores and add formaldehyde or glutaradehyde to the sample to obtain a final concentration of 1.5%.
- 4. Add 60µl of FDA solution in the tubes and homogenize (vortex).
- 5. Incubate the sample at room temperature for 2 to 24 hours (ideally 25°C) until a visible yellow green color develops.
- 6. Extract the fluorescein by adding 4mL of acetone and centrifuge (2800xg; 5 min). If there are particles in suspension make a second centrifugation.
- 7. Measure absorbance at 490 nm.
- 8. Calculate the amount of fluorescein (i.e., reduced FDA) produced per unit of time with the Beer Lambert Law: A = E*1*C (A: measured absorbance; E: molar absorption coefficient (73 350 L.mol⁻¹.cm⁻¹); l: length of the cuvette (1cm)).

Annex 6 Triphenyltetrazolium (TTC) assay protocol

Allow the spectrophotometer to warm up for at least 15 minutes prior to taking measurements.

TTC solution

Dissolve 0.4g of TTC in 50ml solution equivalent to the natural environment of your sample (for example "Instant Ocean", saline solution, or sterile filtered site water).

Method:

- 1. Take 2 grams of sample ideally in glass tubes for later extraction with organic solvents. Make triplicate in order to have good data.
- 2. Add 2ml of TTC solution. Adapt your TTC solution from the natural environment of your sample (for example "Instant Ocean", saline solution, or sterile filtered site water). Final concentration of TTC is 0.4%.
- 3. Incubate the sample at room temperature (ideally 25°C) for 1 to 3 hours until a visible violet or red color develops.
- 4. Centrifuge the sample (2800xg; 10 min) and throw the supernatant.
- 5. Extract the TTC by adding 2mL propanol and centrifuge (2800xg; 5 min). If there are particles in suspension make a second centrifugation.
- Measure absorbance at 480 nm. Calculate the amount of formazan (i.e., reduced TTC) produced per unit of time with the Beer Lambert Law: A = E*l*C (A: measured absorbance; E: molar absorption coefficient (14 320 L.mol⁻¹.cm⁻¹); l: length of the cuvette (1cm)).

Annex 7 Sediments preparation for analysis and experiment

Always wear gloves to avoid sample contamination.

Sand preparation

- 1. Sieve your quartz sand through 100-200 µm filter and wash it with 1 M HCl during 2h.
- 2. Throw the liquid and rinse 3 time the sand by centrifuging with DI water.
- 3. Put the sand on a 30% H₂O₂ solution during 24h and repeat the DI water washing.
- 4. Dry in the oven at 120°C until completed desiccation.

Clay preparation

- 1. Grind the clay standard as thin as possible to make a powder and put the powder on the crusher (10min, 400 rpm).
- 2. Wash it with 1 M HCl during 2h then, throw the liquid and rinse 3 time the clay by centrifuging with DI water.
- 3. Put the clay on a 30% H₂O₂ solution during 24h and repeat the DI water washing.
- 4. Dry in the oven at 40°C until completed desiccation.
- 5. For clay solution, put the wanted amount of clay (e.g. 1g in 100 mL DI water for 10 g.L⁻¹ solution). Made the solution a 1 g.L⁻¹ NaCl in order to have a good ionic strength balance.

Annex 8 Sediments preparation for Granulometry

This protocol allows having an obscuration rate be between 2% and 25% to guarantee a good quality measurement.

Method:

- 1. Make a first sieving (1200 μm) with DI water in order to remove big particles. Granulometer is not able to measure elements of this size. Pour DI water over the sample in order to carry the fine particles to the recipient under the sieve.
- 2. The mixture recovered in the beaker is left to decant then dried in the oven at 40°C.
- 3. To prepare the sample add very little water to make a paw that ensures a good particle size distribution.
- 4. Take a drop of this paste in the most representative way possible with a metal spatula and dispose it in the container of the e-sampler. Do this step in triplicate.
- 5. Program the sequence and measure the grain size.
- 6. The program calculates and displays directly the particle size distribution of each sample.



Figure 2.144: Mastersizer 2000 laser granulometer. The inset on the left shows the typical sample used.

Annex 9 Diatom F/2 medium

Always wear lab coat and gloves to avoid any contamination. Use sterile DI water.

Artificial SeaWater solution:

- 4. Dissolve 1 g of 'Instant Ocean' in 1L of DI water (For 1 g.L⁻¹ salinity solution).
- 5. Put under stirring until salt complete dissolution.

Stock solutions:

- 1. Prepare the three stock solutions dissolving weight of the major nutrient powder as write on the table below.
- 2. After complete dissolution, autoclave the NaNO₃ and NaH₂PO₄ solutions.
- 3. Filter Na_2SiO_3 9H₂O solution (0.2 μ m).
- 4. Store at fridge (4°C) until processing.

Major	Stock s	Concentration	
nutrients	in 1 L H ₂ O	in 100 mL H ₂ O	(M)
NaNO ₃	75 g	7.5 g	8.82.10-1
NaH ₂ PO ₄	5 g	0.5 g	3.62.10 ⁻²
Na ₂ SiO ₃ ·9H ₂ O	30 g	3 g	1.06.10-1

Metal Stock solutions:

- 1. Prepare the rare metal stock solutions dissolving weight of the metal powder as write on the table below.
- 2. Prepare the global solution ("Metal solution") from the five 1st stock solutions and the two powders as write on the table below.
- 3. After complete dissolution, filter stock and metal solutions (0.2 μ m).
- 4. Store at fridge (4°C) until processing.
| | | | | | Concentration |
|---|-------------------------|---------------------------|-------------------------|----------------------------|-----------------------|
| Trace metals | 1st Stock solutions | | *Metal solution | | (M) |
| | in 1 L H ₂ O | in 50 mL H ₂ O | in 1 L H ₂ O | in 100 mL H ₂ O | |
| Na ₂ ·EDTA·2H ₂ | | | | | |
| 0 | - | - | 4.36 g | 0.436 g | 1.17.10 ⁻² |
| FeCl ₃ ·6H ₂ O | - | - | 3.15 g | 0.315 g | 1.17.10 ⁻² |
| CuSO ₄ ·5H ₂ O | 9.8 g | 0.49 g | 1 ml | 0.1 ml | 3.93.10-5 |
| ZnSO ₄ ·7H ₂ O | 22 g | 1.1 g | 1 ml | 0.1 ml | 7.65.10 ⁻⁵ |
| CoCl ₂ ·6H ₂ O | 10 g | 0.5 g | 1 ml | 0.1 ml | 4.2.10 ⁻⁵ |
| MnCl2 [·] 4H2O | 180 g | 9 g | 1 ml | 0.1 ml | 9.1.10 ⁻⁴ |
| Na ₂ MoO ₄ ·2H ₂ O | 6.3 g | 0.315 g | 1 ml | 0.1 ml | 2.6.10-5 |

Vitamins Stock solutions:

- 1. Prepare the stock solutions dissolving weight of the vitamin powder as write on the table below.
- 2. Prepare the global solution from the two 1st stock solutions and thiamin HCL powder as write on the table below.
- 3. After complete dissolution, filter solutions (0.2 μ m).
- 4. Store at freezer (-20°C) until processing.

	1st Stock solutions		Vitamin solution		
Vitamins				in 100 mL	Concentration (M)
	in 1 L H ₂ O	in 100 mL H ₂ O	in 1 L H ₂ O	H ₂ O	
Thiamin ⁻ HCl					
(B ₁)	-		200 mg	20 mg	2.96.10 ⁻³
Biotin (H)	1 g	10 mg	1 ml	0.1 ml	2.05.10-5
B ₁₂	1 g	10 mg	1 ml	0.1 ml	3.69.10-6

Method:

- 8. Prepare the F/2 medium by adding stock solutions as write on the table below. Note vitamin solutions are added after the autoclave.
- 9. Adjust pH between 7.8 and 8.2 with NaOH (1M) and HCL (1M).
- 10. Autoclave and let cold down at ambient temperature.
- 11. Add vitamins.
- 12. Complete volume to 1 L.
- 13. Store at fridge (4°C) until processing.

Stack solutions	Final m	Concentration				
Stock solutions	in 1 L ASW	in 2 L ASW	(M)			
Stock NaNO ₃	1 ml	2 ml	8.82.10-4			
Stock NaH ₂ PO ₄ ·H ₂ O	1 ml	2 ml	3.62.10-5			
Stock Na ₂ SiO ₃ ·9H ₂ O	1 ml	2 ml	1.06.10-4			
Metal solution*	1 ml	2 ml				
 Na₂ EDTA·2H₂O FeCl₃·6H₂O CuSO₄·5H₂O ZnSO₄·7H₂O CoCl₂·6H₂O MnCl₂·4H₂O Na₂MoO₄·2H₂O 						
Α	djust pH arou	nd 7.8-8.2				
Autoclave						
Vitamin solution	0.5 ml	1 ml				
• Thiamin [·] HCl (B ₁)						
• Biotin (H)						
• B ₁₂						



Chapter 3: Production and preservation of Exopolymeric Substances in the Gironde estuary sediments



Table of contents

Chap	ter 3: Production and preservation of Exopolymeric Substances in the Gironde estuary sediments	95
3.1	Characterization of microphytobenthic biofilm community	
3.2	Preservation of exopolymeric substances in estuarine sediments	
	ABSTRACT	106
	INTRODUCTION	107
	Sedimentological settings	109
	MATERIALS AND METHODS	112
	Field site description, sample location, and core sampling	112
	Core processing and sampling	112
	Sediment properties	113
	Cryo-Scanning Electron Microscopy (Cryo-SEM)	113
	Radiocarbon dating	114
	EPS extraction and purification	114
	Physicochemical properties of EPS: wet assays and acid base titrations	114
	Fourier Transform Infrared spectroscopy	115
	Statistical analyses	116
	RESULTS	116
	Vertical evolution of sedimentary facies and sediment properties	116
	Microscopic characterization of biofilms, EPS, and sediments	122
	Wet chemical assays: protein, phenol-sulfuric, and Alcian Blue	125
	Fourier-Transform-Infrared spectroscopy (FT-IR)	126
	Statistical analyses	129
	DISCUSSION	131
	EPS constituents, extraction, and quantification	131
	Relationships between EPS and sediment grain size	132
	Production and consumption of EPS in the sediment	
	EPS-mineral interactions and EPS preservation	136
	Potential tide versus river flood control on EPS preservation	138
	CONCLUSION	144
	ACKNOWLEDGEMENTS	145
	REFERENCES	146
	SUPPLEMENTARY MATERIAL (for Duteil et al. submitted in Frontier in Microbiology)	151
3.3	Annexes	157
	3.3.1 Cryo-SEM and EDX complementary data for BXN-Long Core	157
	3.3.2 Cryo-SEM and EDX complementary data for BXN-Box and BXN-Short Core	163

3.1 Characterization of microphytobenthic biofilm community

In the field, biofilms were observed at the surface of sediments mainly on the intertidal mudflat and in the muddy chute channel. Biofilms form a thin millimeter-thick layer of cohesive gel (Figure 1) with a golden brown color characteristic of diatom biofilms. On one occasion in spring 2020, the color was green due to a bloom of green algae. Biofilms were also visible on solid surfaces such as trunks or stones and more rarely on the mud between reeds on the supratidal marsh (Figure 3.1).



Figure 3.1: Field pictures of diatom biofilm. (A) Diatom biofilm covering on the crest of a mud relief on the mud flat. Diatom biofilms were also observed on rocks (B) or trunks (C).

Biofilms were collected on the Bordeaux Nord point bar at several periods of the year between 2018 and 2021, but mostly during spring and summer when the biofilm was highly active. The sampling is done at low tide by scrapping the sediment surface. Subsequent microscopic observations allowed to observe some of the biofilm-forming microorganisms. Details about technical aspects of microbiological preparations are available in chapter 2, material and

methods. Microphytobenthic biofilms were largely dominated by benthic diatoms (Figures 3.2 and 3.3). Other microorganisms were observed low abundance, e.g., euglenids, cyanobacteria (coccoid and filamentous; Figure 3.3), green algae and microscopic worms. In order to determine the main diatom genera, a few preparations were made following the protocol of Lavoie et al. (2008). For this, 4 mL biofilm is diluted in 20 mL of 30% H₂O₂ during a day to digest cells and EPS. Then the solution is centrifuged to remove the supernatant. DI water is added to the pellet to have a ready observable diatom frustule solution. The results of these characterizations showed a predominance of these genera in order: *Nitzschia, Navicula* and *Pleurosigma* (Figure 3.2). Other genera were also found in a punctual way as for example *Achnanthes* genera (Figure 3.3). Variation in diatom communities were observed along the year, with a predominance of a certain genus over the others (e.g., *Nitzschia* or *Pleurosigma* genera). For example, a large morphotype of *Nitzschia* often predominated in late summer-early autumn (Figure 3.3).



Figure 3.2: Optical microscopic pictures of cleaned frustules. (A) and (B) *Nitzschia* sp. frustules. (C), (D) and (E) *Navicula* sp. frustules. (F) *Achnanthes* sp. frustule.



Figure 3.3: Polarizing microscope photographs of diatom biofilm samples. The sampling date is also indicated. Na: sample *Navicula* sp., Ni: *Nitzschia* sp. and Pl: *Pleurosigma* sp. (A) and (B) sample dominated by *Navicula* sp. (C), (D) and (E) samples dominated by *Nitzschia* sp. (F) Sample dominated by *Pleurosigma* sp. (G) and (H) Diatoms (*Navicula* and *Nitzschia*) are predominant, but rare euglenids (G) or filamentous cyanobacteria (H) can be observed.

Most organisms in microphytobenthic biofilms are able to secrete EPS, but diatoms are the most efficient group for EPS production (Underwood et al., 1995). Microbiological preparations from Bordeaux Nord showed aggregates between diatoms and fine detrital particles (clay and silts). The diatoms and the sediments were held together by a translucent organic matrix (Figure 4A). Staining with Alcian Blue (which binds to acidic EPS molecules) revealed the EPS between the cells (Figure 4B). Translucent expansions were also visible around the frustules of Nitzschia sp. (Figure 4C, 4D). They could correspond to the extracellular strands responsible for the gliding of these diatoms in the sediment. Cryo-SEM observations showed details of the EPS, form an organic matrix around the cells and between the clay and silt particles (Figure 4E, 4F). Within the biofilm, the microorganisms secrete a significant amount of EPS which seems to adhere to the sedimentary particles (see chapter 5 for a detailed analysis of the mechanism of formation of EPS-sediment complexes). The following section will describe the composition, properties and preservation of these EPS with depth.



Figure 3.4: Interactions between diatoms and sedimentary particles in the Gironde diatom biofilms. (A) Polarizing microscope image showing three genera of diatom: *Nitzschia* (Ni), *Pleurosigma* (Pl) and *Navicula* (Na). Diatoms form aggregates with silt and clay, which are held together by the EPS matrix. (B) Alcian Blue staining of acidic EPS strands surrounding diatoms. (C) and (D) Polarizing microscope images showing small expansions outside the frustules of *Nitzschia* sp, which could correspond to EPS strands involved in diatom gliding. (E) and (F) Cryo-scanning electron microscope images of frustules coated with EPS. EPS can form an alveolar network (E) or occur as individual filaments (F). Part of the EPS secretion occurs through the pores visible in the frustule. Image (F) also displays clay particles bound to EPS.

3.2 Preservation of exopolymeric substances in estuarine sediments

This part is the subject of an article submission in the journal Frontiers in Microbiology currently in revision.

Résumé en français :

La surface des sédiments estuariens intertidaux est couverte de biofilms de diatomées excrétant des substances exopolymériques (EPS) par photosynthèse. Ces EPS sont très réactives et augmentent la cohésion des sédiments notamment par des interactions organo-minérales. Dans la plupart des environnements sédimentaires, les EPS sont partiellement ou totalement dégradées par des bactéries hétérotrophes dans les premiers millimètres du sédiment et on pense donc qu'elles sont pratiquement absentes plus profondément dans la colonne sédimentaire. Nous présentons ici la première preuve de la préservation des EPS et des agrégats minéraux-EPS dans une carotte sédimentaire de 6 mètres de long extraite d'une barre tidale estuarienne de l'estuaire de la Gironde. Les EPS ont été extraites à 18 profondeurs le long de la carotte et leurs propriétés physico-chimiques ont été caractérisées par (i) des tests colorimétriques pour mesurer les concentrations de polysaccharides et de protéines, ainsi que la densité des sites acides sur les EPS, (ii) des titrations acide-base, et (iii) la spectroscopie infrarouge à transformée de Fourier. Les complexes EPS-sédiments ont également été imagés par cryo-microscopie électronique à balayage. Les résultats ont été comparés aux propriétés des sédiments, notamment les faciès sédimentaires, la taille des grains et le carbone organique total. Nos résultats ont montré une diminution attendue des concentrations d'EPS (protéines et polysaccharides) et de la réactivité des EPS, du biofilm de surface jusqu'à une profondeur de 0,5 m. Cette diminution est probablement liée à une dégradation hétérotrophe. Les concentrations sont restées relativement faibles jusqu'à environ 4,3 m de profondeur. Étonnamment, à cette profondeur, l'abondance des EPS est comparable à celle du biofilm de surface. Les EPS montrent ensuite une nouvelle diminution jusqu'au pied de la carotte à 6,08 m. Le cryo-MEB a montré que les complexes EPS-sédiments étaient abondants à toutes les profondeurs étudiées. La composition des EPS ne change pas substantiellement de la surface au fond de la carotte et les propriétés des EPS ne montrent aucune corrélation statistique avec la taille des grains, le TOC ou la profondeur. Cependant, les concentrations maximales d'EPS ont été trouvées au sommet des séquences sédimentaires dominées par la marée, et de très faibles concentrations ont été trouvées dans les séquences sédimentaires dominées par les crues fluviales. Sur la base de cette observation, nous proposons un scénario où le développement du biofilm et la production d'EPS sont maximaux lorsque (i) la barre tidale et les zones intertidales étaient les plus étendues, c'est-à-dire au toit des séquences dominées par la marée et (ii) le dépôt dominé par la marée a été suivi d'un enfouissement rapide sous les sédiments de crue fluviale.

Preservation of exopolymeric substances in estuarine sediments

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ABSTRACT

The surface of intertidal estuarine sediments is covered with diatom biofilms excreting exopolymeric substances (EPS) through photosynthesis. These EPS are highly reactive and increase sediment cohesiveness notably through organo-mineral interactions. In most sedimentary environments, EPS are partly to fully degraded by heterotrophic bacteria in the uppermost millimeters of the sediment and so they are thought to be virtually absent deeper in the sedimentary column. Here we present the first evidence of the preservation of EPS and EPS-mineral aggregates in a 6 m-long sedimentary core obtained from an estuarine point bar in the Gironde Estuary. EPS were extracted from 18 depth intervals along the core and their physicochemical properties were characterized by (i) wet chemical assays to measure the concentrations of polysaccharides and proteins, and EPS deprotonation of functional groups, (ii) acid-base titrations, and (iii) Fourier transform infrared spectroscopy. EPS-sediment complexes were also imaged using cryo–scanning electron microscopy. The results were compared to sediment properties including facies, grain size, and total organic carbon. Our

results showed a predictable decrease in EPS concentrations (proteins and polysaccharides) and reactivity from the surface biofilm to a depth of 0.5 m, possibly linked to heterotrophic degradation. Concentrations remained relatively low down to ca. 4.3 m deep. Surprisingly, at that depth EPS abundance was comparable to the surface, and showed a downward decrease to 6.08 m. Cryo-SEM showed that the EPS complexes with sediment were abundant at all studied depth. EPS composition did not change substantially from the surface to the bottom of the core. EPS properties showed no statistical correlation with grain size, TOC, or depth. However, maximum EPS concentrations were found at the top of tide-dominated sedimentary sequences, and very low concentrations were found in river flood-dominated sedimentary sequences. Based on this observation, we propose a scenario where biofilm development and EPS production is maximal when (i) the point bar and the intertidal areas were the most extensive, i.e., tide-dominated sequences and (ii) the tide-dominated deposit was succeeded by rapid burial beneath river flood sediments.

INTRODUCTION

Diatoms are a major constituent of microphytobenthic biofilms covering intertidal or shallow subtidal coastal and estuarine sediments (Smith & Underwood, 1998). These biofilm communities excrete copious amounts of exopolymeric substances (EPS), forming a hydrated matrix between the cells and the sediments (De Winder et al., 1999). By secreting EPS, benthic diatoms establish a microenvironment that protects cells against desiccation, allows motility through the sediment, and supports communication and exchange of metabolites among cells (Underwood & Paterson, 2003; Decho & Gutierrez, 2017). Diatom EPS are composed of 40 to 90% polysaccharides, but also include proteins, nucleic acids, lipids, and low-molecular weight, non-carbohydrate compounds such as pyruvate and succinate (Underwood & Paterson, 2003). The negatively-charged reactive functional groups provide the EPS a cation binding capacity

to sequester metal ions or metalloids (Braissant et al., 2007). EPS also possess electrostatic, hydrophilic and hydrophobic properties allowing their sorption to mineral surfaces (Decho, 2000; Duteil et al., 2020). As a consequence, EPS promote the cohesiveness and stability of the sediment (Tolhurst et al., 2002), impacting bedform morphology (De Winder et al., 1999; Malarkey et al., 2015). In estuaries, EPS also bind clay and sand particles, resulting in the formation of thin clay envelopes, also referred to as detrital clay coats, that surround sand grains (Wooldridge et al., 2017; Virolle et al., 2019; Duteil et al., 2020; Virolle et al., 2021).

The production and consumption of EPS in subsurface sediments, as well as their composition and properties (e.g., negatively charged groups), are poorly understood. EPS are mainly produced by photoautotrophic microorganisms at the surface (e.g., cyanobacteria, purple- and green sulfur bacteria, benthic diatoms; Taylor et al., 2013). Below the sediment photic zone, heterotrophic communities (e.g., sulfate-reducing bacteria) are also able to produce EPS, albeit at lower production rates than photosynthetic communities (Braissant et al., 2007).

The amount of EPS typically decreases exponentially in the first millimeters to centimeters of estuarine mudflats (Paterson et al., 2000), but also in hypersaline stromatolites and microbial mats (Decho et al., 2005; Braissant et al., 2009; Pace et al., 2018), riverine sediments (Gerbersdorf et al., 2009a), or dryland cyanobacterial crusts (Mager, 2010). This decrease could result from their consumption by heterotrophic microorganisms. While labile forms of EPS such as low molecular weight components are preferentially consumed by heterotrophs, refractory high molecular weight polymers (e.g., proteins) have a higher preservation potential (Smith & Underwood, 1998; Kleber et al., 2007). The preservation of EPS in the sediment could be enhanced by three mechanisms: (i) inherent recalcitrance of specific EPS moieties against enzymatic microbial degradation, (ii) chemical stabilization by interactions between minerals and reactive groups in EPS, and (iii) physical protection through the formation of aggregates

with minerals (Kleber et al., 2015). However, it is not well understood to which degree the EPS persist with depth in sediments (Kleber et al., 2015).

In this study, we analyzed EPS extracted from a 6 m-long sedimentary core drilled in an estuarine point bar in the Gironde Estuary (southwest France). The composition including the physicochemical properties of EPS are determined at several depths by a combination of wet chemical assays, acid-base titrations, Fourier Transform-Infrared spectroscopy (FT-IR), and cryo-electron microscopy. EPS characteristics are compared to sediment properties (e.g., grain size, total organic carbon) and sedimentary facies (e.g., tidal and river flood deposits), in order to understand the factors potentially controlling EPS production and preservation in estuaries.

Sedimentological settings

The Bordeaux North point bar is located in the Garonne estuarine channel, 94 km upstream from the estuary mouth. The accretion of this point bar was probably started less than 300 years ago (Virolle et al., 2021). The bar is 1.1 km long (north–south axis) and 0.2 km wide (east–west axis). The Gironde Estuary has a turbidity maximum zone (TMZ) that forms a dynamic turbid cloud in the water column (Sottolichio et al., 2011). The TMZ has a high concentration of suspended particulate matter (concentration between 1 and 10 g.L⁻¹), spreading for 70 to 80 km along the estuary (Allen & Castaing, 1973). Salinity near the Bordeaux North point bar is about 1 g.L⁻¹ (Sottolichio et al., 2011). The suspended matter is composed of clay minerals (60%), fine quartz grains (<10 μ m; 25%), calcite (5%), organic matter (3%), and feldspars (6%). The average suspended clay assemblage is composed of illite (49%), smectite (22%), kaolinite (14%), and chlorite (15%) (Jouanneau & Latouche, 1981). In the sandy sediments of the point bar, the clay-fraction reached on average 20% of the total sediment volume, with about 7% of smectite, 9% of illite, 2% of chlorite, and 2% of kaolinite (Virolle et al., 2021). At low tide, the exposed part of the point bar can be divided into three zones (Figure 1), from the channel bank

to the estuarine point bar: (i) an intertidal mud flat (Figure 1A), (ii) a muddy chute channel extensively covered by gold-colored diatom biofilms (Virolle et al., 2019a; Duteil et al., 2020), and (iii) a low relief (<1 m) formed by the sandy point bar relative to the surface of the chute channel. The surface of the point bar is covered by tidal sand dunes. The point bar is heterolithic, being composed of sand dunes and muddy tidal rythmites (Allen, 1991). Details of the point bar stratigraphy can be found in Virolle et al. (2021).

111



Figure 1: (A) Map of the Gironde estuary showing the location of the Bordeaux North point bar, and satellite image of the point bar at low tide (Google Earth Pro) showing the location of the studied core (BXN Long Core) and surface samples. Three sedimentary domains are visible in the intertidal zone of the point bar: the mud flat, the chute channel, and the sand dunes. (B) Photographs and X-Ray image (SCOPIX) of the principal facies found in the BXN long core.

MATERIALS AND METHODS

Field site description, sample location, and core sampling

The field campaigns to the Bordeaux North point bar were conducted in June 2019. A 6.23 mlong vertical core (BXN Long Core; taken at 44°53'47.40"N, 0°32'24.80"W) was extracted with the EPOC portable vibro-corer (Bordeaux, France) from the chute channel (Figure 1). To protect sediment structures from deformation and fluidization due to water escape, the core barrel was hammered without any rotation and the piston was maintained with a cable. The core was stored at 4 °C until opening.

Core processing and sampling

Cores were photographed upon opening. Two-dimensional X-ray images of the cores were obtained with a SCOPIX device (EPOC laboratory, Bordeaux; Migeon et al., 1998), allowing for identification of sedimentary fabrics with a non-destructive approach at high resolution (125 μ m). Sixteen 20 cm-long samples (10 cm above and below the target depth) were taken along the core. Samples were homogenized and a subsample was weighed before and after drying in the oven (24 hr at 30 °C), in order to determine sediment wet and dry weights. A second subsample was kept for measurements of grain size and TOC content, and for cryofixation. The remaining part of the sample was used for EPS extraction, purification, and characterization.

Biofilms were collected by scraping from the surface of the intertidal muddy area (N 44°53'39.10", W 0°32'22.70") in the zone of drilling for comparison with core samples (Figure 1A). Surface samples were then split into two portions that were analyzed separately: (i) EPS extracted from the mud-poor supernatant were called water EPS; (ii) EPS extracted from the mud-rich sediment were referred to as mud EPS.

Sediment properties

Grain size was measured with a Mastersizer 2000 laser granulometer (Malvern, United Kingdom), with an obscuration rate of 2–25% at the EPOC laboratory (Bordeaux, France). The 16 sub-samples (approximately 1 g) were first sieved to remove large particles (>1200 μ m). Each sample was then measured in triplicate. The median grain size (D₅₀, in μ m) and the proportion of sand-, silt-, and clay-sized particles were calculated for each sample.

Total organic carbon analyses of sediments were performed with a TOC-LCSH analyzer (Shimadzu, Japan) at the IC2MP laboratory (Poitiers, France) using the Non Purgeable Organic Carbon method on the 16 core samples. This method consists in adding 2M HCl to the sample, and then removing dissolved inorganic carbon by air bubbling (2 min 30 s, flow rate: 150 mL.min⁻¹, purified air 5.0 quality). Then 50 μ L of sample was placed in a furnace at 720 °C for complete catalytic combustion and the CO₂ formed was detected by Infra-Red absorption. TOC concentrations of the liquid samples are expressed relative to a calibration curve established with sodium hydrogenophtalate standard solutions (range 0.2 to 10 mg-C.L⁻¹). Analyses were performed in duplicate.

Cryo-Scanning Electron Microscopy (Cryo-SEM)

For cryo-fixation, small fractions of each of the eight samples (seven core samples plus one sample from the surface biofilm) were placed in gold or silver cups (0.9–1 mm in diameter; 0.3 mm deep) and cryo-fixated under high-pressure freezing (HPF; pressure: 2050 bars) with an EM HPM 100 device (Leica; Switzerland) at the Bordeaux Imaging Center (BIC; Bordeaux, France). Cryo-fixation prevents the collapse of EPS, allowing optimal observations of three-dimensional organo-mineral relationships. HPF is rapid (freezing occurs within milliseconds) and ensures the simultaneous immobilization of all the EPS macromolecular components. Samples were stored in a cryo-bank (liquid nitrogen; -196 °C) until processing. They were

subsequently observed under cryo-SEM with a Gemini SEM 300 (Zeiss; Germany) at the BIC. Sublimation, coating, and transfer of the sample to the SEM chamber were done with a cryotransfer system PP3010T (Quorum Technologies).

Radiocarbon dating

Radiocarbon ages (Δ^{14} C) were measured on two gastropod shells (*Viviparus sp.*) found in the core (2.6 and 6.4 m) at the Atmosphere and Ocean Research Institute (University of Tokyo, Japan), using Single Stage Accelerator Mass Spectrometry. Sample preparations and analytical conditions vary depending on the sample size and are described in detail in Yokoyama et al. (2010).

EPS extraction and purification

EPS were extracted from the core and surface samples by diluting one volume of sample with two volumes of DI water. The solution formed was then put into an ultrasonic bath for 10 minutes then gently stirred for 4 hours. Centrifugation was performed (2500xg, 10 min) to remove the sediments (silt, clay, and sand) and cells. The recovered supernatant was filtered through polycarbonate filters (0.22 μ m). The filtrate was precipitated using three volumes of cold propanol (<0 °C) per volume of filtrate for a day. The precipitate was recovered by centrifugation (2800xg, 20 min), placed in dialysis tubing (10 kDa), and dialyzed for three days in a buffer solution (2 L of DI water at 1mM EDTA) and three more days against 2 L of DI water (>18 M\Omega). Liquid EPS solutions were then stored in the refrigerator at 4 °C.

Physicochemical properties of EPS: wet assays and acid base titrations

The composition of EPS was estimated using three colorimetric assays: (i) phenol-sulfuric acid assay (Dubois et al., 1956), (ii) the alcian blue assay (Passow & Alldredge, 1995) and (iii) the Pierce-modified Lowry assay (Lowry et al., 1951). The three assays provide an estimate of (i) the amount of sugar monomers in the sample (i.e., the amount of neutral sugars in EPS); (ii) the

amount of cationic dye binding sites (i.e., the amount of negatively-charged reactive sites in the EPS); and (iii) the amount of proteins. Triplicates (100 μ l) were used for each assay. The standards were xanthan for the phenol-sulfuric and alcian blue assays, and bovine serum albumin for the Pierce-modified Lowry assay. Absorptions were measured on a Helios Epsilon spectrophotometer (Thermo scientific; cat: 9423UVE100CE).

Acid-base titrations were performed on two EPS samples (surface water EPS and 6.08 m depth) to determine the potential types and densities of the functional groups present in the EPS. For that, 3 mL of EPS were acidified with 100 μ L HCL (1 M), and 100 μ L of KCL (1 M) were added to provide sufficient ionic concentration. Once acidified, the solution was left under constant nitrogen flow for 20 minutes to avoid potential CO₂ dissolution and carbonate or bicarbonate formation during the titration. The solution was titrated with NaOH (0.1 M) added stepwise in 10 μ L increments and 5 μ L increments when the pH rise was fast. The pH was recorded up to 11 with a pH microelectrode (Sentix 60, A005106A030) connected to a Consort C561 meter (De Bruyne, Belgium). Control titrations were effected with DI water under the same experimental conditions. The titration curves were analyzed using PROTOFIT 2.1 software (Turner & Fein, 2006).

Fourier Transform Infrared spectroscopy

Fourier Transform Infrared Spectroscopy (FT-IR) analyses of the 18 EPS samples (16 core samples and 2 surface samples) were performed on a Nicolet IS50 (ThermoScientific) Spectrometer in Attenuated Total Reflection (ATR) mode at the IC2MP laboratory (Poitiers, France). One drop of EPS solution was placed on the diamond ATR element and evaporated. Spectra were acquired between 650 cm⁻¹ and 4000 cm⁻¹, with a 4 cm⁻¹ spectral resolution and 100 scans were co-added for each spectrum.

Statistical analyses

Statistical analyses were performed with the Matlab® R2018b statistic toolbox software. Normality of data was assessed by the Kolmogorov-Smirnov test. As non-parametric distributions were observed, the relationships between EPS and sediment properties were assessed using Spearman rank correlation as well as principal components analysis (PCA).

RESULTS

Vertical evolution of sedimentary facies and sediment properties

Four facies were defined in the BXN Long Core (Figure 1B):

Facies 1 (F1; fluvial flood facies) consisted of centimeter- to decimeter-thick horizons of coarse-grained sand mixed with detrital gravels, generally exhibiting a fining-upward trend. These deposits were intercalated with floating centimetric- to multi-centimetric-sized rounded to sub-angular soft mud clasts, some armored with quartz grains (Figures 1 and 2). Mud clasts were more abundant at the base, and were locally totally amalgamated. Facies 1 could also be recorded locally as a single erosive surface marked by sparse granule pavements or local accumulation of soft mud clasts over a few centimeters (e.g., between 0.9 and 1 m depth, Figures 1 and 2).

Facies 2 (F2; tidal sand dunes facies) was composed of well- to moderately-sorted fine-tomedium sands, organized in 10 to 25 cm-thick beds of coarse sands with trough cross-bedding and reactivation surfaces. Dune foresets were locally draped by muds forming mud couplets and were mostly oriented seaward indicating a predominant ebb current (Figures 1 and 2). Facies 3 (F3; heterolithic fine sands and laminated mud facies) was made up of decimeter to meter-thick laminated heterolithic intervals composed of grey to brown laminated clay

117

(lamination was more clearly visible in ScopiX images; Figures 1 and 2) and well- to moderately-sorted fine-to-medium micaceous sands with occasional centimetric current ripples draped by muds.

Facies 4 (F4; fluid and laminated mud facies) consisted of centimeter to decimeter-thick fluid mud layers alternating with millimeter-thick layers of very fine-grained sands to silty lenses and slack-water clay drapes.

Figure 2 shows the vertical evolution of sedimentary facies, median grain size (D_{50}), and the proportion of particle class sizes. The core is dominated by silt (23–78%) and sand (17–90%), while clay-sized particles account for 0 to 7% of the sediment. Four sequences (Sequence 1 to Sequence 4) could be defined based on sedimentary facies, grain size, and bed thickness trends:

- (i) sequence 1 (depth 6.46 m to 4.25 m). The base of sequence 1 (6.46–5.95 m) was dominated by fluid mud (F4). Three sand and mud pebble-rich flood deposits (F1) were found, respectively at 6.4 m, 6.10 m, and 6.00 m. A gastropod found at 6.40 m yielded a radiocarbon age of 2939±24 BP. The top of sequence 1 (Figure 2) was made up of decimeter-thick sand dunes (F2) alternating with fine-grained silty heterolithic layers exhibiting centimeter-thick current ripples (F3). Tidal dunes showed a thickening-upward trend, and the proportion of sand increased from 55 to 98% between 6.08 and 4.33 m. The D₅₀ followed approximately the same trend, increasing from 174±93 to 473±7 µm. This sequence is tide-dominated.
- (ii) sequence 2 (depth 4.25 m to 2.80 m). The base of sequence 2 (4.25 m to 3.50 m) was dominated by silty heterolithic layers displaying centimeter-thick current ripples (F3). From 3.50 to 3.65 m, the mud showed a reddish stain. A decimeter-thick fluvial flood layer rich in mud pebbles (F1) was found at 4.05 m and a sandy layer with ebb-oriented dune foresets was found at 3.90 m. The top of sequence 2 (3.50 m to 2.80 m) consisted of successive fluvial flood deposits rich in sand and

118

mud pebbles and bounded by erosive bases (F1). The proportion of sand and the D_{50} increased respectively from 61 to 82% and from 371±3 to 487±3 µm in the same depth interval. The uppermost fluvial flood layer was 25 cm thick. This sequence is dominated by river flood deposits.

- (iii) sequence 3 (depth 2.80 m to 1.30 m). The base of sequence 3 (1.75 m to 2.80 m) is made up of fine grained heterolithic layers with current ripples (F3). The proportion of sand reached its lowest relative content (17%) at 2.59 m, while D₅₀ reached 20±0.7 µm at 2.16 m. Two decimeter-thick fluvial flood layers with sand and mud pebbles (F1) were found at the top of the sequence (1.70 m and 1.40 m). The proportion of sand and D₅₀ increased respectively to 75% and 485±5 µm at 1.63 m, and decreased to 66% and 367±79 µm at 1.3 m. A gastropod shell found at 2.59 m had a 1459±24 BP radiocarbon age. This sequence is dominated by river flood deposits.
- (iv) sequence 4 (depth 1.30 m to the sediment surface). The base of sequence 4 consisted in a 5 cm thick homogeneous fluid layer (F4), overlain by fine-grained heterolithic deposits (F3) from 1.25 to 0.75 m. Two river flood events (F1) were recorded at 1.00 m and 0.93 m as centimeter-thick coarse sand to granules and mud pebble layers with erosive bases. An ebb-oriented sand dune (F2) was found between 0.75 and 0.5 m. The top of sequence 2 was dominated by silty heterolithic deposits (F3). A second ebb-oriented sand dune (F2) was observed between 0.25 and 0.35 m. The proportion of sand increased from 17% to 90% between 0.93 and 0.5 m, and decreased to 50% at 0.06 m. The median grain size follows approximately the same trends, increasing from 24±0.4 to 274±2 µm, then decreasing to 69±19 µm. This sequence is tide-dominated.

TOC fluctuated between 0.17 and 10.84 milligrams of carbon per gram of dry sediment (mg.g⁻¹ DW). TOC decreased from 7.92 to 0.17 mg.g⁻¹ DW between 6.08 m and 4.33 m, which was followed by an increase to 5.97 mg.g⁻¹ DW at 4.12 m. It subsequently dropped to 1.83 mg.g⁻¹ DW at 3.68 m, and then progressively increased and reached a maximum of 10.84 mg.g⁻¹ DW at 2.16 m. In the upper part of the core, TOC was below 0.8 mg.g⁻¹ DW at 1.63 m, 1.30 m, and 0.5 m, and exhibited high values of 7.58 mg.g⁻¹ DW and 4.72 mg.g⁻¹ DW at 0.93 m and 0.06 m respectively.



Figure 2: Evolution of exopolymeric substances (EPS) and sediment properties with depth along the 6 m-long BXN Long Core (see location Figure 1). First column to the left: sedimentary description. (1) X-Ray image (SCOPIX); (2) core photographs; (3) sketch; (4) facies; (5) sedimentary log, and (6) sedimentary sequences. Sample depths are written in red. Second column: median grain size (D₅₀; yellow dots). Third column: particle size class percentage: clay (brown), silt (grey), and sand (yellow). Fourth column: Sugar and protein concentrations: phenol-sulfuric assay (yellow) and protein assay (green). Fifth column: EPS acidic site density measured with the alcian blue assay (blue). Sixth column: total organic carbon (TOC) in the sediment (black).

Microscopic characterization of biofilms, EPS, and sediments

The biofilm formed a golden layer, approximately one-millimeter-thick, at the surface of the sediment (Figure 3A). Microscopic inspection revealed abundant diatom presence (Figure 1B) and also contained a few green microalgae (e.g., *Euglena* sp.). Three main genera of diatoms were identified: *Nitzschia* sp., *Pleurosigma* sp., and *Navicula* sp (Lange-Bertalot et al., 2017). Exopolymeric substances (EPS) occurred as a transparent gel around diatoms and/or within and around clay and silt aggregates. Alcian blue staining showed that the EPS were acidic (Figure 3B). Cryo-SEM observations revealed the fine alveolar or filamentous texture of EPS surrounding diatoms covered with clay- to silt-sized particles (Figures 3C and 3D).

Diatoms were scarce below the surface biofilm (Figure 4), but EPS were found at all sampled depths within the core (Figures 2, 4, S1 and S2). Sand and large silt grains were covered by EPS alone (Figures 4A and 4B), or by a detrital coat composed of clay to fine silt attached to EPS (Figure 4C to 4F). Similar coats were observed in all sand-dominated layers (4.76, 4.33, 2.80, and 0.50 m). In mud-rich layers, EPS formed an alveolar network between clay and silt particles (Figure S1). The resulting organo-mineral complex was observed in all muddy layers sampled (Figures 4, S1, and S2).



Figure 3: Field and microscope observations of the surface biofilm. Exopolymeric substances (blue arrow), diatoms (green arrow), and clay-EPS complexes (brown arrow). (A) Field picture of the diatom biofilm at the surface of the sediment. The biofilm forms a millimeter-thick golden lamina at the surface of the sediment. (B) Transmitted light microscopic image of the biofilm colored with alcian blue. The blue coloration shows acidic EPS fibers surrounding diatoms. Three diatom genera were identified: *Nitzschia* sp., *Pleurosigma* sp., and *Navicula* sp. (C) Cryo-SEM picture of the surface biofilm showing two diatoms surrounded by a mixture of clay and EPS. EPS fibers form an organo-mineral complex with clay platelets. (D) Cryo-SEM picture of a diatom. Cryo-fixation preserves the 3D structure of EPS and allows the observation of very thin EPS fibers. The clay-EPS complex displays a typical alveolar structure.

0.06m depth:



Figure 4: Cryo-SEM images of subsurface sediments from the BXN Long Core at three depths. Exopolymeric substances (blue arrow), quartz grain (yellow arrow), mica (red arrow), and clay-exopolymeric substance (EPS) complexes (brown arrow). (A) and (B) Depth of 0.06 m. Alveolar EPS cover a large part of a quartz grain. EPS are locally complexed to clay particles, forming a detrital clay coat. (C) and (D) Depth of 4.33 m. A clay-quartz-EPS complex covers a large part of a mica (C). A sand quartz grain is fully covered by a clay-rich detrital coat (D). (E) and (F) Depth of 6.08 m. EPS fibers are partially covered with clay platelets and coat both quartz and mica grains (E). The surface of a quartz sand grain is partially coated by a dense clay-EPS complex (F).

Wet chemical assays: protein, phenol-sulfuric, and Alcian Blue

The amount of EPS measured with the three colorimetric assays (i.e., protein, phenol-sulfuric, and alcian blue) exhibited large variations with depth (Figures 2 and S3). For comparison, the results of the assays are expressed as micrograms of EPS per gram of dry sediment (µg.g⁻¹DW), relative to albumin (protein assay) or xanthan (phenol-sulfuric and Alcian Blue assays) standards. The biofilm at the surface of the sediment had the highest EPS value for the phenolsulfuric assay ($23\pm3 \mu g.g^{-1}$ DW), and high values for the protein and Alcian Blue assays $(37\pm5 \ \mu g.g^{-1} DW and 27\pm1 \ \mu g.g^{-1} DW respectively)$. The EPS extracted from the surface mud showed a more than a twofold decrease in EPS amount ($14\pm0.3 \ \mu g.g^{-1}$ DW). They also had the highest acidic site concentration (32±0.5 µg.g⁻¹ DW) but small amounts of proteins $(7\pm1 \text{ }\mu\text{g.g}^{-1} \text{ }\text{DW})$. At 0.06 m, the amount of EPS neutral sugars showed a slight decrease as well as proteins and acidic sites density (4.9±0.05 µg.g⁻¹ DW, 13.6±0.5 µg.g⁻¹ DW, and $16.6\pm0.1 \,\mu g.g^{-1}$ DW respectively). This was followed at 0.5 m by a seven-fold decrease for the phenol-sulfuric and protein assays and by a 24-fold decrease for the Alcian Blue assay. EPS concentrations increased at 0.93 m to $1.2\pm0.7 \ \mu g.g^{-1}$ DW for neutral sugars, $11\pm0.5 \ \mu g.g^{-1}$ DW for proteins and $11\pm0.05 \ \mu g.g^{-1}$ DW for the acidic sites density. Between 1.3 and 2.16 m, EPS amounts were low, below 1 µg.g⁻¹ DW for the neutral sugars, 3 µg.g⁻¹ DW for proteins and $2 \mu g.g^{-1}$ DW for acidic sites density. At 2.59 m, EPS concentrations increased to $3\pm 3 \mu g.g^{-1}$ DW for neutral sugars, $8\pm0.2 \ \mu g.g^{-1}$ DW for proteins and $18\pm0.7 \ \mu g.g^{-1}$ DW for acidic sites density. Between 2.8 and 4.12 m, EPS concentrations were below 2 µg.g DW⁻¹ for the phenol-sulfuric assay, 3 µg.g⁻¹ DW for the protein assay, and 5 µg.g⁻¹ DW for the Alcian Blue assay. At 4.33 m, concentrations were high, reaching $6.6\pm0.2 \ \mu g.g^{-1}$ DW for neutral sugars, $35.7\pm9 \ \mu g.g^{-1}$ DW for protein, and $13\pm0.24 \ \mu g.g^{-1}$ DW for acidic sites. Between 4.33 m and 6.08m, EPS concentrations decreased progressively to around 2.2 µg.g⁻¹ DW for the phenol-sulfuric assay, $8 \,\mu g.g^{-1}$ DW for the protein assay, and $8.4 \,\mu g.g^{-1}$ DW for the Alcian Blue assay (Table S3).

Fourier-Transform-Infrared spectroscopy (FT-IR)

EPS extracted from the core displayed several major infrared absorption peaks typical of polysaccharides and proteins, and also of nucleic acids and lipids (Braissant et al., 2007; Braissant et al., 2009; Duteil et al., 2020; Figures 5, S4, S5 and S6): (i) the vibrational bands in the spectral range 2800-3000 cm⁻¹ represent the symmetric and antisymmetric stretching of C-H from CH₂ and CH₃ groups; (ii) the peak at 1710 cm⁻¹ could be attributed to protonated carboxylic acid groups (iii) the peak at 1630 cm⁻¹ was likely due to amide I C=O stretching; (iv) the peak at 1535 cm⁻¹ could be attributed to the amide II C-N stretching and NH bending; (v) the peak at 1450 cm⁻¹ represented the CH₂ scissoring vibration; (vi) the doublet at 1404 and 1365cm⁻¹ was likely due to C-OO⁻ stretching; (vii) the peaks at 1235 cm⁻¹ could be attributed to stretching of P=O bond in phosphate and the C-O-C group in esters, and at 1155 cm⁻¹ to S=O stretches vibration from sulfate; (viii) the peak at 1024 cm⁻¹ represented the carbohydrate C-O stretching vibrations. While present in the surface biofilm EPS, protonated carboxylic acid groups disappeared at 0.06 m and 4.33 m. This group could be characterized again by a very small peak at 6.08 m (Figure 5). The observed frequency variation of amide I between samples (1619-1651 cm⁻¹) can be explained by the angular deformation of water from EPS or smectite (Chukanov & Chervonnyi, 2014). The amide II band exhibited a shift with depth between the surface and the bottom of the core (from 1535 to 1521 cm⁻¹).

While absent from the surface biofilm, clay stretches (Figure 5) of various heights were identified in the 0.06 m sample and below. They could be attributed to one or more of the four clay mineral species found in the estuary, i.e., chlorite, smectite, kaolinite, and illite (Virolle et al., 2019a; Duteil et al., 2020). The four minerals have common around 3620 (structural OH stretching) and 1020 cm⁻¹ (SiO stretching) that were identified in all samples. Chlorite presents specific peaks at 3550, 3400, and 990 cm⁻¹, smectite at 3400 cm⁻¹, and kaolinite at 3627, 1113, and 914 cm⁻¹ (Bergaya & Lagaly, 2013; Chukanov & Chervonnyi, 2014). Some of these peaks

(e.g., Si-O at 1020 cm⁻¹) masked EPS peaks, e.g., carbohydrates C-O at 1024 cm⁻¹. The broad peak between 3500 and 2800 cm⁻¹ were assigned to water H₂O stretching vibrations.



Figure 5: Fourier transform infrared spectroscopy spectra of four exopolymeric substances samples recovered from different depths along the BXN Long Core. Colored lines highlight the EPS absorption bands, while black dotted lines indicate clay mineral absorption bands. The red arrows show the position of the protonated carboxylic acid group stretches, which attenuate or disappear at a certain depth. Green arrows show the shift of the amide II stretches.
EPS acid-base titrations

Acid-base titrations performed on EPS extracted from the surface biofilm (water EPS) and from the bottom of the core (6.08 m depth) exhibited similar patterns and could be described using a model assuming three main buffering zones (Figure 6). These buffering zones correspond to the capacity of the EPS to counteract the pH changes induced by the addition of NaOH through a release of protons. The first buffering capacity was encountered at a pH close to 2 and could be attributed to carboxyl or sulfate groups. The second buffering zone, found at pH 3.4 in the surface EPS and at pH 5.52 for the 6.03 m EPS, could be attributed to carboxyl groups. The third buffering area observed around pH 10 in both samples could be attributed to amine groups. Typical pK values range below 2.8 for sulfates, between 1 and 5 for carboxylic acids, and between 8.5 and 12.5 for amines (Stumm & Morgan, 1996; Braissant et al., 2007). Using Protofit 2.1 software (Turner & Fein, 2006), and assuming three non-electrostatic binding sites, the pKa of the proton-binding sites as well as their densities could be estimated (Figure 6). A first pKa (pK1) between 1.9 and 1.98 was attributed to either carboxyl or sulfate groups and accounted respectively for 73% and 72% of site density in the surface and 6.08 m EPS. The second buffering zone (pK2) was located at 3.4 and represented 23% of site density in surface EPS. In 6.08 m EPS, pK2 was found at 5.32, accounting only for 5% of site density. The third buffering zone (pK3) located at 9.28 (surface EPS) and 10.53 (6.08 m EPS) was attributed to amino groups, with a higher site density at 6.08 m (21%) than at the surface (4%). These results showed that EPS predominantly included low pKa sites in both surface and deep sediment EPS. However, a higher amino group density indicated a potential for deep sediment EPS to deprotonate at higher pH.



Figure 6: Acid-base titration curves from exopolymeric substances (EPS) extracted from the surface biofilm and the bottom of the BXN Long Core (depth=6.08 m). The titration curves obtained were analyzed using PROTOFIT 2.1 software, and both show three buffering zones. The first zone located around pH 2 is attributed to carboxyl or sulfate groups, the second zone located near pH 5 is attributed to carboxyl groups, and the third buffering zone near pH 9.5 is attributed to amino groups. Control titration was performed using the same volume of deionized water adjusted to the same initial pH with HCl 1M.

Statistical analyses

The correlation matrix (Figure 7) reveals significant correlations between sediment grain size and TOC and EPS properties (based on three assays). The first axis of the plot explains 47% of the variation and the second 35%. D₅₀ and silt and clay are strongly anti-correlated (n=16 samples, p-value ≤ 0.05 , r=-0.91). TOC is inversely correlated with D₅₀ (n=16, r=-0.6; p-value ≤ 0.05) while being positively correlated with silt+clay (n=16, r=-0.57, p-value ≤ 0.05), confirming that finer sediments contain more organic carbon (Figure 2). The amount of sugars in EPS (phenol-sulfuric assay) is positively correlated with the amount of proteins (n=16, r=0.83, p-value ≤ 0.05). The alcian blue assay (density of acidic sites in EPS) is positively correlated with the phenol-sulfuric and the protein assays (n=16, r=0.70; p-value ≤ 0.05 and r=0.57; p-value ≤ 0.05 , respectively). There were no statistical correlations between sediment grain size and EPS abundance/reactivity (p-values >0.05), and depth was not a discriminating factor either (p-values >0.05).



Figure 7: Left, Principal Components Analysis circle plot of correlation between the samples from the BXN Long Core. Right, correlation matrix of Pearson's r coefficients between EPS properties, sedimentological factors, and depth for the 16 samples. Green boxes indicate p-value ≤ 0.05 .

DISCUSSION

EPS constituents, extraction, and quantification

Exopolymeric substances are composed of numerous molecules from gels to fully dissolved states that provide the particular physical properties and critical functions of the biofilm (Decho, 2000; Flemming et al., 2016). The EPS matrix is usually separated into two fractions, the bound EPS and the colloidal EPS (also called soluble EPS; Nielsen & Jahn, 1999). Bound EPS (e.g., capsular polymers, condensed gel) are closely associated with cell surfaces, while colloidal EPS (e.g., soluble macromolecules, "slimes") are mainly dissolved and interact more with sediments and/or water (Underwood & Paterson, 2003). The two fractions usually have different properties. The colloidal EPS were particularly targeted in our study due to their reactivity toward sediments (De Brouwer et al., 2002), including the capacity to form organomineral complexes with clay minerals (Duteil et al., 2020). Physical methods, particularly centrifugation, are generally efficient for the extraction of colloidal carbohydrates and proteins (Comte et al., 2006). EPS can be isolated from cells by centrifugation, a method that minimizes cell lysis and contamination by intracellular material (Klock et al., 2007). Due to the large amount of fine sediments in our samples, we added a short ultrasonication step in order to break bonds between EPS and minerals (Dignac et al., 1998). Furthermore, ultrasonic treatment increases the EPS yield by 1.17 on average (Comte et al., 2006). Chemical extractions including propanol as a solvent ensure that the extracted EPS are not contaminated by the intracellular material (Comte et al., 2006). EPS precipitation in cold propanol is particularly effective for extracting polysaccharides and proteins with limited structural modification of these molecules (D'Abzac et al., 2010). This precipitation method mostly selects polymers larger than 100 kD (De Brouwer & Stal, 2001), which are key polymers for the physical and chemical properties of biofilm (Decho & Gutierrez, 2017) or interactions with sediment (De Brouwer et al., 2002;

Xu et al., 2020). Based on our protocol, we assume that the EPS extracted in our study include mostly the colloidal fraction and possibly (a small part of) the bound fraction of EPS.

The concentrations of sugars in EPS are commonly measured with the phenol-sulfuric acid assay. In estuarine or intertidal mudflats the colloidal EPS concentration for the surface biofilms ranged from 8–10 μ g.g⁻¹ sediment DW (Orvain et al., 2014; Paterson et al., 2000 respectively) to 3 mg.g⁻¹ DW (Yallop et al., 2000). In these studies, glucose is used as a standard for the phenol-sulfuric assay. In our work, EPS concentrations were measured using xanthan as a standard and were between 0.7 μ g.g⁻¹ DW in some deep sediment horizons and 17 μ g.g⁻¹ DW in the surface biofilm. Xanthan was chosen because it is a large polysaccharide closely related to the molecules naturally present in EPS (Chenu & Robertson, 1996; Braissant et al., 2008). Xanthan is composed of five hexoses, i.e., it is five times larger than glucose. Even multiplied by 5, the sugar concentrations measured in this study remain largely below 100 μ g.g⁻¹ DW (in glucose equivalent), which places our results in the low to medium range of EPS concentrations in similar environments. Such EPS concentrations could reflect moderate EPS production in the studied area possibly related to seasonal variations. However, the very high sedimentation rates on the Bordeaux North point bar (up to 4 cm.yr⁻¹; Virolle et al., 2021) could dilute EPS both in surface and subsurface sediments.

Relationships between EPS and sediment grain size

Estuarine microphytobenthic biofilms grow in the intertidal zone of estuaries, preferentially on muddy and stable substrates (e.g., Underwood & Paterson, 2003; Orvain et al., 2012). However, EPS components show a differential response to sediment grain size. In surface sediments or short sedimentary cores (<30 cm), the amount of colloidal carbohydrates is often positively correlated to the proportion of mud (i.e., particles <63 um; Paterson et al., 2009; Hope et al., 2020), while proteins or bound carbohydrates are not (Gerbersdorf et al., 2009a). Similar

observations were made in laboratory experiments, with greater secretion of colloidal carbohydrates by diatoms on mud than on sand, while bound proteins were more abundant in sand-mud mixtures (Ubertini et al., 2015). Our results show no correlation between grain size, and the amounts of sugars, proteins, or the acidity of EPS in the 6 m-long core BXN-LC (Figures 2 and 7), indicating that grain size does not exert a major influence on EPS preservation in subsurface sediments.

Light attenuation is greater in muddy sediments than in sands. While light penetrates just the uppermost millimeter of muds, it can persist up to several millimeters or centimeters in sands (Kühl et al., 1994; Cartaxana et al., 2011). Distinct assemblages, of epipelic and epipsammic diatoms, develop on mud and sand. Epipelic diatoms are able to migrate vertically through the sediment as a possible adaptation to lower and more fluctuating light conditions, and/or to avoid resuspension (Jesus et al., 2009). A high level of production of EPS in mud and sand mixtures could reflect a balance between light and nutrient availability (Morelle et al., 2020). Biofilm development could be less efficient at the surface of sands, but persist deeper, attenuating the difference in EPS concentrations between sandy and muddy facies. Besides, erosion of EPS by tidal currents could be intensified in sands due to their greater permeability (Ubertini et al., 2015). Underwood and Krompkamp (1999) estimated that 30 to 50% of the EPS produced in intertidal biofilms could be transported during a semi-diurnal cycle. Strong tidal currents and wind waves cause resuspension and redistribution of sediments including EPS-sediment complexes (e.g., Underwood, 2010; Redzuan & Underwood, 2021), which could contribute to the homogenization of EPS concentrations in low-energy muddy and high-energy sandy areas (Duteil et al., 2020).

Production and consumption of EPS in the sediment

Our results show that the photosynthetically active diatom biofilm has the highest concentrations in colloidal EPS (both sugars and proteins) in the intertidal zone of the Gironde estuary (Figures 2 and 4). We also show for the first time that significant amounts of EPS can be preserved several meters deep in estuarine sediments (Figure 4). Benthic diatoms produce EPS with functions (e.g., motility in the sediment, adhesion to the sediment, protection against desiccation) allowing their establishment and sustainability on the estuary sedimentary bottom, despite highly fluctuating light conditions, hydrodynamics or frequent emersions (Decho, 2000). In sedimentary environments, EPS are highly concentrated and reactive in the biofilms developing at the surface of the sediment and within the first millimeters of the sediment, owing to photoautotrophic production (Braissant et al., 2009; Pace et al., 2018). The diatom genera present in the Gironde epibenthic biofilms (e.g., *Navicula*, *Nitzchia*) are efficient EPS producers, as observed in cultures (Underwood & Paterson, 2003).

EPS quantities generally decrease exponentially with depth, as documented in several sedimentary environments such as river sediments (Gerbersdorf et al., 2009a), estuarine mudflats (Paterson et al., 2000; De Brouwer & Stal, 2001), hypersaline lake microbial mats (Pace et al., 2018; Braissant et al., 2009), and tropical soil crusts (Mager, 2010). The decrease in the amount of EPS and in their reactivity with depth is interpreted as the result of (i) lowered photoautotrophic EPS production below the photic zone of the biofilm/mat and (ii) increased heterotrophic consumption associated with aerobic and anaerobic respiration (Decho et al., 2005; Braissant et al., 2009; McKew et al. 2013). Within the sediment, heterotrophs rapidly (within days) consume the smaller and more labile fraction of the EPS (also LMWOC), while larger and refractory molecules are degraded more slowly (Decho et al., 2005; Braissant et al., 2009). In intertidal sediments, anaerobes are more efficient than aerobes at degrading the

large and refractory EPS molecules produced by diatoms into smaller molecules (i.e., LMWOC; McKew et al., 2013).

In the Gironde estuary, we observe a decrease in the amount and acidity of EPS near the surface and at a depth of 0.5 m (Figure 2), which was combined with a disappearance of the carboxylic acid peak in FT-IR from 0.06 m (Figures 5, S4 and S5). Diatoms are known to produce sulfaterich polysaccharides (Bhaskar et al., 2005). The presence of sulfate groups in the Gironde water EPS (Figure 5) could indicate a greater contribution of diatoms to the surface EPS pool. The disappearance of the sulfate peak in deeper horizons (Figure 5) could result from the consumption of these sulfate-rich EPS by heterotrophs (Battersby et al., 1984; Visscher et al., 1999). The consumption of low-molecular-weight organic compounds (LMWOC) reduces the number of acidic functional groups in EPS (Braissant et al., 2009). Such a phenomenon could explain the decreasing EPS concentration and reactivity observed from the surface biofilm to a depth of 0.5 m, but also the low values observed between 1.30 and 4.12 m (except for 2.59 m). By contrast, some deeper horizons contained EPS in comparable amounts to the surface biofilm (e.g., 4.33 m; Figure 2). The relatively high acidic sites densities observed at 0.93 m, 2.59 m, 4.33 m, or 5.20 m (Figure 2) could indicate a relatively low degree of EPS degradation in these horizons. To our knowledge, this is the first report of reactive EPS several meters deep in sediment. These EPS could be remnants of former diatom biofilms. The degradation of photosynthetically produced EPS by heterotrophs may result in the formation of more refractory EPS compounds (Decho et al., 2005). Some extracellular proteins produced by bacteria or diatoms, such as amyloid fibrils, are also highly resistant to heterotrophic degradation (Decho & Gutierrez, 2017), which could explain the high protein concentrations in some deep horizons from the studied core (e.g., 0.93 m, 4.33 m, 4.76 m; Figure 2).

Aerobic and anaerobic heterotrophs inhabiting sediments also secrete EPS, including abundant extracellular enzymes (Marvasi et al., 2010; Decho & Gutierrez, 2017). For instance, sulfate-

reducing bacteria (SRB) produce EPS rich in polysaccharides in culture and probably within hypersaline microbial mats (Braissant et al., 2007). Being abundant in estuarine sediments (Leloup et al., 2005), SRB could be significant contributors to the subsurface EPS pool of the Gironde estuary. Other anaerobes such as methanogens are known to produce polysaccharide and protein-rich EPS (Veiga et al., 1997), and are also present within estuarine sediments (McKew et al., 2013).

EPS-mineral interactions and EPS preservation

Exopolymeric substances play a major role in the interactions between sediments and microorganisms, although the ecological functions of EPS differ between biofilm-forming groups. In intertidal biofilms, most benthic diatoms secrete EPS for motility on the sediment in response to semi-diurnal cycles, while some bacteria use EPS to attach to the substratum, i.e., sedimentary particles (Smith & Underwood, 1998; Lubarsky et al., 2010). As a consequence, some EPS molecules exhibit high reactivity toward mineral surfaces (Dontsova & Bigham, 2005; Kleber et al., 2007; Ghashoghchi et al., 2017; Hope et al., 2020). EPS can complex to mineral surfaces through hydrogen-bonding, van der Waals forces, hydrophobic/hydrophilic bonding, and cation bridges (Kleber et al., 2015; Duteil et al., 2020). In the Gironde estuary, EPS acidity peaked in the mud-rich part of the surface biofilm, indicating a potential strong cation binding capacity (Braissant et al., 2009; Pace et al., 2018). Acid-base titrations and FT-IR show that the same EPS included functional groups that could interact with mineral surfaces, e.g., carboxylic acids, sulfate and amino groups, and also hydrophobic parts of proteins (Figure 5 and 6; Duteil et al., 2020). For example, carboxylic acid groups may interact with the hydroxyl groups or the metal cations on the edge of the clay minerals through hydrogen-bonding (Kleber et al., 2007). The proportion of functional groups varied through the studied core (Figures 2, 5, and 6). The contribution of amino groups is of 4% in the surface biofilm (Figure 6), but increased to 21% at 6.08 m (21%; Figure 6). In contrast, the contribution of carboxylic acid and/or sulfate groups was more pronounced at the top than at the bottom of the core (Figure 6). Given the range of porewater pH measured in the core (7.9–8.3; unpublished data), carboxyl and sulfate groups could be deprotonated at all sediment depths and available for interactions with metallic cations and with clay mineral surfaces through cation bridging (Duteil et al., 2020).

In the surface biofilm, EPS formed aggregates together with clay and silt-sized particles, mainly clay minerals, micas, and quartz (Figure 3B to 3D). Similar aggregates were observed at all depths in the core (Figure 4). In sand-rich horizons, EPS or EPS-clay aggregates covered large parts of the surface of sand grains (quartz or micas), forming detrital clay coats. Clay-coated grains account for 30% of sands in the point bar (Virolle et al., 2021), for 17 to 25% of sands in the tidal bars from the bay-head delta in the same estuary (Virolle et al., 2019b), and up to 50 to 90% of sands in the Ravenglass estuary (UK; Wooldridge et al., 2017; Worden et al., 2020). Wooldridge et al. (2017) reported a positive correlation between carbohydrate concentrations and the proportion of clay-coated grains in the sediment, which was not the case in the present study. Detrital clay coats were formed experimentally at ambient surface conditions by mixing purified diatom biofilm EPS with quartz and clay minerals, confirming a prominent role of EPS in the aggregation of mud and sand (Duteil et al., 2020). Natural diatom biofilms include numerous satellite bacteria, and experiments have shown that mixed diatombacteria assemblages produce EPS with higher cohesive properties than axenic diatom cultures or pure prokaryotic cultures (Lubarsky et al., 2010). The EPS secreted by anaerobic heterotrophic bacteria also promote clay aggregation. Jaisi et al. (2007) demonstrated experimentally that the combination of iron (III) reduction and exopolysaccharide production by iron-reducing bacteria induces the aggregation of nontronite. Some molecules of the EPS could also be integrated within the interlayer space of dioctahedral smectites (Duteil et al., 2020), which represent an average of 7% of the mineralogical assemblage in the point bar studied by Virolle and coworkers (2021). Interactions of proteins and polysaccharides with minerals result in significantly stronger adhesion of organisms constituting the biofilm and in increased biofilm and sediment stability (Gerbersdorf et al., 2009b; Flemming & Wingender, 2010).

Clay-EPS aggregation may reduce EPS consumption through an occlusion of the porosity within aggregates, particularly through hydrophobic interactions (Kleber et al., 2015). EPS extracted from the Gironde sediments contained proteins (Figures 2, 5, and 6) including molecules prone to such interactions (Lubarsky et al., 2010). The shift of the amide II peaks (Figure 5) could indicate a structural change in the secondary structure of the proteins upon stronger interactions with mineral surfaces (Dousseau & Pezolet, 1990; Kleber et al., 2007). Aggregates could form exclusion zones with mineral surfaces protecting EPS against degradation by heterotrophs (Marshman & Marshall, 1981; Kleber et al., 2015; Figure 8). Long-term interactions during sediment burial (over centuries in the present study) may have consolidated the bonds between EPS and mineral surfaces, initially formed at the surface (Duteil et al., 2020; Figure 8). The reduction of porosity and mechanical reorganization of grains due to sediment compaction probably had very little influence, given the shallow burial of the sediments in the core under study (Rebata-Land & Santamarina, 2006).

Potential tide versus river flood control on EPS preservation

Both historic maps of the Gironde estuary and radiometric ages measured in sedimentary cores indicate that the Bordeaux North point bar is less than 300 years old (Virolle et al., 2021). The radiocarbon ages measured on gastropods in this study are older (Figure 2), but reworking of older sedimentary material is common within point bars. During the last century, the Bordeaux North point bar experienced a rapid lateral accretion rate (1 m.yr⁻¹ on average), with an average

sedimentation rate of between 1 and 4 cm.yr⁻¹ (Virolle et al., 2021). In the studied core (BXN Long Core), sedimentary sequences 1 and 4 are dominated by muddy (Facies F3) and sandy (Facies F2) tidal facies whereas sequences 2 and 3 exhibit fine-grained tidal facies (Facies F3) overlain by river flood deposits (Facies F1; Figures 1B and 3). Similar vertical successions have been described in tidal bars from the Gironde bay-head delta, ca. 30 km downstream from the study area. In the Plassac area of the estuary, thick fine-grained heterolithic layers similar to facies F3 could result from low-water discharge during dry periods, while it is thought that thick sands rich in mud-pebbles similar to facies F1 accumulate during subsequent peak floods (Chaumillon et al., 2013). In the Gironde estuary, the dimensions and migration (e.g., longitudinal vs. lateral accretion) of tidal bars and point bars (including the Bordeaux North point bar) responds to multi-annual to decadal variations of river discharge (Fenies & Tastet, 1998; Billy et al., 2012; Gascuel, 2017). A stratigraphic correlation with a core located ca. 100 m south of the investigated area and dated using Cs-Pb (core Bo-2016-W; Virolle et al., 2021) indicates that the uppermost 1.5 m of the BXN Long Core, i.e., sequence 4, could have been deposited during the last seven decades. From the 1950s until the 2010s, the point bar displays relative stability, with a near-constant volume and no major migration (Gascuel, 2017). Over the same period, river discharges have been relatively low compared to the first half of the 20th century (Billy et al., 2012), leading to the turbidity maximum zone (TMZ) migrating several kilometers upstream in the Garonne channel (Jalón-Rojas et al., 2021). Decreasing river discharges could have led to an increase of fine sediment trapping (e.g., Virolle et al., 2019a) and to the deposition of the tide-dominated sediments described in sequence 4 (Figure 2). Frequent and intense river floods can result in significant erosion, especially in the inner parts of estuaries (Luan et al., 2016). River discharge was high from the 1900s to the 1940s, with numerous episodes of river floods (Billy et al., 2012), some of which might be recorded in sequences 2 and 3. Over the same period, the Bordeaux North point bar underwent significant longitudinal accretion (Gascuel, 2017). Based on these observations, the sedimentary succession described in this study (Figure 2) could record successive phases of point bar development alternatively dominated by tides (sequences 1 and 4) and by river floods (sequences 2 and 3). We propose a conceptual model of the sedimentary evolution of the point bar during both types of sequences, and of subsequent biofilm development, EPS production, and EPS preservation (Figure 8).

Tide-dominated sequences show higher average EPS concentrations (4.93 μg xanthan eq.g⁻¹ DW for neutral sugars; 15.68 µg albumin eq.g⁻¹ DW for proteins; Figure 2) than river flood-dominated sequences (1.87 µg xanthan eq.g⁻¹ DW for neutral sugars; 3.35 µg albumin eq.g DW⁻¹ for proteins; Figure 2). EPS concentrations are very high at the top of tidedominated sequences, and decrease exponentially with depth (Figure 2). River flood-dominated sequences show no obvious vertical trends. EPS acidity is almost twice as high on average in tide-dominated sequences as in river flood-dominated sequences (12.6 vs. 6.67 µg xanthan eq. g DW⁻¹ respectively; Figure 2). However, while acidic site density decreases overall with depth in sequence 1, it shows no particular vertical trend in sequence 4 (Figure 7). In river flood-dominated sequences, acidity tends to increase slightly with depth (Figure 2).

We propose a scenario describing potential interactions between the sedimentary dynamics of the point bar and the production and preservation of EPS (Figure 8). Tide-dominated sequences (sequences 1 and 4) could be characterized by a stable point bar and by a substantial extension of the muddy intertidal zones (Figure 8A1). The stability of the sediment substrate could favor the development of biofilms, leading to significant photoautotrophic EPS production at the surface. With increasing depth, EPS concentration decreases exponentially (Figure 2) due to heterotrophic degradation (Figure 8D). During river flood-dominated sequences (sequences 2 and 3), sustained erosion of the mudflats and migration of the point bar could result in reduced development of intertidal biofilms and in lower EPS surface production (Figure 8A2).

Sediment-EPS aggregates, including clay coats, form at the surface of the sediment (Virolle et al., 2019a; Duteil et al., 2020; Figure 8B). This aggregation could create exclusion zones protecting EPS from heterotrophic degradation (Figure 8C). The preservation of significant amounts of EPS, as observed at the transition between sequences 1 and 2 (4.33 m deep) could result from the combination of: (i) high EPS production at the top of a tidedominated sequence, followed by (ii) a rapid burial by river flood deposits, preventing aerobic degradation of EPS. Rapid burial of surface EPS could strengthen the chemical links between EPS and mineral surfaces and promote the formation of stronger and less degradable aggregates (Figure 8C1). In contrast, river flood-dominated deposits overlain by tide-dominated deposits could result in reduced EPS preservation, as observed at the transition between sequences 3 and 4. Lower initial EPS production followed by increased dismantling of aggregates and EPS degradation due to low sedimentation rates could explain the low EPS concentrations observed in sequences 2 and 3 (Figure 8C2). A single flood event within a tide-dominated sequence could also carry biofilm material and EPS from upstream, explaining the slightly higher EPS concentration at 0.93 m (Figure 2). More generally, our results demonstrate a potential strong sedimentary and hydroclimatic control on EPS production and preservation in estuaries. Our proposed model could be tested in other estuaries, as well as in other sedimentary environments.



Figure 8: Multiscale scenario explaining the role of the hydro-sedimentary context and organomineral interactions in the production and preservation of exopolymeric substances (EPS) in estuaries. (A) Sketch of the Bordeaux North point bar during a tide-dominated period (A.1; brown) and a river flood dominated period (A.2; blue). Biofilm development and EPS production are optimal during tide-dominated intervals, when the point bar is stable and the muddy intertidal domain well developed. During river flood-dominated periods, the migration of the point bar coupled to the erosion of the intertidal zone results in lower EPS production. (B) Formation of clay-EPS complexes: clay-EPS aggregates and detrital coats in surface sediments. (C) The preservation of clay-EPS complexes and EPS during burial depends on the sedimentation rate: (C.1) rapid burial following, e.g., a river flood protects EPS from heterotrophic degradation; and (C.2) normal sedimentation rate leads to increased EPS degradation. (D) Vertical trends of EPS concentrations depend on production and sedimentation. Tide-dominated periods followed by rapid burial create the optimum conditions for EPS preservation, while river flood-dominated periods followed by tide-dominated periods are associated with low EPS preservation.

CONCLUSION

Exopolymeric substances (EPS) are often considered as degraded below the first millimeters of the sediment. In this study, we extracted and characterized EPS from a 6 m-long core drilled in an estuarine point bar. EPS composition and physicochemical properties were compared to sediment grain size, total organic carbon (TOC), sedimentary facies, and depth. The core could be subdivided into four sedimentary sequences, S1 to S4 from older to younger. S1 and S4 exhibited a predominance of tidal facies (e.g., tidal sedimentary dunes, flaser bedding), while S2 and S3 displayed abundant river flood facies (e.g., erosive, mudclast rich facies). The Gironde surface and subsurface EPS were rich in polysaccharides and proteins, including reactive functional groups, mainly carboxylic acids and amino groups, but also possibly sulfates. Such EPS were present in variable concentrations down to 6 m deep in the sediment. EPS were abundant and highly reactive in the surface diatom biofilm, and decreased sharply in the first decimeters of the sediment. However, we measured EPS concentrations comparable to the surface biofilm 4.33 m deep in the sediment. This is the first report of EPS at such depth. FTIR spectroscopy and acid-base titrations showed minor changes in the composition of EPS with depth: (i) a local disappearance of carboxylic acid groups in some horizons, (ii) a shift of the amide II band with depth possibly linked to the complexation of proteins with mineral surfaces, and (iii) an increase in amino groups relative to carboxyl or sulfate groups at depth.

Sedimentary particles (e.g., sand, silt, and clay) and EPS formed organo-mineral complexes (e.g., detrital clay coats) at the surface of the sediment and these aggregates were preserved at all depths in the core. The concentration of EPS was not statistically correlated to sediment grain size or TOC. EPS concentrations are higher overall in sedimentary sequences dominated by tidal facies than in sequences dominated by river flood facies. Such sequences could form over several decades. Based on these results, we proposed a scenario including a combination of factors that could explain the presence and preservation of EPS several meters below the

sediment-water interface. EPS production could be maximum at the end of tide-dominated periods, when the estuarine point bar is stable and the muddy intertidal zone is most extensive. In contrast, river flood periods could be associated with significant point bar migration and erosion of the intertidal zones. This instability could prevent significant development of biofilms. The preservation of EPS could be enhanced by the formation of EPS-sediment aggregates protecting EPS against heterotrophic degradation. Rapid burial by river flood deposits following a tide-dominated sequence could provide the best conditions for EPS preservation. A part of the subsurface EPS could also be produced *in situ* by heterotrophs. In brief, the quantity of EPS preserved in the sediment could reflect the changes in the sedimentary dynamics of the estuary (i.e., tide vs. river floods). Highly reactive preserved EPS could play a major role in early diagenetic organo-mineral reactions.

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REFERENCES

- Allen, G.P., 1991, Sedimentary Processes and Facies in the Gironde Estuary: A Recent Model for Macrotidal Estuarine Systems, p. 29–39.
- Allen, G.P., and Castaing, P., 1973, Suspended sediment transport from the Gironde estuary (France) onto the adjacent continental shelf: Marine Geology, v. 14, p. 47–53, doi:10.1016/0025-3227(73)90011-X.
- Battersby, N., Stewart, D., and Sharma, A., 1984, Effect of xanthan on the growth of sulphate-reduction bacteria in marine sediments., in 2. Colloque International de Bacteriologie Marine, Brest (France), 1-5.
- Bergaya, F., and Lagaly, G., 2013, Handbook of Clay Science: Newnes.
- Bhaskar, P.V., Grossart, H.-P., Bhosle, N.B., and Simon, M., 2005, Production of macroaggregates from dissolved exopolymeric substances (EPS) of bacterial and diatom origin: FEMS Microbiology Ecology, v. 53, p. 255–264, doi: 10.1016/j.femsec.2004.12.013.
- Billy, J., Chaumillon, E., Féniès, H., and Poirier, C., 2012, Tidal and fluvial controls on the morphological evolution of a lobate estuarine tidal bar: The Plassac Tidal Bar in the Gironde Estuary (France): Geomorphology, v. 169–170, p. 86–97, doi: 10.1016/j.geomorph.2012.04.015.
- Braissant, O., Decho, A.W., Dupraz, C., Glunk, C., Przekop, K.M., and Visscher, P.T., 2007, Exopolymeric substances of sulfate-reducing bacteria: interactions with calcium at alkaline pH and implication for formation of carbonate minerals: Geobiology, v. 5, p. 401–411.
- Braissant, O., Decho, A.W., Przekop, K.M., Gallagher, K.L., Glunk, C., Dupraz, C., and Visscher, P.T., 2009, Characteristics and turnover of exopolymeric substances in a hypersaline microbial mat: FEMS microbiology ecology, v. 67, p. 293–307.
- Cartaxana, P., Ruivo, M., Hubas, C., Davidson, I., Serôdio, J., and Jesus, B., 2011, Physiological versus behavioral photoprotection in intertidal epipelic and epipsammic benthic diatom communities: Journal of Experimental Marine Biology and Ecology, v. 405, p. 120–127, doi: 10.1016/j.jembe.2011.05.027.
- Chaumillon, E., Féniès, H., Billy, J., Breilh, J.-F., and Richetti, H., 2013, Tidal and fluvial controls on the internal architecture and sedimentary facies of a lobate estuarine tidal bar (The Plassac Tidal Bar in the Gironde Estuary, France): Marine Geology, v. 346, p. 58–72, doi: 10.1016/j.margeo.2013.07.017.
- Chenu, C., and Roberson, E.B., 1996, Diffusion of glucose in microbial extracellular polysaccharide as affected by water potential: Soil Biology and Biochemistry, v. 28, p. 877–884.
- Chukanov, N.V., and Chervonnyi, A.D., 2016, Infrared Spectroscopy of Minerals and Related Compounds: Springer.
- Comte, S., Guibaud, G., and Baudu, M., 2006, Relations between extraction protocols for activated sludge extracellular polymeric substances (EPS) and EPS complexation properties: Part I. Comparison of the efficiency of eight EPS extraction methods: Enzyme and microbial technology, v. 38, p. 237–245.
- D'Abzac, P., Bordas, F., Van Hullebusch, E., Lens, P.N., and Guibaud, G., 2010, Extraction of extracellular polymeric substances (EPS) from anaerobic granular sludges: comparison of chemical and physical extraction protocols: Applied microbiology and biotechnology, v. 85, p. 1589–1599.
- De Brouwer, J.F.C., Ruddy, G.K., Jones, T.E.R., and Stal, L.J., 2002, Sorption of EPS to sediment particles and the effect on the rheology of sediment slurries: Biogeochemistry, v. 61, p. 57–71.
- De Brouwer, J.F.C., and Stal, L., 2001, Short-term dynamics in microphytobenthos distribution and associated extracellular carbohydrates in surface sediments of an intertidal mudflat: Marine Ecology Progress Series, v. 218, p. 33–44, doi:10.3354/meps218033.
- De Winder, B., Staats, N., Stal, L.J., and Paterson, D.M., 1999, Carbohydrate secretion by phototrophic communities in tidal sediments: Journal of Sea Research, v. 42, p. 131–146.
- Decho, A.W., 2000, Microbial biofilms in intertidal systems: an overview: Continental shelf research, v. 20, p. 1257–1273.
- Decho, A.W., and Gutierrez, T., 2017, Microbial extracellular polymeric substances (EPSs) in ocean

systems: Frontiers in microbiology, v. 8, p. 922.

- Decho, A.W., Visscher, P.T., and Reid, R.P., 2005, Production and cycling of natural microbial exopolymers (EPS) within a marine stromatolite: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 219, p. 71–86, doi: 10.1016/j.palaeo.2004.10.015.
- Dignac, M.-F., Urbain, V., Rybacki, D., Bruchet, A., Snidaro, D., and Scribe, P., 1998, Chemical description of extracellular polymers: implication on activated sludge floc structure: Water Science and Technology, v. 38, p. 45–53.
- Dontsova, K.M., and Bigham, J.M., 2005, Anionic Polysaccharide Sorption by Clay Minerals: Soil Science Society of America Journal, v. 69, p. 1026–1035, doi:10.2136/sssaj2004.0203.
- Dousseau, F., and Pezolet, M., 1990, Determination of the secondary structure content of proteins in aqueous solutions from their amide I and amide II infrared bands. Comparison between classical and partial least-squares methods: Biochemistry, v. 29, p. 8771–8779, doi:10.1021/bi00489a038.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., and Smith, F., 1956, Colorimetric Method for Determination of Sugars and Related Substances: Analytical Chemistry, v. 28, p. 350–356, doi:10.1021/ac60111a017.
- Duteil, T., Bourillot, R., Grégoire, B., Virolle, M., Brigaud, B., Nouet, J., Braissant, O., Portier, E., Féniès, H., and Patrier, P., 2020, Experimental formation of clay-coated sand grains using diatom biofilm exopolymers: Geology, v. 48, p. 1012–1017.
- Fenies, H., and Tastet, J.-P., 1998, Facies and architecture of an estuarine tidal bar (the Trompeloup bar, Gironde Estuary, SW France): Marine Geology, v. 150, p. 149–169, doi:10.1016/S0025-3227(98)00059-0.
- Flemming, H.-C., Neu, D.T.R., and Wingender, D.J., 2016, The Perfect Slime: Microbial Extracellular Polymeric Substances (EPS): IWA Publishing.
- Flemming, H.-C., and Wingender, J., 2010, The biofilm matrix: Nature reviews microbiology, v. 8, p. 623–633.
- Gascuel, V., 2017, Analyse de l'évolution morphologique de barres de marée de l'estuaire de la Gironde: Etude de l'évolution des barres de Bordeaux, Plassac, Trompeloup et Richard en relation avec les processus sédimentaires et anthropiques: Stage de deuxième année d'école ingénieur à l'ENSEGID, Bordeaux INP, p. 85.
- Gerbersdorf, S.U., Bittner, R., Lubarsky, H., Manz, W., and Paterson, D.M., 2009a, Microbial assemblages as ecosystem engineers of sediment stability: Journal of Soils and Sediments, v. 9, p. 640–652, doi:10.1007/s11368-009-0142-5.
- Gerbersdorf, S.U., Westrich, B., and Paterson, D.M., 2009b, Microbial Extracellular Polymeric Substances (EPS) in Fresh Water Sediments: Microbial Ecology, v. 58, p. 334–349, doi:10.1007/s00248-009-9498-8.
- Ghashoghchi, R.A., Hosseini, M.R., and Ahmadi, A., 2017, Effects of microbial cells and their associated extracellular polymeric substances on the bio-flocculation of kaolin and quartz: Applied Clay Science, v. 138, p. 81–88, doi: 10.1016/j.clay.2017.01.002.
- Hope, J.A. et al., 2020, Interactions between sediment microbial ecology and physical dynamics drive heterogeneity in contextually similar depositional systems: Limnology and Oceanography, v. 65, p. 2403–2419, doi:10.1002/lno.11461.
- Jaisi, D.P., Dong, H., and Liu, C., 2007, Influence of biogenic Fe(II) on the extent of microbial reduction of Fe(III) in clay minerals nontronite, illite, and chlorite: Geochimica et Cosmochimica Acta, v. 71, p. 1145–1158, doi: 10.1016/j.gca.2006.11.027.
- Jalón-Rojas, I., Dijkstra, Y.M., Schuttelaars, H.M., Brouwer, R.L., Schmidt, S., and Sottolichio, A., 2021, Multidecadal Evolution of the Turbidity Maximum Zone in a Macrotidal River Under Climate and Anthropogenic Pressures: Journal of Geophysical Research: Oceans, v. 126, doi:10.1029/2020JC016273.
- Jesus, B., Brotas, V., Ribeiro, L., Mendes, C.R., Cartaxana, P., and Paterson, D.M., 2009, Adaptations of microphytobenthos assemblages to sediment type and tidal position: Continental Shelf Research, v. 29, p. 1624–1634, doi: 10.1016/j.csr.2009.05.006.
- Jouanneau, J.M., and Latouche, C., 1981, The Gironde Estuary, https://www.schweizerbart.de/publications/detail/isbn/9783510570102 (accessed January 2022).

- Kleber, M., Eusterhues, K., Keiluweit, M., Mikutta, C., Mikutta, R., and Nico, P.S., 2015, Chapter One Mineral–Organic Associations: Formation, Properties, and Relevance in Soil Environments, in Sparks, D.L. ed., Advances in Agronomy, Academic Press, v. 130, p. 1–140, doi: 10.1016/bs.agron.2014.10.005.
- Kleber, M., Sollins, P., and Sutton, R., 2007, A conceptual model of organo-mineral interactions in soils: self-assembly of organic molecular fragments into zonal structures on mineral surfaces: Biogeochemistry, v. 85, p. 9–24, doi:10.1007/s10533-007-9103-5.
- Klock, J.-H., Wieland, A., Seifert, R., and Michaelis, W., 2007, Extracellular polymeric substances (EPS) from cyanobacterial mats: characterisation and isolation method optimisation: Marine Biology, v. 152, p. 1077–1085.
- Kühl, M., Lassen, C., and Jørgensen, B.B., 1994, Light penetration and light intensity in sandy marine sediments measured with irradiance and scalar irradiance fiber-optic microprobes: Marine Ecology Progress Series, v. 105, p. 139–148.
- Lange-Bertalot, H., Hofmann, G., Werum, M., and Cantonati, M., 2017, Freshwater benthic diatoms of Central Europe: over 800 common species used in ecological assessment: v. 942.
- Lavoie, I., and Hamilton, P.B., 2008, Guide d'identification des diatomées des rivières de l'Est du Canada: PUQ.
- Leloup, J., Petit, F., Boust, D., Deloffre, J., Bally, G., Clarisse, O., and Quillet, L., 2005, Dynamics of Sulfate-Reducing Microorganisms (dsrAB genes) in Two Contrasting Mudflats of the Seine Estuary (France): Microbial Ecology, v. 50, p. 307–314, doi:10.1007/s00248-004-0034-6.
- Lowry, O., Rosebrough, N., Farr, A., and Randall, R., 1951, Protein measurement with the Folin phenol reagent: Journal of Biological Chemistry, v. 193, p. 265–275, doi:10.1016/S0021-9258(19)52451-6.
- Luan, H.L., Ding, P.X., Wang, Z.B., Ge, J.Z., and Yang, S.L., 2016, Decadal morphological evolution of the Yangtze Estuary in response to river input changes and estuarine engineering projects: Geomorphology, v. 265, p. 12–23, doi: 10.1016/j.geomorph.2016.04.022.
- Lubarsky, H.V., Hubas, C., Chocholek, M., Larson, F., Manz, W., Paterson, D.M., and Gerbersdorf, S.U., 2010, The Stabilisation Potential of Individual and Mixed Assemblages of Natural Bacteria and Microalgae: PLOS ONE, v. 5, p. e13794, doi: 10.1371/journal.pone.0013794.
- Mager, D.M., 2010, Carbohydrates in cyanobacterial soil crusts as a source of carbon in the southwest Kalahari, Botswana: Soil Biology and Biochemistry, v. 42, p. 313–318.
- Malarkey, J., Baas, J.H., Hope, J.A., Aspden, R.J., Parsons, D.R., Peakall, J., Paterson, D.M., Schindler, R.J., Ye, L., and Lichtman, I.D., 2015, The pervasive role of biological cohesion in bedform development: Nature communications, v. 6, p. 1–6.
- Marshman, N.A., and Marshall, K.C., 1981, Bacterial growth on proteins in the presence of clay minerals: Soil Biology and Biochemistry, v. 13, p. 127–134, doi:10.1016/0038-0717(81)90008-0.
- Marvasi, M., Visscher, P.T., and Casillas Martinez, L., 2010, Exopolymeric substances (EPS) from Bacillus subtilis: polymers and genes encoding their synthesis: EPS from Bacillus subtilis: FEMS Microbiology Letters, v. 313, p. 1–9, doi:10.1111/j.1574-6968.2010.02085.x.
- McKew, B.A., Dumbrell, A.J., Taylor, J.D., McGenity, T.J., and Underwood, G.J.C., 2013, Differences between aerobic and anaerobic degradation of microphytobenthic biofilm-derived organic matter within intertidal sediments: FEMS Microbiology Ecology, v. 84, p. 495–509, doi:10.1111/1574-6941.12077.
- Migeon, S., Weber, O., Faugeres, J.-C., and Saint-Paul, J., 1998, SCOPIX: A new X-ray imaging system for core analysis: Geo-Marine Letters, v. 18, p. 251–255, doi:10.1007/s003670050076.
- Morelle, J., Claquin, P., and Orvain, F., 2020, Evidence for better microphytobenthos dynamics in mixed sand/mud zones than in pure sand or mud intertidal flats (Seine estuary, Normandy, France): PLOS ONE, v. 15, p. e0237211, doi: 10.1371/journal.pone.0237211.
- Nielsen, P.H., and Jahn, A., 1999, Extraction of EPS, in Microbial extracellular polymeric substances, Springer, p. 49–72.
- Orvain, F., De Crignis, M., Guizien, K., Lefebvre, S., Mallet, C., Takahashi, E., and Dupuy, C., 2014, Tidal and seasonal effects on the short-term temporal patterns of bacteria, microphytobenthos and exopolymers in natural intertidal biofilms (Brouage, France): Journal of Sea Research, v. 92, p. 6–18, doi: 10.1016/j.seares.2014.02.018.

- Orvain, F., Lefebvre, S., Montepini, J., Sébire, M., Gangnery, A., and Sylvand, B., 2012, Spatial and temporal interaction between sediment and microphytobenthos in a temperate estuarine macro-intertidal bay: Marine Ecology Progress Series, v. 458, p. 53–68, doi:10.3354/meps09698.
- Pace, A., Bourillot, R., Bouton, A., Vennin, E., Braissant, O., Dupraz, C., Duteil, T., Bundeleva, I., Patrier, P., and Galaup, S., 2018, Formation of stromatolite lamina at the interface of oxygenic– anoxygenic photosynthesis: Geobiology, v. 16, p. 378–398.
- Passow, U., and Alldredge, A.L., 1995, A dye-binding assay for the spectrophotometric measurement of transparent exopolymer particles (TEP): Limnology and Oceanography, v. 40, p. 1326–1335, doi:10.4319/lo.1995.40.7.1326.
- Paterson, D.M., Tolhurst, T.J., Kelly, J.A., Honeywill, C., de Deckere, E.M.G.T., Huet, V., Shayler, S.A., Black, K.S., de Brouwer, J., and Davidson, I., 2000, Variations in sediment properties, Skeffling mudflat, Humber Estuary, UK: Continental Shelf Research, v. 20, p. 1373–1396, doi:10.1016/S0278-4343(00)00028-5.
- Rebata-Landa, V., and Santamarina, J.C., 2006, Mechanical limits to microbial activity in deep sediments: MICROBES: Geochemistry, Geophysics, Geosystems, v. 7, p. n/a-n/a, doi:10.1029/2006GC001355.
- Redzuan, N.S., and Underwood, G.J.C., 2021, The importance of weather and tides on the resuspension and deposition of microphytobenthos (MPB) on intertidal mudflats: Estuarine, Coastal and Shelf Science, v. 251, p. 107190, doi: 10.1016/j.ecss.2021.107190.
- Smith, D.J., and Underwood, G.J.C., 1998, Exopolymer production by intertidal epipelic diatoms: Limnology and Oceanography, v. 43, p. 1578–1591, doi:10.4319/lo.1998.43.7.1578.
- Sottolichio, A., Hurther, D., Gratiot, N., and Bretel, P., 2011, Acoustic turbulence measurements of near-bed suspended sediment dynamics in highly turbid waters of a macrotidal estuary: Continental Shelf Research, v. 31, p. S36–S49, doi: 10.1016/j.csr.2011.03.016.
- Stumm, W., and Morgan, J.J., 1996, An Introduction Emphasizing Chemical Equilibria in Natural Waters, p. 2.
- Taylor, J.D., McKew, B.A., Kuhl, A., McGenity, T.J., and Underwood, G.J., 2013, Microphytobenthic extracellular polymeric substances (EPS) in intertidal sediments fuel both generalist and specialist EPS-degrading bacteria: Limnology and Oceanography, v. 58, p. 1463–1480.
- Tolhurst, T.J., Gust, G., and Paterson, D.M., 2002, The influence of an extracellular polymeric substance (EPS) on cohesive sediment stability, in Proceedings in marine science, Elsevier, v. 5, p. 409–425.
- Turner, B.F., and Fein, J.B., 2006, Protofit: A program for determining surface protonation constants from titration data: Computers & Geosciences, v. 32, p. 1344–1356, doi: 10.1016/j.cageo.2005.12.005.
- Ubertini, M., Lefebvre, S., Rakotomalala, C., and Orvain, F., 2015, Impact of sediment grain-size and biofilm age on epipelic microphytobenthos resuspension: Journal of Experimental Marine Biology and Ecology, v. 467, p. 52–64, doi: 10.1016/j.jembe.2015.02.007.
- Underwood, G.J.C., 2010, Microphytobenthos and phytoplankton in the Severn estuary, UK: Present situation and possible consequences of a tidal energy barrage: Marine Pollution Bulletin, v. 61, p. 83–91, doi: 10.1016/j.marpolbul.2009.12.015.
- Underwood, G.J.C., and Kromkamp, J., 1999, Primary Production by Phytoplankton and Microphytobenthos in Estuaries, in Advances in Ecological Research, Elsevier, v. 29, p. 93– 153, doi:10.1016/S0065-2504(08)60192-0.
- Underwood, G.J.C., and Paterson, D.M., 2003, The importance of extracellular carbohydrate productionby marine epipelic diatoms, in Advances in Botanical Research, Elsevier, v. 40, p. 183–240, doi:10.1016/S0065-2296(05)40005-1.
- Underwood, G.J.C., Paterson, D.M., and Parkes, R.J., 1995, The measurement of microbial carbohydrate exopolymers from intertidal sediments: Limnology and Oceanography, v. 40, p. 1243–1253.
- Veiga, M.C., Jain, M.K., Wu, W., Hollingsworth, R.I., and Zeikus, J.G., 1997, Composition and role of extracellular polymers in methanogenic granules: Applied and Environmental Microbiology, v. 63, p. 403–407, doi:10.1128/aem.63.2.403-407.1997.
- Virolle, M., Brigaud, B., Bourillot, R., Féniès, H., Portier, E., Duteil, T., Nouet, J., Patrier, P., and

Beaufort, D., 2019a, Detrital clay grain coats in estuarine clastic deposits: Origin and spatial distribution within a modern sedimentary system, the Gironde Estuary (south-west France): Sedimentology, v. 66, p. 859–894.

- Virolle, M., Brigaud, B., Féniès, H., Bourillot, R., Portier, E., Patrier, P., Derriennic, H., and Beaufort, D., 2021, Preservation and distribution of detrital clay coats in a modern estuarine heterolithic point bar in the Gironde estuary (Bordeaux, France): Journal of Sedimentary Research, v. 91, p. 812–832, doi:10.2110/jsr.2020.146.
- Virolle, M., Brigaud, B., Luby, S., Portier, E., Féniès, H., Bourillot, R., Patrier, P., and Beaufort, D., 2019b, Influence of sedimentation and detrital clay grain coats on chloritized sandstone reservoir qualities: Insights from comparisons between ancient tidal heterolithic sandstones and a modern estuarine system: Marine and Petroleum Geology, v. 107, p. 163–184, doi: 10.1016/j.marpetgeo.2019.05.010.
- Visscher, P.T., Gritzer, R.F., and Leadbetter, E.R., 1999, Low-molecular-weight sulfonates, a major substrate for sulfate reducers in marine microbial mats: Applied and Environmental Microbiology, v. 65, p. 3272–3278.
- Wooldridge, L.J., Worden, R.H., Griffiths, J., Thompson, A., and Chung, P., 2017, Biofilm origin of clay-coated sand grains: Geology, v. 45, p. 875–878, doi:10.1130/G39161.1.
- Worden, R.H., Griffiths, J., Wooldridge, L.J., Utley, J.E.P., Lawan, A.Y., Muhammed, D.D., Simon, N., and Armitage, P.J., 2020, Chlorite in sandstones: Earth-Science Reviews, v. 204, p. 103105, doi: 10.1016/j.earscirev.2020.103105.
- Xu, H., Li, F., Kong, M., Lv, X., Du, H., and Jiang, H., 2020, Adsorption of cyanobacterial extracellular polymeric substance on colloidal particle: Influence of molecular weight: Science of The Total Environment, v. 715, p. 136959.
- Yallop, M.L., Paterson, D.M., and Wellsbury, P., 2000, Interrelationships between rates of microbial production, exopolymer production, microbial biomass, and sediment stability in biofilms of intertidal sediments: Microbial ecology, v. 39, p. 116–127.
- Yokoyama, Y., Koizumi, M., Matsuzaki, H., Miyairi, Y., and Ohkouchi, N., 2010, Developing Ultra Small-Scale Radiocarbon Sample Measurement at the University of Tokyo: Radiocarbon, v. 52, p. 310–318, doi:10.1017/S0033822200045355.

SUPPLEMENTARY MATERIAL (for Duteil et al. submitted in Frontier in Microbiology)

<u>- 0.50 m depth:</u>



Figure S1: Cryo-SEM images of sediments from the BXN Long Core at three depths. Exopolymeric substances (blue arrow), quartz grain (yellow arrow), mica (red arrow), and clay-EPS complexes (brown arrow). (A) and (B) Depth of 0.5 m. Alveolar EPSs cover a large part of a quartz grain (A). EPSs are complexed to clay particles, forming a detrital clay coat which includes quartz grains (B). (C) and (D) Depth of 0.93 m. Quartz grain is covered by a clay-rich detrital coat (C). A sand quartz grain is trapped by a clay-EPS organo mineral coat (D). (E) and (F) Depth of 1.63 m. A centric diatom frustule on a quartz grain covered by clay and mica (E). The surface of a quartz sand grain is partially coated by a dense clay-EPS complex (F).

- 2.805 m depth:



Figure S2: Cryo-SEM images of sediments from the BXN Long Core at three depths. Exopolymeric substances (blue arrow), quartz grain (yellow arrow), mica (red arrow), and clay-EPS complexes (brown arrow). (A) and (B) Depth of 2.805 m. Part of a frustule is deposited on a dense clay-EPS matrix that glue also a mica particle (A). Quartz grain displaying a detrital clay coat (B). (C) and (D) Depth of 3.68 m. The quartz silt is covered by a EPS-clay organo mineral detrital coat (C). A centric diatom frustule is engulfed in the clay-EPS complex that also traps a silt and mica particles (D). (E) and (F) Depth of 4.76 m. Quartz grain covered by clay-EPS organo-mineral complex (E). The surface of a quartz sand grain is partially coated by a dense clay-EPS complex (F).

EPS properties							Sediments properties					
Depth	Lowry assay	Std dev	Phenol sulfuric assay	Std dev	Alcian blue assay	Std dev	Depth	тос	Mean grain size	Sand	Silt	Clay
ст	mg eq. Albumin/ g dry sed	NA	mg eq. Xanthan/ g dry sed	NA	mg eq. Xanthan/ g dry sed	NA	cm	mg C/ g dry sed	μm	%	%	%
0 (Water EPS)	3,76E-02	4,02E-03	1,65E-02	2,47E-03	2,74E-02	6,09E-04	0 (Water EPS)	NA	NA	NA	NA	NA
0 (Mud EPS)	1,42E-02	3,34E-04	7,18E-03	9,07E-04	3,25E-02	5,13E-04	0 (Mud EPS)	NA	NA	NA	NA	NA
6	i 1,37E-02	5,41E-04	4,99E-03	4,93E-05	1,67E-02	1,35E-04	6	4,72	69,55	50,42	47,48	2,10
50	1,74E-03	1,37E-04	7,34E-04	1,06E-04	6,72E-04	1,91E-04	50	0,68	274,46	90,26	9,23	0,52
93	1,15E-02	4,96E-04	1,25E-03	7,70E-04	1,10E-02	5,24E-05	93	7,58	24,67	17,72	77,74	4,54
130	3,24E-03	1,04E-04	1,39E-03	6,03E-05	2,39E-03	1,01E-03	130	0,79	367,07	66,25	31,90	1,85
163,5	, 6,16E-04	5,26E-04	1,56E-03	1,34E-04	2,56E-03	8,17E-04	163,5	0,74	485,36	75,69	23,04	1,27
216	1,63E-03	3,97E-05	9,28E-04	2,37E-04	2,32E-03	9,36E-05	216	10,84	20,83	17,48	77,01	5,51
259,5	, 8,77E-03	1,65E-04	3,47E-03	2,99E-03	1,80E-02	7,73E-04	259,5	9,66	21,80	17,39	77,67	4,95
280,5	, 3,18E-03	6,00E-05	1,89E-03	2,79E-04	5,69E-03	1,54E-04	280,5	7,58	487,13	82,10	16,65	1,25
325	, 2,98E-03	1,77E-04	7,69E-04	5,23E-04	5,69E-03	4,94E-06	325	2,26	371,10	61,26	35,93	2,80
368	3,14E-03	4,91E-04	2,11E-03	6,22E-04	5,39E-03	1,12E-04	368	1,83	17,21	17,70	74,91	7,38
412	3,51E-03	3,89E-05	2,82E-03	6,45E-04	1,03E-02	1,81E-04	412	5,97	175,06	55,13	42,10	2,77
433	3,57E-02	8,51E-03	6,63E-03	2,25E-04	1,30E-02	2,42E-04	433	0,17	463,31	. 98,41	1,52	0,08
476	i 1,41E-02	3,95E-04	5,33E-03	1,04E-04	1,02E-02	3,29E-05	476	0,65	473,14	95,39	4,26	0,34
520	9,06E-03	3,82E-04	3,42E-03	5,33E-04	1,56E-02	3,76E-04	520	5,29	283,80	66,23	31,31	2,46
564	9,70E-03	4,85E-04	2,29E-03	7,42E-05	9,05E-03	6,57E-05	564	3,53	349,43	65,23	32,36	2,40
608	7,65E-03	8,10E-04	2,26E-03	3,87E-04	8,44E-03	3,33E-05	608	7,92	174,41	55,65	41,94	2,41

 Table S1: Data used in this study: colorimetric assays and sediment properties.



Figure S3: FTIR spectra of four exopolymeric substances samples recovered from different depths along the BXN Long Core. Colored lines highlight the EPS absorption bands, while black dotted lines indicate clay mineral absorption bands.

155



Figure S4: FTIR spectra of four exopolymeric substances samples recovered from different depths along the BXN Long Core. Colored lines highlight the EPS absorption bands, while black dotted lines indicate clay mineral absorption bands.



Figure S5: FTIR spectra of four exopolymeric substances samples recovered from different depths along the BXN Long Core. Colored lines highlight the EPS absorption bands, while black dotted lines indicate clay mineral absorption bands.

3.3 Annexes

3.3.1 Cryo-SEM and EDX complementary data for the BXN-Long Core

This section includes additional pictures and EDX probe measurements obtained on samples from BXN-LC. The samples are the same as in section 3.2. For all pictures, exopolymeric substances are indicated by a blue arrow, quartz grains by a yellow arrow, micas by a red arrow, clay platelets by a dark brown arrow and clay-EPS complexes by a light brown arrow.





Figure 3.5: Cryo-SEM images and corresponding EDX profiles of the 0.93 m depth sample. (A) Two silt grains (points 1 and 3) and clay particles (point 2) were analyzed. (B) A quartz grain (point 1) is coated by clay particles (points 2 and 3).

2.805 m depth:



Figure 3.6: Cryo-SEM images and corresponding EDX profiles of the 2.805 m depth sample. (A) A quartz grain (area 1) is coated by clay particles (points 2 and 3). (B) Two mica particles (area 1) and a silt grain (point 2) are embedded in an EPS-clay complex (point 3).



3.68 m depth:

Figure 3.7: Cryo-SEM images and corresponding EDX profiles of the 3.68 m depth sample. (A) A quartz grain (area 1) coated by a mica particle (area 2) and a clay platelet (area 3). (B) A quartz grain (point 1) and a mica (point 2) are embedded in EPS-clay complex (point 3).

4.33 m depth:



Figure 3.8: Cryo-SEM images and corresponding EDX profiles of the 4.33 m depth sample. (A) A mica (area 1) is coated by a clay-EPS complex (point 2) containing a quartz silt grain (point 3). (B) A quartz grain (point 1) is coated by clay (point 2).

4.76 m depth:



Figure 3.9: Cryo-SEM images and corresponding EDX profiles of the 4.76 m depth sample. (A) Quartz grain (point 1) coated by a clay-EPS complex (points 2 and 3). (B) A quartz grain (point 1) is coated with a clay-EPS organo mineral complex (point 2).

6.08 m depth:



Figure 3.10: Cryo-SEM images and corresponding EDX profiles of the 6.08 m depth sample. (A) and (B) additional pictures displaying mica and quartz grain coated by clay-EPS organo mineral complex. (C) A quartz grain (area 2) and a mica (area 1) are stuck in clay-EPS complex (point 3). (D) A quartz grain (point 1) is covered with a clay-EPS organo-mineral complex (points 2 and 3).

3.3.2 Cryo-SEM and EDX complementary data for BXN-Box and BXN-Short Core

This section includes additional pictures and EDX probe measurements obtained on samples from BXN-Box and BXN-SC. These two shallow cores (28 cm and 70 cm long respectively) will be described in chapter 4. As in BXN-LC, Sediment-EPS complexes including clay coats are observed at all depth between the surface and a depth of 60 centimeters. For all pictures, exopolymeric substances are indicated by a blue arrow, quartz grains by a yellow arrow, micas by a red arrow, clay platelets by a dark brown arrow and clay-EPS complexes by a light brown arrow.


Figure 3.11: Cryo-SEM images and corresponding EDX profiles of the 2 cm depth sample from BXN-Box. (A) A quartz grain (point 1) is coated by a clay-EPS organo mineral complex (point 4) including a silt grain (point 2) and a mica (point 3). (B) A quartz grain (point 1) is covered with a clay-EPS organo-mineral complex (points 2 and 3).

3 Energy (KeV)

2

3



5 cm depth:

Figure 3.12: Cryo-SEM images and corresponding EDX profiles of the 5 cm depth sample from BXN-Box. (A) and (B) pictures showing a quartz grain coated by a clay-EPS organo-mineral complex. (C) A quartz grain (areas 1 and 2) is coated by a clay-EPS complex (point 3). (D) Two silt grains (points 2 and 3) embedded in EPS-clay complex (area 1).

0.5

Energy (KeV)

15 cm depth:



Figure 3.135: Cryo-SEM images and corresponding EDX profiles of the 15 cm depth sample from BXN-Box. Pictures displaying a mica (A) and quartz silts (B) coated by a clay-EPS organo mineral complex. (C) EDX profile of mica (area 1) and clay particles (point 2 and 3) stuck on the quartz by EPS. (D) A quartz silt grain (area 1) is coated by clay particles (points 2 and 3).



25 cm depth:

Figure 3.14: Cryo-SEM images of the 25 cm depth sample from BXN-Box. (A) and (B) Alveolar EPS matrix embedding clay platelets and covers a large part of a quartz sand. (C) A quartz grain is fully covered by a clay-EPS detrital coat. (D) Zoom on (C). (E) Quartz sand grains and micas partially coated by a dense clay-EPS complex.



Figure 3.156: Cryo-SEM images and corresponding EDX profiles of the 25 cm depth sample from BXN-Box. (A and B) Pictures displaying quartz grains coated by clay-EPS organo mineral complex. EDX profiles show quartz (profiles 1, 2 on image A; profile 1 on image B) and clay (profile 3 on image A; profile 2 on image B) characteristic signature. Profile 3 on image B show a typical carbonate profile (dolomite?).





Figure 73.16: Cryo-SEM images and corresponding EDX profiles of the 40 cm depth sample from BXN-SC. Pictures displaying a quartz sand (A and B) partially coated by a clay-EPS organo mineral complex. (C) EDX profiles shows quartz (point 1) coated by clay particles (points 2 and 3). (D) A quartz grain (point 1) is coated by a clay-EPS complex (points 2 and 3).

60 cm depth:



Figure 3.178: Cryo-SEM images and corresponding EDX profiles of the 60 cm depth sample from BXN-SC. Pictures showing quartz grains (A and B) coated by a clay-EPS organo mineral complex. (C) EDX profiles of a quartz (area 1) coated by an EPS-clay complex (points 2 and 3) embedding silt grains. (D) A quartz grain (area 1) is coated by a clay-EPS complex (points 2 and 3).

Chapter 4: Impact of microbial activity on biogeochemical cycles and Exopolymeric Substances in estuarine sediments



Table of contents

Chapter 4: Impact of microbial activity on biogeochemical cycles and Exopolymeric Substances in estuarine sediments 171

4.1 Manuscript article: Impact of microbial activity on biogeochemical cycles and	174
Exopolymeric Substances in estuarine sediments.	174
Introduction	175
Materials and methods	180
Results	187
Discussion	204
Geochemical gradients as a response to the layering of microbial communities	204
The impact of microbial activity on early diagenesis	211
A possible conceptual geomicrobiological model	215
Conclusion	220
References	222
Supplementary material	231

4.1 Manuscript article: Impact of microbial activity on biogeochemical cycles and Exopolymeric Substances in estuarine sediments.

This part is the subject of a paper soon to be submitted to the journal Geobiology. Due to time constraints, the interpretation of the data is at a preliminary stage and will evolve between now and the final version of the paper. The objective of this paper is to link the preservation of EPS in sediments described in the previous section to microbial activity via the measurement of biogeochemical cycles.

Impact of microbial activity on biogeochemical cycles and exopolymeric

substances in estuarine sediments

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Introduction

Estuaries are transition zones at the interface of continental and oceanic environments with specific geochemical cycles (Chiffoleau et al., 1994; Kraepiel et al., 1997; Stecko & Bendell-Young, 2000). Estuarine mixing of seawater and freshwater leads to increased metal release and accumulation in the sediments, and estuaries are important sinks for many persistent pollutants and their subsequent accumulation (de Souza Machado et al., 2016; Schäfer et al., 2022). Fine-grained sediments (i.e., silt and clay) are especially prone to trace metal accumulation because of their high adsorption properties (Chatterjee et al., 2007).

Sedimentary biofilms and microbial mats are particularly efficient in the cycling of key elements, e.g., C, N, S, Fe. In the sediment, microorganisms are organized in functional groups, which are stratified according to the source of acceptor they use for their metabolism (i.e., energy-generating redox reactions; e.g., Dupraz et al., 2009). The cycling of elements by microorganisms results in coupled reactions of oxidation and reduction, producing steep vertical geochemical gradients (e.g., dissolved oxygen, pH) that could influence metal speciation in the sediment (Visscher & Stolz, 2005). Photoautotrophic benthic biofilms

typically develop at the water-sediment interface (Underwood & Paterson, 2003; De Winder et al., 1999). In estuarine intertidal zones, these biofilms are typically dominated by diatoms, and could be responsible for up to 50% of the autotrophic production and 1:3 of the carbon fixation in estuaries (Underwood & Kromkamp, 1999). Diatom biofilms perform oxygenic photosynthesis during daylight, in the process producing oxygen (Glud et al., 1992) and organic carbon, including exopolymeric substances (EPS; Decho, 2000; Underwood & Paterson, 2003). The organic carbon produced by photoautotrophs can be consumed and transformed into CO₂ by heterotrophs through various respiratory and fermentative pathways. Heterotrophs are generally stratified according to a gradient of electron acceptors with decreasing free energy yield: oxygen, nitrate, manganese and iron oxides, sulfate and methane (Sagemann et al., 1996; Visscher & Stolz, 2005; Konhauser, 2009, Martin, 2018). Aerobic respiration results in an almost complete consumption of oxygen at shallow depth in the sediment (i.e., millimeters to centimeters; Sagemann et al., 1996; Jørgensen & Nelson, 2004; Hensen et al., 2006). The subsequent formation of anoxic microenvironments allows the predominance of anaerobic heterotrophs performing denitrification and sulfate reduction (Burdige & Zheng; 1998; Jorgensen & Nelson, 2004; Mortimer et al., 2004; Audry et al., 2006). Due to their sensitivity to redox conditions, dissolved iron and manganese profiles typically show more complex patterns. For example, in the oxic zone, Fe(II) oxidation by iron oxidizing bacteria results in ferrihydrite precipitation in Fe(II) and Fe(III) rich environment (Mc Allister et al., 2015). In the anoxic zone, the mineralization of organic carbon coupled with the reduction and solubilization of solid Mn (III/IV) and Fe (III) species could result from both chemical and microbial reactions (Tugel et al., 1986; Burdige, 2011). This reduction is often coincident with sulfate and sulfur respiration (Sundby et al., 1986; Burdige, 1993; Canfield et al., 1993; Fiket et al., 2019) and leads to the precipitation of reduced manganese and iron phases, such as sulfides (e.g., mackinawite, pyrite; Sorensen & Jorgensen, 1987). When preserved from oxidation, these mineral phases can be considered as mineral markers of reducing porewaters in ancient sedimentary rocks (Marin Carbonne et al., 2014). Although absent in the first cm of sediments, iron reduction generally occurs in the upper few dm of the sediments and ferrous iron cation (Fe^{2^+}) concentration increases with depth (Burdige, 1993; Canfield et al., 1993; Sorensen & Jorgensen, 1987). Finally, when sulfate is depleted methanogenesis becomes predominant metabolism for the mineralization of organic carbon in the tidal flat sediments (O'Sullivan et al., 2013; Webster et al., 2010).

Through their combined metabolic activity, microorganisms modify the concentration profiles of these electron acceptors, but also indirectly of other elements such as trace metals. For example, cadmium can be released in estuarine pore water through organic matter degradation associated with Mn and Fe reduction (Audry et al., 2005). This general model of microbial stratification may vary depending on microbial communities or environmental conditions (Visscher & Stolz, 2005). For example, sulfate reduction or methanogenesis can be active in the zone of oxygenic photosynthesis of microbial mats (Visscher et al., 1992; Hoehler et al., 2002. Pace et al., 2018). Coupled reduction reactions or overlap of these have been documented; for example, denitrification and manganese reduction may co-occur due to close ΔG^{o} values (Klinkhammer et al., 1980; Burdige, 1993). These biogeochemical models are also locally exacerbated by hydrological and sedimentary properties such as surface or groundwater flow, and sedimentary architecture (McAllister et al., 2015; Beck et al., 2017). For example, low river discharge can enhance carbon remineralization in estuaries by displacing the salinity gradient upstream (Meiggs & Taillefert, 2011).

In addition to aqueous geochemical properties, the role of EPS is rarely considered in such conceptual models. EPS possess negatively charged reactive groups, which are capable of chealting metals (Braissant et al., 2007; Decho & Gutierrez, 2017). Although primarily produced by photoautotrophs, EPS may also be secreted by heterotrophs such as sulfate-

reducing bacteria at depth (SRB; Braissant et al., 2007). In estuarine sediments, significant concentrations of EPS can be preserved several meters below the sediment surface (Duteil et al., submitted; Chapter 3). Such preservation could be explained by a protection from heterotrophic degradation by a rapid burial during river floods or through the link with clay mineral surfaces. It is possible that the ratio of consumption to production of EPS in estuarine sediments has a major role in metal availability and/or precipitation. This is for example, freshly-produced EPS in microbial mats inhibits carbonate mineral precipitation, while degradation of EPS releases Ca^{2+} and promotes precipitation (Braissant et al., 2007).

Many studies focused on early diagenesis and the link this with microbial metabolisms but only to depths of around 20 cm (Burdige & Gieskes, 1983; Sorensen & Jorgensen, 1987; Hensen et al., 2006). For example, in the Gironde estuary, gradients of several elements (O₂, NO₃⁻, SO₄⁻, Fe, Mn) were studied from the water column down in the sediments to 50 cm depth (Audry et al., 2006). These studies did not include direct measurements of metabolic activities and the role of microbes was only assumed. To date, no study has combined sediment and porewater chemistry, EPS physicochemical properties and microbial activity to characterize early diagenetic geomicrobiological processes in estuarine sediments.

In the current study, we investigated the relationships between microbial activity, the cycling of key metals and the physicochemistry of exopolymeric substances (EPS) in estuarine sediments. Sediment cores and box samples were collected in an estuarine point bar from the Gironde Estuary (southwest France). The changes in microbial activities with depth was measured with metabolic assays coupled to microcalorimetry. Porewater concentrations of O₂, pH, HS⁻, SO4²⁻, NO3⁻, Fe (II/III), Mn, Ca, Mg, Na, Cl were combined with microelectrode measurements, liquid chromatography, Inductively Coupled Plasma atomic emission spectroscopy and colorimetric assays. We extracted exopolymeric substances (EPS) from the sediments and characterized the physicochemical properties using wet chemical analyses. The

potential for metal dissolution and precipitation was evaluated based on core-scale X-Ray Fluorescence and porewater geochemical modeling. Following statistical analyses of this dataset, we finally propose an integrative model of estuarine sediment geomicrobiology that includes early diagenesis.

Materials and methods

Field site description, sample location, and core sampling

Field campaigns were held at the Bordeaux Nord point bar, located in the Garonne estuarine channel, in June 2019. A 6.23m long vertical core (BXN Long Core or BXN-LC; 44°53'47.40"N, 0°32'24.80"O) was extracted from the chute channel with a portable vibro-corer (refer to part 4.2 of this thesis for more details about BXN-LC). In order to preserve sediment structures from deformation and fluidization due to water escape, the core barrel is hammered without any rotation and the piston is maintained with a cable. A second, 0.7 m long core (BXN Short Core or BXN-SC; 44°53'45.00"N, 0°32'23.40"O; Figure 1) was extracted with a handheld core sampler. Finally, a sediment box core (BXN box or BXN-B; 44°53'45.00"N, 0°32'23.40"O; Figure 1) was sampled in the same area with a steel sampling box (length: 28cm, width: 17.5cm, depth: 6.2cm). After extraction, the cores were sealed and kept vertical to avoid water mixing and stored at 4°C until porewater sampling and opening.



Figure 1: (A) Map of the Gironde estuary with location of the Bordeaux Nord point bar and satellite image of the point bar at low tide (Google Earth Pro) showing the location of the studied cores (BXN-LC, BXN-SC and BXN-B) and surface biofilm samples. Three sedimentary domains are visible in the intertidal zone of the point bar: the mud flat, the chute channel (low energy), and the sand dunes (high energy). (B) Image of BXN-B showing a change is sediment color from orange to grey at ca. 14 cm. The main facies is composed of small ripples alternating with clay drapes (wavy bedding) and is typical of an intertidal zone with moderate tidal currents. (C) Image, (D) mapping of sand vs. clay and (E) sedimentary log from BXN-SC. The top 40 centimeters of BXN-SC are similar to BXN-B, but the bottom 25 centimeters show high energy tidal dunes. In order to simplify the presentation of results, BXN-B and BXN-SC are merged into one synthetic core in the following figures.

Porewater chemistry: microelectrode measurements

Porewater dissolved oxygen (O_2) concentrations were measured in the field down to a depth 1.4 cm with a vertical resolution of 250 µm using a polarographic electrode. Subsequently, the concentrations of O_2 , sulfide (H_2S/S^{2-}), and the pH were measured in the laboratory by inserting microelectrodes in the sediment through holes in the core tubing (vertical resolution: 0.2 mm). The measurements for O_2 , H_2S/S^{2-} and pH were taken using polarographic and ion-specific needle microelectrodes in combination with a field microsensor multimeter (Unisense, Aarhus, Denmark; Visscher et al., 2000) and measurements for S^{2-} using a high-impedance millivolt meter (Microscale Measurements, The Hague, The Netherlands). The [HS⁻] was calculated by combining H_2S or S_2^- readings with pH measurements at each depth (Visscher et al., 1991). Detection limits for electrode measurements were better than 1 µm for O_2 , and better than 5 µm for H_2S , and 2 µm for the S^{2-} electrode. Depth profiles were determined three times for each analyte. The temperature and conductivity of the overlying water were determined with an Accumet AP-75 handheld meter and the pH using a Mettler Toledo GoFive meter.

Porewater chemistry: water extraction

Porewater was sampled with rhizons (pore diameter of 0.1 µm; Rhizosphere Research Products; Wageningen) inserted in holes drilled in the core tubing. A total of 34 pore water samples were extracted, 12 for BXN-B (*ca.* 1 sample every 5 cm), 6 for BXN-SC (ca. 1 sample every 12 cm) and 16 for BXN-LC (ca. 1 sample every 40 cm). In order to improve water recovery, rhizons were saturated with deionized water for 30 minutes before use. 25 mL syringes were connected with the Luer-Lock system to the Rhizons with a wooden retainer to create a vacuum. Water sampling was performed over 24 hours, then syringes were closed with Luer-Lock plugs and stored on the fridge (4°C) until processing.

Porewater chemistry: major dissolved elements measurements and modelling

Major cation (Ca²⁺, Mg²⁺, Mn²⁺, Na⁺, K⁺) and anion (Cl⁻, NO₃⁻, and PO₄³⁻) concentrations were determined by ion chromatography (IC; Dionex ICS-1500) in the 34 porewater samples, with an analytical precision of 0.2 mg.L⁻¹. Metal concentrations were measured by ICP-OES (Cd²⁺, Ca²⁺, Mg²⁺, dissolved Mn, dissolved Fe, Pb²⁺, Zn²⁺) on the same samples at Eurofins laboratory (Bordeaux). Two depth intervals did not yield enough water for analyses (Table S1), and thus did not allow for ICP measurements on 5 samples from BXN-B and BXN-SC. Iron speciation (Fe(II)/Fe(III)) was determined using the 1,10-phenantroline colometric test (ref LCK 321; Hach Lange) with a Hach DR 3900 spectrophotometer (Hach Lange, CO, USA). The concentrations were entered in the geochemical modeling software Phreeqc to determine the saturation index of oxides, sulfides and carbonates using the Pitzer model (Pitzer, 1979).

Core opening, processing and sampling

Cores were immediately photographed upon opening. Two-dimensional X-ray images of the cores were obtained with a SCOPIX device (EPOC laboratory, Bordeaux; Migeon et al., 1998), allowing for identification of sedimentary fabrics with a non-destructive approach at high resolution (125 µm). Core sedimentology was subsequently analyzed in order to localize the main facies changes. Sixteen 20 cm-long samples (10 cm above and below the target depth) were taken along the core. Samples were homogenized and a subsample was weighed before and after drying in the oven (24 hr at 30 °C), in order to determine sediment wet and dry weights. A second subsample was kept for measurements of grain size and TOC content, and for cryofixation. The remaining part of the sample was used for (i) metabolic assays and (ii) EPS extraction, purification, and characterization. X-ray fluorescence spectrometric analysis (XRF) was done for BXN-SC and BXN-LC with a XRF core scanner (AVAATECH XRF Core Scanner; Avaatech; Netherlands).

Metabolic assays

The TTC assay was used as a proxy for monitoring metabolically active cells in the sediment. The total reductase activity was measured in 34 samples (in triplicates) through the reduction of triphenyltetrazolium chloride (TTC) to triphenylformazan to characterize metabolic active cells (Relexans, 1996; Braissant et al., 2020). For this, 2 grams of sediment sample were homogenized and mixed with 2 mL of 0.8% TTC in Instant Ocean (pH 7.1) to obtain a final concentration of 0.4% TTC. The samples were incubated for 3 h at 30°C. Following the incubation, the samples were centrifuged (3000 g, 10 min) and the pellet was resuspended in 4mL of acetone to extract the triphenylformazan. After 5 min, the sample was centrifuged (3000 g, 3 min) and the absorbance of the supernatant at 490 nm was measured with a spectrophotometer (Helios Epsilon, Thermo Fisher Scientific). The concentration of formazan was calculated with the Beer-Lambert law with a molar absorption coefficient of 14 320 L.mol⁻¹.cm⁻¹. Blanks were prepared by adding 2 mL of 1.5% glutaraldehyde to the sediment. Active diatom cells were used as positive control.

The FDA assay was used as a proxy for monitoring the enzymatic activity in the sediment. The hydrolytic activity of the ubiquitous lipase, protease, and esterase enzymes (non-specific hydrolases) was measured in 34 samples (in triplicates) by the hydrolysis of the fluorescein diacetate (FDA) into fluorescein (Green et al., 2006; Braissant et al., 2020). For this, 2 grams of sediment were homogenized in 4 mL of Instant Ocean (pH 7.1) and 60µL of FDA solution (1 mg.mL⁻¹ in acetone). The samples were incubated for one day at 30°C. Following incubation, the fluorescein was extracted by adding 4mL of acetone. After 5 min, the sample was centrifuged (3000 g, 3 min) and the absorbance of the supernatant at 490 nm was measured with a spectrophotometer. The concentration of fluorescein was calculated with the Beer-Lambert law considering a molar absorption coefficient of 73,350 L.mol⁻¹.cm⁻¹. Blanks were prepared by adding 2 mL of 1.5% glutaraldehyde to the sediment. Active diatom cells were used as positive control.

Isothermal calorimetry (ITC)

Two grams of sediments were put in the calorimeter (TAM air; TA Instruments, New Castle, DE) with 200 μ L of R2A culture medium. Autoclaves sediment samples (20 min; 121°C) were used as blank. For each depth, sampling was carried out in triplicate. The heat release was measured during 24h to quantify the microbial activity. The growth rate (μ), the lag phase (λ) and the heat produced (Q) were calculated by fitting the heat over time curve with the modified Gompertz model (Gil et al., 2006; Braissant et al., 2013). The growth rate (μ) predicts how fast the bacterial community could grow using the experimental substrate (h). The lag time (λ) corresponds to the time before the bacterial community is active. The maximum heat flow was used to estimate the number of active cells, assuming a heat production rate of 3pW.cell⁻¹. For aerobic microbial plate counts, 1 g of sample was dissolved in 10 ml of PBS then subjected to 10 folds dilution series. The appropriate dilutions where then poured into a petridish containing R2A medium and 1.5% agar. Colonies were counted after 1 (CFU 1), 7 (CFU 7) and 30 (CFU 30) days and aerobic microbial numbers were calculated using the sample dilutions.

EPS extraction and physico-chemical characterization

EPS were extracted from the BXN-LC by diluting one volume of sample with two volumes of DI water. The solution formed was then put into an ultrasonic bath for 10 minutes and then gently stirred for 4 hours. Centrifugation was performed (2500xg, 10 min) to remove the sediments (silt, clay, and sand) and cells. The supernatant was filtered through polycarbonate filters (0.22 μ m) and the filtrate was precipitated using three volumes of cold propanol (<0 °C) per volume of filtrate during 24h. The precipitate was recovered by centrifugation (2800 g, 20 min), placed in dialysis tubing (10 kDa), and dialyzed for three days in a buffer solution (2 L of DI water at 1mM EDTA) and three more days against 2 L of DI water (>18 M\Omega). Liquid EPS solutions were then stored in the refrigerator at 4 °C. The composition of EPS was

determined using three colorimetric assays: (i) the phenol-sulfuric acid assay (Dubois et al., 1956), (ii) the Alcian Blue assay (Passow & Alldredge, 1995) and (iii) the Pierce-modified Lowry assay (Lowry et al., 1951) measured with a Helios Epsilon spectrophotometer (Thermo scientific). These three assays provide an estimate of (i) the amount of sugar monomers in the sample (i.e., the amount of neutral sugars in EPS); (ii) the amount of cationic dye binding sites (i.e., the amount of negatively-charged reactive sites in the EPS); and (iii) the amount of protein. Triplicates (100 μ l) were used for each assay. The amount of EPS measured with the three colorimetric assays (i.e., protein, phenol-sulfuric, and Alcian Blue) are expressed as micrograms of EPS per gram of dry sediment (μ g.g⁻¹ DW), using albumin (protein assay) or xanthan (phenol-sulfuric and Alcian Blue assays) as standards.

Statistical analyses

All the statistical analyses were performed using the R software (R Core Team, 2016). PCA were achieved using the *prcomp* function and visualized with the factoextra package and *fviz pca* function. Correlation matrices were calculated with the function *cor* and visualized with the package corrplot. Finally, function *rcorr* of the package Hmisc was used to calculate the level of significance for the Pearson and Spearman correlations.

Results

BXN-box and BXN short core were sampled in close proximity (5 cm). As they show a very similar sedimentary succession, and in order to simplify the results, BXN-Box and BXN Short Core were grouped together in the figures. All data are available as part of the supplementary material (Tables S1 and S2).

Porewater analysis

In BXN-B and BXN-LC, the concentration of dissolved oxygen (O₂) increased from 223 to 476 μ M between 0 and 3 mm depth, and decreased exponentially to 5 μ M at 13 mm depth (Figure 2). The O₂ concentration remained very low between 2 and 30 cm (between 0.25 and 11 μ M), and reached 0 at 35 centimeters. In the same core, pH increased from 7.9 to 8.2 between 0 and 12 cm depth, and stabilized around 8.2 below 12 cm (Figure 2).

Sulfide concentrations (HS⁻) were not considered at the top and the bottom of the cores BXN-B and BXN-SC (0, 26 and 65 cm deep), because of the potential of oxygen diffusion. [HS⁻] was not detected in the upper 2 cm after which it increased to 240 μ M at 20 cm depth (Figure 2). This was followed by a decrease to 157 μ M at 24 cm, after which it remained relatively constant (152-163 μ M) to 40 cm and decreased further to 129 μ M at 50 cm, before finally increasing to 165 μ M at 60 cm.

The sulfate concentration (SO_4^{2-}) was of 8.39 mg.L⁻¹ at 2 cm below the sediment surface and decreased to 4.9 mg.L⁻¹ (Figure 2) at 6 cm. $[SO_4^{2-}]$ increased to 9.24 mg.L⁻¹ at 12 cm and reached zero at 18 cm. At 20 cm, the concentration increased again to 2 mg.L⁻¹, and was low between 22 and 26 cm (0-0.71 mg.L⁻¹). The concentration increased to 7.8 mg.L⁻¹ at 30 cm, rapidly decreased to 0.24 mg.L⁻¹ at 35 cm, rose again to 5.9 mg.L⁻¹ at 40 cm. $[SO_4^{2-}]$, and remained low, around 0-0.1 mg.L⁻¹ between 50 and 65 cm. In BXN-LC, sulfate concentration was at 0.54 mg.L⁻¹ at 60 cm, and decreased to approximately 0.15 mg.L⁻¹ between 50 cm and

1.3 m (Figure 3). It increased to 1.03 mg.L⁻¹ between 1.3 and 1.6 m. No sulfate was detected between 2.16 and 2.59 m, and concentrations remained low below 2.8 m (0-0.3 mg.L⁻¹), except at 3.25 m (0.55 mg.L⁻¹).

Nitrate was absent between 0 and 6 cm, after which the concentration increased to 6 mg.L⁻¹ at 8 cm (Figure 2). The $[NO_3^{-7}]$ dropped to 1.86 mg.L⁻¹ at 10 cm and increased to 2.8 mg.L⁻¹ at 12 cm. $[NO_3^{-7}]$ fell to 0 mg.L⁻¹ at 16 cm and until 65 cm depth. In BXN-LC, the concentration rose from 1 to 1.4 mg.L⁻¹ between 0.06 and 0.5 m depth (Figure 3). The nitrate concentration decreased to zero at 1.63 m and remained absent down to 2.59 m depth. Low nitrate concentrations of 0.14 and 0.17 mg.L⁻¹ were found at 2.8 and 3.25 m before decreasing to zero near the bottom of the core bottom.

In BXN-B and BXN-SC, no dissolved iron (Fe) was present from the sediment surface down to 10 cm depth. Iron concentrations reached 0.15 mg.L⁻¹ at 18 cm before decreasing to zero at 24 cm depth (Figure 2). Iron concentration reached 0.14 mg.L⁻¹ at 26 cm before decreasing to to 4.1 μ g.L⁻¹ at 35 cm. The concentration went up to 67 μ g.L⁻¹ at 50 cm before reaching zero at 65 cm. Concentrations were below the detection limit of the assay in BXN-B and BXN-SC.

The concentrations of dissolved iron, Fe(II) and Fe(III), were measured in BXN-LC (Figure 3). Fe(II) and Fe(III) increased to 0.94 and 0.67 mg.L⁻¹ at 0.06 and 0.5 m, respectively with higher Fe(II) concentrations than Fe(III) concentrations. These concentrations decreased to 0.06 mg.L⁻¹ for Fe(II) and 0.62 mg.L⁻¹ for Fe(III), respectively at 0.93 m, and then increased to 0.22 mg.L⁻¹ and 1.32 mg.L⁻¹ at 1.3 m. This was followed by a decrease to zero for both species at 1.63 m. Both concentrations increased to 0.22-0.29 mg.L⁻¹ for Fe(II) and 1.75-1.84mg.L⁻¹ for Fe(III) between 2.16 and 2.59 m. Below this depth, Fe (II) was only measured at 4.76 m. Fe(III) decreased to 0.53 mg.L⁻¹ at 2.8 m and increased to 1.35 mg.L⁻¹ at 3.25 m. A similar pattern was observed between 3.68 and 4.76 m, with an increase from 0.97 to 1.54 mg.L⁻¹, and between 5.68 and 6.08 m with an increase from 0.43 to 2.39 mg.L⁻¹ (Figure 3). Dissolved Manganese (Mn) increased from 45 μ g.L⁻¹ to 0.29 mg.L⁻¹ between 2 and 6 cm, then stabilized around 0.3 mg.L⁻¹ between 6 and 16 cm in BXN-B and BXN-SC (Figure 2). A minor increase to 0.4 mg.L⁻¹ occurred at 18 cm, followed by a major decrease to 14 μ g.L⁻¹ at 24 cm. The concentration reached 0.49 mg.L⁻¹ at 26 cm, and subsequently decreased to 0.3 mg.L⁻¹ at 35 cm. Another increase to 0.46 mg.L⁻¹ occurred at 50 cm, followed by a decrease to 68 μ g.L⁻¹ at 65 cm. In BXN-LC, the concentration peaked to 1.4 mg.L⁻¹ at 0.06 m, followed by a decrease to 2.1 mg.L⁻¹ at 6.03 m.

In BXN-B and BXN-SC, dissolved magnesium (Mg^{2+}) concentration remained the same from 2 to 6 cm depth with values around 7 mg.L⁻¹ before decreasing to 2.56 mg.L⁻¹ at 8 cm (Figure 2). Then, the concentration increased to approximately 9.5 mg.L⁻¹ between 10 and 12 cm before decreasing to 5.68 mg.L⁻¹ at 20 cm. After a slight increase at 6.85 mg.L⁻¹ at 22 cm, the $[Mg^{2+}]$ decreased to 2.28 mg.L⁻¹ at 40 cm. Finally, the concentration increased to 6.63 mg.L⁻¹ at 65 cm depth. In BXN-LC, the magnesium concentration increased from 9.71 at 0.06 m to 31.3 mg.L⁻¹ at 4.76 m depth (Figure 3). This followed by a small decrease at 26.75 mg.L⁻¹ at 5.2 m depth and a little increase to 31.75 mg.L⁻¹ at 5.64 m depth. Finally the Mg²⁺ concentration was equal to 27.57 mg.L⁻¹ at 6.08 m depth.

Calcium (Ca²⁺) concentrations in the BXN-B and BXN-SC both showed a minor increase from 32 to 35.79 mg.L⁻¹ between 2 and 4 cm, before decreasing to 21 mg.L⁻¹ at 8 cm depth (Figure 2). Then, concentration increased to 35.21 mg.L⁻¹ at 10 cm depth just before decreased to 16.39 mg.L⁻¹ at 20 cm depth, before a slight increase to 25.10 mg.L⁻¹ at 22 cm depth. This was followed by a small decrease to 18.87 mg.L⁻¹ at 24 cm depth, followed by an increase to 22.28 mg.L⁻¹ at 35 cm. A major decrease to 8.87 mg.L⁻¹ at 40 cm depth was followed by a realtively constant concentration of approximately 18 mg.L⁻¹ between 50 and 65 cm depth. In BXN-LC, the Ca²⁺ concentration showed a similar pattern with depth: approximately 37 mg.L⁻¹ between

0.06 and 0.5m (Figure 3). This followed by a small decrease at 23.58 mg.L⁻¹ at 0.93 m and an increase to 42.74 mg.L⁻¹ at 1.63 m depth. The concentration stabilized around 20 mg.L⁻¹ between 2.16 and 2.8 m before decreasing to 11.04 mg.L⁻¹ until 6.08m depth.

In BXN-B and BXN-SC, sodium (Na⁺) and chloride (Cl⁻) concentrations were constant from 2 to 6 cm depth at 10.5 and 17 mg.L⁻¹ respectively, before decreasing to 4 and 10 mg.L⁻¹ at 8 cm (Figure 2). Then, both concentrations increased to 91 mg.L⁻¹ for Na⁺ and 102 mg.L⁻¹ for Cl⁻ at a depth of 26 cm before slightly decreasing to 88 and 86 mg.L⁻¹ at 30 cm. This was followed by a small increase to 97 mg.L⁻¹ for Na⁺ and 110 mg.L⁻¹ for Cl⁻ at 35 cm depth, followed by a drop to 63 and 72 mg.L⁻¹, respectively, at 40 cm depth. Between 40 and 65 cm, the concentration increased to 116 mg.L⁻¹ for Na⁺ and 138 mg.L⁻¹ for Cl⁻.

In BXN-LC, the concentration was of 40 mg.L⁻¹ for Na⁺ and 54 mg.L⁻¹ for Cl⁻ at 0.06 m (Figure 3). A maximum concentration of 86 mg.L⁻¹ for Na⁺ and 191 mg.L⁻¹ for Cl⁻ was observed at 0.5 m. This was followed by a decrease to 46 and 102 mg.L⁻¹ at 0.93 m. Both concentrations progressively decreased, reaching their minima of 27 mg.L⁻¹ at 2.59 meters for Na⁺ and of 58 mg.L⁻¹ for Cl⁻ at 3.68 m. [Na⁺] increased from 2.59 m down to the bottom of the core, reaching a value of 99 mg.L⁻¹ at 6.08 m depth. The chloride concentration [Cl⁻] increased between 3.68 and 6.08 m depth, reaching a maximum of 114 mg.L⁻¹ at the bottom of the core.



Figure 2: Porewater concentration profiles of major elements in BXN-B and BXN-SC in function of depth. Left part, picture of BXN-B (circled in blue) merged with BXN-SC (circled in red). Profiles of dissolved oxygen (in blue), sulfide (in yellow-orange) and pH (in green) measured using microelectrodes. The red square shows a detail of the oxygen profile in the upper 1.5 cm of the sediment. Profiles of sulfate (yellow), nitrate (purple), sodium (red), chloride (green), magnesium (blue) and calcium (green) concentrations measured by ion chromatography. Profiles of total dissolved iron (brown) and manganese (grey) measured by ICP-OES.



Figure 3: Porewater concentration profiles of major elements in BXN-LC in function of depth. Left part, picture and sedimentological interpretation of BXN-LC (see Duteil et al., submitted and chapter 3 for details). Sample locations are marked by red dots. Profiles of sulfate (yellow), nitrate (purple), sodium (red), chloride (green), magnesium (blue) and calcium (green) concentrations measured by ion chromatography. Profiles of species Fe(II) and Fe(III) measured by a colorimetric assay. Profile of manganese (grey) measured by ICP-OES.

Microbial and enzymatic activities

Microbial activity tests in surface films of diatom cells were positive, indicated by TTC-stained metabolically active organelles (e.g., mitochondria) and FDA-stained areas near the nucleus as well as the lipid droplets (Figure S1). TTC reduction was high in BXN-B and BXN-SC at approximately 6 cm depth, displaying rates of 1.44.10⁻⁴ mol.h⁻¹.g sed⁻¹ and 1.5810⁻⁴ mol.h⁻¹.g sed⁻¹ wet weight at depth of 1 and 2 cm depth, respectively (Figure 4). Deeper into the cores, the activity decreased to 2.63. 10⁻⁵ mol.h⁻¹.g sed⁻¹ wet weight at 3 cm depth, and increased to 1.91. 10⁻⁴ mol.h⁻¹.g sed⁻¹ wet weight between depths of 4 and 25 cm. Metabolic activity measured with TTC decreased to 2.87. 10⁻⁵ mol.h⁻¹.g sed⁻¹ WW between 28 and 40 cm, and increased slightly to 7.3.10⁻⁵ mol.h⁻¹.g sed⁻¹ WW at 50 cm. The metabolic activity remained constant around 2.2.10⁻⁵ mol.h⁻¹.g sed⁻¹ WW at 60 and 65 cm.

In BXN-LC, TTC reduction rates were high at 6 cm depth with a rate of 1.44.10⁻⁴ mol.h⁻¹.g sed⁻¹ WW. It decreased to 2.19.10⁻⁵ mol.h⁻¹.g sed⁻¹ WW at 50 cm (Figure 5). The metabolic activity subsequently increased to 1.56.10⁻⁴ mol.h⁻¹.g sed⁻¹ WW between 50 and 130 cm. The rate of TTC reduction was low at 163 cm depth with a value of 2.77.10⁻⁵ mol.h⁻¹.g sed⁻¹ WW, and slightly increased to 7.32.10⁻⁵ mol.h⁻¹.g sed⁻¹ WW at 216 cm. The metabolic activity decreased to 4.46.10⁻⁵ mol.h⁻¹.g sed⁻¹ WW at 259 cm and again to 1.33.10⁻⁴ mol.h⁻¹.g sed⁻¹ WW at 2.8 m. The reduction of TTC then stabilized around 6.5.10⁻⁵ mol.h⁻¹.g sed⁻¹ WW between 3.25 and 3.68 m and decreased again to 1.79.10⁻⁵ until 4.76 m. The metabolic activity subsequently increased to 4.2.10⁻⁵ mol.h⁻¹.g sed⁻¹ WW between 4.76 and 5.64 m depth, and to 1.1.10⁻⁵ mol.h⁻¹.g sed⁻¹ WW at 6.08 m.

The enzymatic activity as measured with the FDA assay was high at the top of BXN-B and BXN-SC, comprised between 2.93.10⁻⁵ mol.h⁻¹.g sed⁻¹ WW and 3.32.10⁻⁵ mol.h⁻¹.g sed⁻¹ WW between 1 and 5 cm depth (Figure 4). Then the activity progressively decreased to 1.2.10⁻⁵ mol.h⁻¹.g sed⁻¹ WW between 10 and 35 cm. The rate of FDA hydrolysis increased

and remained approximately constant around 1.8.10⁻⁵ mol.h⁻¹.g sed⁻¹ WW between 40 and 65 cm. In BXN-LC, the enzymatic activity was relatively high at 6 cm, reaching 2.98.10⁻⁵ mol.h⁻¹.g sed⁻¹ WW, and subsequently decreased to 1.34.10⁻⁵ mol.h⁻¹.g sed⁻¹ WW at 93 cm (Figure 5). The hydrolysis rate increased to 1.87 10⁻⁵ mol.h⁻¹.g sed⁻¹ WW at 130 cm, remained relatively stable approximately 1.4.10⁻⁵ mol.h⁻¹.g sed⁻¹ WW between 163 cm and 259 cm, before dropping to 1.12.10⁻⁵ mol.h⁻¹.g sed⁻¹ WW at 280 cm. The enzymatic activity increased and remained constant around 1.95.10⁻⁵ mol.h⁻¹.g sed⁻¹ WW between 325 cm and 433 cm, decreased to 8.43.10⁻⁶ mol.h⁻¹.g sed⁻¹ WW at 476 cm. The rate of FDA hydrolysis increased to 1.21.10⁻⁵ mol.h⁻¹.g sed⁻¹ WW at 520 cm and stabilized at 1.77.10⁻⁵ mol.h⁻¹.g sed⁻¹ WW between 564 and 608 cm.



Figure 4: Profiles of metabolic and enzymatic activities in the sediment in BXN-B and BXN-SC. The depth of the samples is indicated along the ruler. The rate TTC reduction (red) is a proxy for metabolic activity. The rate of hydrolyzed FDA (yellow) is a proxy for hydrolytic enzymes activity.



Figure 5: Profiles of metabolic and enzymatic activities in the sediment in BXN-LC. The depth of the samples is indicated along the ruler. The rate TTC reduction (red) is a proxy for metabolic activity. The rate of hydrolyzed FDA (yellow) is a proxy for hydrolytic enzymes activity. EPS profiles are plotted to the right: concentrations of neutral sugars (yellow), proteins (green) and density of acidic sites (blue).

Isothermal calorimetric measurements of microbial activity are presented in Table 1. μ value was of 0.17 h⁻¹ at 6 cm, increased to 0.24 h⁻¹ at 50 cm and decreased to 0.13 h⁻¹ at 93 cm. The lag time was equal to zero at 6 and 93 cm, while reaching 3.57 h at 50 cm depth. The total heat released during the experiment (Q), was equal to 5.42 J at 6 cm depth, 4.21 J at 50 cm and 2.61 J at 93 cm. maxHF the peak of thermal activity corresponds to the maximum of metabolic activity was equal to 75 μ W at 6 cm, 89.5 μ W at 50 cm and 62.5 μ W at 93 cm depth. Finally CFU 30, the counting of bacterial colonies after 30 days of culture was of 5,3.10⁸ at 6 cm depth, 2,8.10⁷ at 50 cm and 2,9.10⁸ at 93 cm.

Depth		mu	Lag	Q	maxHF	CFU 30
(cm)		h -1	h	J	μW	No unit
	6	0,17	0	5,42	75	5,3.10 ⁸
	50	0,24	3,57	4,21	89,5	$2, 8.10^{7}$
	93	0,13	0	2,61	62,5	2,9.10 ⁸

Table 1: Measurements of microbial activity with isothermal calorimetry. These measurements were done on sediments from three depths in BXN-LC. Mu corresponds to the growth rate, i.e., how fast the bacterial community can grow using the substrate. Lag is the time before the bacterial community is active (negative value = 0). Total heat Q is the total heat released during the experiment, maxHF is the peak of thermal activity and CFU 30 the counting of bacterial colonies after 30 days of culture.

Exopolymeric Substances quantity in the sediment

The biofilm at the surface of the sediment had the highest values for the phenol-sulfuric assay $(23\pm3 \ \mu g.g^{-1} \ EPS \ dry \ weight)$, and high values for the protein and Alcian Blue assays $(37\pm5 \ \mu g.g^{-1} \ EPS \ DW \ and \ 27\pm1 \ \mu g.g^{-1} \ DW \ respectively; Figure 5)$. At 6 cm, the amount of neutral sugars in EPS showed a slight decrease $(4.9\pm0.05 \ \mu g.g^{-1} \ DW)$ as well as proteins $(13.6\pm0.5 \ \mu g.g^{-1} \ DW)$ and acidic sites density $(16.6\pm0.1 \ \mu g.g^{-1} \ DW)$. This was followed at 50 cm by a seven-fold decrease for the phenol-sulfuric and protein assays and by a 24-fold decrease for the Alcian Blue assay. At 93 cm, the neutral sugar content increased to $1.2\pm0.7 \ \mu g.g^{-1} \ DW$, the protein concentration to $11\pm0.5 \ \mu g.g^{-1} \ DW$ and Alcian Blue values to $11\pm0.05 \ \mu g.g^{-1} \ DW$.

Between 130 and 216 cm, properties characterizing EPS were low: neutral sugars were below 1 μ g.g⁻¹ DW, the protein content was 3 μ g.g⁻¹ DW and acidic sites concentration 2 μ g.g⁻¹ DW. At 259 cm, neutral sugar content in EPS increased to 3±3 μ g.g⁻¹ DW, the protein concentration to 8±0.2 μ g.g⁻¹ DW and density of acidic sites to 18±0.7 μ g.g⁻¹ DW. Between 280 and 412 cm, amount of neutral sugars in EPS were below 2 μ g.g DW⁻¹, amount of proteins below 3 μ g.g⁻¹ DW, and density of acidic sites below 5 μ g.g⁻¹ DW. At 433 cm, the neutral sugar content was high, reaching 6.6±0.2 μ g.g⁻¹ DW, as well as the protein concentration (35.7±9 μ g.g⁻¹ DW), and the acidic sites concentration (13±0.24 μ g.g⁻¹ DW). Between 433 and 608 cm, properties characterizing EPS decreased progressively to around 2.2 μ g.g⁻¹ DW for the neutral sugars, 8 μ g.g⁻¹ DW for protein content, and 8.4 μ g.g⁻¹ DW for the Alcian Blue values.

Statistical analyses of porewater geochemistry and microbial activity

The first axis of the PCA for porewater constituents in both BXN-B and BXN-SC cores explained 58% of the variation and the second axis account for 16.3% of the variation (Figure 6). The correlation analyses revealed three separate groups: (i) sulfate, nitrate and oxygen were opposite to (ii) sulfide, chloride, sodium, pH and depth on axis 1, while (iii) Fe and Mn were grouped on the negative side of axis 2. The correlations are considered as significant when $p \le 0.05$. The correlation matrix of the BXN-B and BXN-SC (Figure 6; n=18 samples) showed significant correlations between core depth and most of the ions analyzed. Sulfate, nitrate and oxygen were anti-correlated with depth (correlation coefficients of r=-0.61, r=-0.34 and r=-0.6 respectively). Sulfide, pH, sodium and chloride were positively correlated with depth (r=0.54, r=0.41, r=0.88 and r=0.89). Oxygen was anti-correlated with the elements that increased with depth (e.g., sulfate, r=0.72). Sulfide followed the opposite trend, being positively correlated with e.g., sodium (r=0.75) and negatively correlated with sulfate (r=-0.69). Sulfide and pH were also

positively correlated (r=0.85). pH was anti-correlated with sulfate (r=-0.52) and correlated with sodium and chloride (r=0.6 and r=0.57). Sulfate was negatively correlated with sodium and chloride (r=-0.79 and r=-0.83) and positively correlated with nitrate (r=0.51). Nitrate was anti-correlated with iron (r=-0.21), manganese (r=-0.32), sodium (r=-0.50) and chloride (r=-0.51). Fe and Mn showed no correlations (p-value>0.05) with other ions, pH or depth, but were positively correlated to each other (r=0.72). Finally, sodium was strongly correlated to chloride (n=18, p-value ≤ 0.05 , r=-0.99).



Figure 6: Left part, PCA correlation circle plot of variables of the porewater composition in BXN-B and BXN-SC. Right part, correlation matrix of Pearson's r coefficients between depth, concentration of the main dissolved elements, oxygen and pH for the 18 samples. Green boxes indicate a p-value ≤ 0.05 .

The first two axes of the PCA for BXN-LC explained respectively 43.4% and 27.8% of the variation (Figure 7). Nitrate, Mn and Fe (II) were grouped on the negative side of axis 1, in contrast to depth. Sodium and sulfate were located on the negative and positive side of axis 2, respectively. The correlation matrix of BXN-LC porewater data (n=16 samples) is shown Figure 7. Sulfate, nitrate, Fe(II) and Mn were anti-correlated with depth (correlation coefficient of r=-0.53, r=-0.7, r=-0.52 and r=-0.56 respectively). Sulfate was anti-correlated with sodium
(r=-0.42). While nitrate was anti-correlated with Fe(III), although with a very low correlation coefficient (r=-0.16), it was correlated with Fe(II), Mn, sodium and chloride (r=0.77, r=0.83, r=0.04 and r=0.56, respectively). Fe(II) was positively correlated with both Mn (r=0.68) and chloride (r=0.56). Finally, Mn was correlated with chloride (r=0.39).



Figure 7: Left part, PCA correlation circle plot of variables of the porewater composition in BXN-LC. Right part, correlation matrix of Pearson's r coefficients between depth, concentration of the main dissolved elements for the 16 samples. Green boxes indicate p-value ≤ 0.05 .

A correlation matrix including metabolic assays and physicochemical properties of EPS was generated for BXN-LC (Figure S2; n=16). The first axis of the PCA explained 47% of the variation, and the second 17.8%. EPS properties and depth were grouped on the positive side of the first axis, in contrast to the TTC assay, which is in the negative part of the first axis. The TTC assay was anti-correlated with the concentrations of neutral polysaccharides (r=-0.38) and the proteins (r=-0.35). Neutral sugars were positively correlated to proteins (r=0.84) and the density of acidic sites in EPS (r=0.71). Finally, proteins were correlated to the number of acidic sites (r=0.58). The FDA assay showed no statistical correlation with the other variables.

Precipitation-dissolution modeling

Saturation indices were calculated independently using Phreeqc for (i) BXN-B plus BXN-SC, which include sulfide measurements (Table 2) and (ii) BXN-LC (Table 3). In BXN-B and BXN-SC, the porewater was undersaturated for ferrihydrite ($Fe^{3+}_{10}O_{14}(OH)_2$; saturation index (SI) -5.19 to -7.35; -6 on average; Table 2). and for goethite (α -Fe³⁺O(OH); SI -1.34 to 0.41; - 0.4 on average). The porewater in both cores was supersaturated for hematite at depth greater than 22 cm (Fe₂O₃; SI 0,67 to 2.78) and alternated between under- and supersaturated a greater depths/deeper in the cores. Porewater was close to equilibrium with calcite (CaCO₃; SI -0.49 to 0.28; 0.07 on average). The saturation was variable for sulfide minerals: mackinawite (Fe_{1+x}S) was undersaturated above 6 cm depth (-2.82 to -3.44) and supersaturated below 6 cm (SI 0.18 to 2.15); pyrite (FeS₂) was supersaturated at all depth, with significantly lower values above 6 centimeters (SI 1.76 to 2.53) than below (SI 8.47 to 10.57). These results are visualized in Figure S3.

Sulfide measurements were lacking from BXN-LC, and thus we calculated saturation indices for iron oxides and carbonates only (Table 3). Porewaters were largely supersaturated for goethite (SI 7.25 to 9.33; 8.6 on average) and hematite (SI 16.47 to 20.62; 19.11 on average), and much less for ferrihydrite (1.65 to 3.73; 3 on average). Porewaters were close to equilibrium for calcite (SI 0.02-0.55; -0.18 on average) and for siderite (FeCO₃) (-1.05 to 0.91; -0.38 on average). Saturation indices were also slightly positive for dolomite (saturation index between 0.15 and 1.08 with 0.74 on average). These results were plotted in the figure S4.

BNX-Box and	Saturation					
BXN-SC	index					
Depth	Ferrihydrite	Goethite	Hematite	Calcite	Mackinawite	Pyrite
cm	Fe(OH) ₃	FeOOH	Fe ₂ O ₃	CaCO ₃	FeS	FeS ₂
2	-5.19	0.41	2.78	0.08	-2.82	2.53
4	-5.32	0.28	2.52	0.09	-2.96	2.42
6	-5.72	-0.13	1.72	0	-3.44	1.76
10	-5.47	0.13	2.22	0.39	1.03	9.03
16	-5.87	-0.27	1.43	0.07	1.59	10.05
18	-5.44	0.15	2.28	0.03	2.09	10.57
22	-6.25	-0.65	0.67	0.28	1.3	9.68
24	-7.35	-1.75	-1.53	0.03	0.18	8.47
26	-5.43	0.17	2.31	0.23	2.15	10.21
35	-6.94	-1.34	-0.71	-0.49	0.62	8.76
50	-5.72	-0.12	1.73	0.09	1.88	9.69
60	-6.77	-1.18	-0.38	0.09	0.93	8.98
65	-6.49	-0.89	0.19	0.03	0.43	8.07

Table 2: Saturation indices of some mineral phases in BXN-B and BXN-SC according to the modeling performed with phreeQc.

BXN- LC	Saturation index						
			Hematit	Siderit	Calcit		Aragonit
Depth	Ferrihydrite	Goethite	e	e	e	Dolomite	e
	-				CaCO	CaMg(CO ₃)	
m	Fe(OH) ₃	FeOOH	Fe ₂ O ₃	FeCO ₃	3	2	CaCO ₃
0.06	2.5	8.09	18.16	0.21	0.26	0.26	0.11
0.5	3.17	8.77	19.52	0.91	0.39	0.72	0.25
0.93	3.14	8.74	19.45	-0.41	0.02	0.15	-0.12
1.3	3.47	9.07	20.11	0.28	0.29	0.74	0.15
1.63	1.65	7.25	16.47	-0.99	0.55	1.08	0.41
2.16	3.61	9.21	20.39	0.21	0.04	0.42	-0.1
2.59	3.59	9.19	20.35	0.39	0.09	0.56	-0.05
2.8	3.07	8.67	19.31	-1.05	0.15	0.62	0.01
3.25	3.48	9.08	20.13	-0.98	0.13	0.83	-0.02
3.68	3.34	8.93	19.84	-0.96	0.16	0.92	0.02
4.12	1.65	7.25	16.47	-0.94	0.06	0.86	-0.08
4.76	3.54	9.13	20.24	0.4	0.17	1.04	0.03
5.2	1.65	7.25	16.47	-0.92	0.2	0.97	0.06
5.64	2.98	8.58	19.13	-0.89	0.22	1.12	0.07
6.08	3.73	9.33	20.62	-0.94	0.04	0.8	-0.1

Table 3: Saturation indices of some mineral phases in BXN-LC according to the modeling performed with phreeQc.

XRF sediment measurement

In BXN-BOX and BXN-SC, XRF results showed a small peak in sediment sulfur amount at approximately 8 cm depth (Figure S5). This followed by a zone between 15 and 42 cm with a high sulfur amount. The amount of aluminum in the sediment was more variable. The aluminum quantity is important in the sediments between 0 and 5 cm depth before being lower between 5 and 16 cm depth. Aluminum amount was then relatively high between 16 and 62 cm depth before decreasing until 69 cm depth. The quantity of manganese in sediments was low between 0 and 16 cm depth before increased. Manganese amount in sediments remained relatively high between 16 and 52 cm depth. Between 52 and 69 cm depth the manganese quantity in sediments was relatively low. Finally, iron amount in sediments was relatively high between 0 and 3 cm depth. Quantity remained low between 3 and 15 cm and was relatively high between 15 and 62 cm depth. Iron quantity decreased at the end of the BXN-SC.

In BXN-LC, XRF results showed that the amount of sulfur in sediments peaked at approximately 0.5, 1.5, 2.8, 5.5 and 6 m depth (Figure S6). A zone with a lower sulfur content was present between 3.25 and 4.25 m depth and the sediments were enriched in sulfur from 4.3 to 6.08 m. The amount of aluminum in sediments seemed rather stable between 0 and 2 m before gradually increasing until the bottom of the core. The manganese content remained relatively constant as well although three peaks were found at 1, 3 and 4.5 m depth. The amount of iron varied greatly with depth, with low amount between 0.6-0.8, 1.3-1.5, 2.5-3.2, 3.6-4.5 and 5-5.3 m depth. Iron content was relatively high between 0.8-1.4, 1.6-2.5, 3.2-4.1, 4.5-5 and 5.3-6.08 m depth.

Discussion

Geochemical gradients as a response to the layering of microbial communities

The peak of [O₂] between 0 and 1 mm was found in the diatom biofilm formed at the surface of the sediment (Figure 2) and indicated active oxygenic photosynthesis (Revsbech et al., 1986). Estuarine diatom biofilms produce a large amount of organic matter, mainly in the form of exopolymeric substances (EPS; Underwood & Kromkamp, 1999; Thornton et al., 2002). EPS was easily extracted down to 6 meters in the sediment cores obtained from the Gironde estuary (Duteil et al., submitted; Chapter 3; Figure 5) and could be used as carbon source for heterotrophic bacteria at depth (Braissant et al., 2009). Degradation of diatom biofilm EPS by heterotrophic bacteria extracted from estuarine sediment was shown in laboratory under both oxic and anoxic conditions (Bohórquez et al., 2017).

In order to simplify the following biogeochemical reactions, EPS or any other source of organic matter is considered as CH₂O. Under oxic conditions, EPS and other carbon sources are consumed by heterotrophic bacteria through aerobic respiration, following the simplified reaction (Stumm & Morgan, 1996; Hulth et al., 1999; Dupraz & Visscher 2005; Visscher & Stolz 2005):

 $CH_2O + O_2 = CO_2 + H_2O; \Delta G^{\circ} = -479 \text{ kJ.mol}^{-1}.$

The depletion of oxygen indicated that this metabolism was mainly active above 4 centimeters in the Gironde sediments (Figure 2). Robert et al. (2004) and Audry et al. (2006) proposed a similar interpretation for the O₂ depletion in other intertidal areas of the Gironde estuary. A depth of approximately 4 cm coincided with the highest FDA hydrolysis rates (Figure 4), indicating potential active enzymatic degradation of organic carbon (Battin, 1997). High TCC reduction rates confirmed that the upper 2 cm were associated with an elevated metabolic activity (Figure 4). Nitrate is generally consumed in the first centimeters of the sediment by heterotrophic bacteria through denitrification. Denitrification was found by several investigators in the water column and at the surface of the sediment of the Gironde estuary (between 0-5 cm depth; Robert et al., 2004; Audry et al., 2006) as well as in other estuarine sediments (e.g., between 0-10 cm depth in the Colne estuary (Dong et al., 2000) and Patuxent estuary (Jenkins & Kemp, 1984). The denitrification reaction is typically found at low oxygen or in anaerobic conditions, and can be formulated as (Stumm & Morgan, 1996; Hulth et al., 1999; Megonigal et al., 2004; Visscher & Stolz, 2005):

 $5 \text{ CH}_2\text{O} + 4 \text{ NO}_3^- + 4 \text{ H}^+ = 5 \text{ CO}_2 + 2 \text{ N}_2 + 7 \text{ H}_2\text{O}$

5 CH₂O + 4 NO₃⁻ = CO₂ + 2 N₂ + 4 HCO₃⁻ + 3 H₂O; Δ G^{o'} = -448 kJ.mol⁻¹

Jenkins & Kemp (1984) had calculated a denitrification rate of *ca.* 125 μ mol N.m⁻².h⁻¹ in estuarine mudflat sediments. Jørgensen (1989) reported that denitrification was the most active in the topmost four centimeters of soft mud intertidal estuarine sediments. Fernandes et al. (2016) found that denitrification was the main process for loss of nitrogen in intertidal sediments from the Arcachon Bay, France. Using isotopically-labelled N compounds in slurry experiments, they measured high denitrification rates (87.3 to 847 μ mol.L⁻¹.d⁻¹). Denitrification was most active under subaerobic conditions and in muddy than in sandy sediments. Consequently, denitrification could explain the absence of nitrate in the top six centimeters in the Gironde sediment (Figure 2). Denitrification under anoxic condition probably occurred between 8 and 16 cm depth, as suggested by the decrease in nitrate concentration (Figure 2). A coeval increase in pH and a high metabolic activity were documented in this depth horizon as well (Figures 2 and 4).

In BXN-B and BXN-SC, the concentration of sulfate strongly decreased below 10 cm and was close to zero at 22 cm, 26 cm and 50 cm (Figure 2). The first sulfate minimum coincided with

or

a peak in sulfides at approximately 20 cm. Similar decreasing trends in porewater sulfate concentration with depth were documented in the Chesapeake Bay (Burdige & Zheng, 1998) and in Southwestern Australian estuaries (Kraal et al., 2013). Sulfate reduction is a common process in sediments under anoxic conditions and can be simplified as follows (Stumm & Morgan, 1996; Hulth et al., 1999; Megonigal et al., 2004; Baumgartner et al., 2006):

 $2CH_2O + SO_4^{2-} + H^+ = 2CO_2 + HS^- + H_2O (\Delta Go' = -175 \text{ kJ.mol}^{-1})$

Sulfate reduction is typically considered as one of the major pathways for organic matter mineralization in coastal and estuarine environments (Thode-Andersen & Jorgensen, 1989; Luther et al., 1992). Sulfate reduction is predominantly performed by sulfate reducing bacteria (SRB), although some Fermicutes and certain archea can also play a role. In the Pearl estuary (China), SRB were present in the sediment at all depth between 0 and 50 centimeters, but their 16SRNA genes were particularly abundant at intermediate depth (6-40 centimeters; Jiang et al., 2009). In the Gironde, sulfate showed a reverse trend to the sulfide concentration and pH between 6 and 22 cm, which could be explained by sulfate reduction (Figure 2). This depth range was characterized by a progressive increase in metabolic activity with activity of TTC reduction reaching a maximum at 20-25 cm (Figure 4). A similar observation was also made by Hong et al. (2019), who measured maximum microbial activity and diversity at the oxic to anoxic transition zone in the Gloucester Beach subterranean estuary. SRB isolated from intertidal sediments from the River Forth estuary (Scotland) can degrade xanthan, which is often used as substitute for EPS (Battersby et al., 1984). The decrease in EPS concentrations observed between 0 and 50 cm in BXN-LC could be explained by (partial) consumption of EPS by sulfate reducers (Duteil et al., submitted; Chapter 3).

Two small sulfate peaks with concentrations tenfold lower than in surface sediment were documented at depth of 1.63 m and 2.80-3.25 m in BXN-LC in mixed sandy and muddy deposits (Figure 3). Small nitrate and sulfate peaks were also found in similar facies at 1.3 m

and 3.25 m (Figure 3). These relatively permeable horizons could be subject to advective porewater intrusion from e.g., the channel bank, locally reoxidizing the sediment (Beck et al., 2008). These nitrate and sulfate peaks were found at the same depth as or just below peaks of TTC reduction (1.30 m and 2.80 m; Figure 5), indicating possible active sulfate and nitrate reduction.

Porewater showed a peak in nitrate concentration at 8 cm, an oxygen-depleted zone (Figure 2). In natural conditions, nitrification generally occurs at the interface between the oxic and anoxic zones, the nitrification process being controlled by the availability of ammonium and oxygen (Dong et al., 2000). Coupled nitrification and denitrification metabolisms are common and have been documented in e.g., in the Patuxent (USA; Jenkins & Kemp, 1984), the Colne (UK; Dong et al., 2000), and the Noosa (Australia; Chen et al., 2020) estuaries. Similarly, coupled nitrification/denitrification reactions were reported in an intertidal sediment of the Arcachon Bay, but were limited to the sediment surface (<2 cm) in which oxygen still may diffuse (Fernandes et al., 2015). The nitrate peak concentration observed in the current study occurred in the anoxic part of the sediment. It coincided with a high manganese concentration and manganese reduction. Such reactions have been reported in marine and estuarine sediments (Hulth et al., 1999; Bartlett et al., 2008), and can be simplified as the following reaction (Hulth et al., 1999):

$$4MnO_2 + NH_4^+ + 6H^+ = NO_3^- + 2Mn_2^+ + H_2O; \Delta G^{\circ} = -175 \text{ kJ.mol}^{-1}$$

The peaks in manganese concentration observed at 2-6 cm, 16-18 cm and 35-50 cm (Figure 2) could also be related to manganese oxide reduction coupled to organic carbon oxidation. Such process occurs under anaerobic conditions (Burdige, 1993) with the following reaction (Stumm & Morgan, 1996; Hulth et al., 1999):

 $CH_2O + 3CO_2 + H_2O + 2MnO_2 = 2Mn^{2+} + 4HCO_3; \Delta G^{\circ} = -190 \text{ kJ.mol}^{-1}$

Manganese-reducing organisms had been found in coastal marine (Burdige & Nealson, 1985) and metal mining polluted estuarine sediments (Pereira, 2017). Microbes were able to couple manganese oxide reduction to other oxidants, for example sulfide, under anoxic conditions (Aller & Rude, 1988), a process that also occurs abiotically (Thamdrup et al., 1994). Therefore, the reduction of manganese oxides may be indirectly mediated by metabolic activity, e.g., the production of reduced compounds such as sulfide may result in a manganese oxide reduction (Burdige, 1993). In the Gironde estuary, the sulfide produced through sulfate reduction could be used for Mn reduction (Figure 2). No dissolved manganese was found below one meter deep (Figure 3), indicating that Mn cycling was mostly active above this depth.

Microbially-mediated iron oxide reduction is a common metabolism in estuarine and coastal sediments (Tugel et al., 1986; Canfield et al., 1993; Kappler et al., 2021), and can be written as follows (Stumm & Morgan, 1996; Hulth et al., 1999):

 $CH_2O + 7CO_2 + 4Fe(OH)_3 = 4Fe^{2+} + 8HCO_3 + 3H_2O; \Delta G^{\circ} = -97 \text{ kJ.mol}^{-1}$

Iron oxide reduction can also be achieved using electron donors such as sulfides (Burdige & Nealson, 1986). Dissolved iron peaks could indicate active iron reduction at 10-18 cm, 24-26 cm and 35-50 cm in BXN-B and BXN-SC (Figure 2). Some of these peaks coincide with high metabolic activities between 10 and 28 cm. Similar observations were made in BXN-LC where increases in concentration of iron can be related to high metabolic activity possibly in association with nitrate and/or sulfate reduction (e.g., at 1.3 m, 2.8 m depth; Figures 4 and 5). Methanogenesis is also a common process in estuarine sediments (O'Sullivan et al., 2013; Hong et al., 2019). In estuaries, methanogenesis can occur in the same zone as sulfate reduction and both metabolisms use similar substrates (e.g., hydrogen or acetate), but SRB have a favorable substrate utilization and outcompete methanogens (Kristjansson et al., 1982; Sela-Adler et al. 2017). Our current data does not allow us to characterize or locate potential zones of methanogenesis, but this metabolism could be dominant outside sulfate reduction zones, i.e.,

below 50 centimeters depth. Hong et al. (2019) showed an increase in methanogenic archaeal gene abundance below 70 centimeters in a subterranean estuary at Gloucester Beach (USA), supporting our thesis that methanogenesis may occur in zones of low sulfate concentration. The comparison of metabolic and enzymatic activities with porewater composition allowed to propose a vertical organization of the predominating metabolisms, as well as of microbial communities, in the cores studied in this study. Studies including molecular data and geochemical measurements also show changes in prokaryotic populations with sediment depth. Estuaries are characterized by surface salinity gradients, with salinity increasing downstream (Kraepiel et al., 1997), and the stratification of microbial communities changes with salinity (e.g., Meiggs & Taillefert, 2011). In the Pearl Estuary (China), Liu et al. (2014) found a stratification of microbial populations in the upper 40 cm of marine sediments, but no stratification in freshwater. The surface water of the Gironde at Bordeaux is typically brackish, with an average salinity of ca. 1 g.L⁻¹ (Sottolicchio et al., 2011). Our results tend to show a well-defined stratification of microbial metabolisms in this low salinity water. O'Sullivan et al. (2013) also discussed the influence of sedimentation on microbial and geochemical gradients in the Colne Estuary (UK). He postulated that geochemical profiles were re-established more quickly than microbial diversity profiles in highly reworked sediments. Estuarine point bars are subject to constant reworking by e.g., river floods. While the deepest part of BXN-LC recorded several river flood events, e.g. at 3.05 and 6.08 meters (Duteil et al., submitted; Chapter 3), the upper meter of the core was characterized by a more continuous tidal sedimentation and limited reworking. This section of the core is characterized by a potentially well-defined stratification of microbial metabolisms, but microbial diversity has yet to be measured.

Isothermal calorimetry corroborated variations in microbial activity with depth. At 6 cm, the microbial community had an average growth rate, but displayed a rapid and high heat release (Table 1). This observation is characteristic of a highly efficient organic matter degradation

(e.g., Wadso et al., 2009; Braissant et al., 2012). The decrease in protein and polysaccharide concentrations between the surface biofilm and 6 cm depth in BXN-LC, suggested that the EPS could be a major source of organic carbon for this microbial community (Duteil et al., submitted). The microbial community at 50 cm depth was the most effective to use the R2A medium substrate, but not the most metabolically active (Table 1). The bacteria present at this depth appeared well-adapted to using R2A as a substrate, that showed a significantly longer lag time, which may explain the low number of bacteria found after 30 days of cultivation (CFU $30=2.8.10^{-7}$; Table 1). These results coincide with the low metabolic activity measured in the sediment at 50 cm (Figure 4). The low EPS concentrations measured at this depth could reflect a lack of easily degradable organic carbon. This in turn could explain the development of a community poorly metabolically specialized with a low effective metabolism for R2A assimilation but a high adaptability to the type organic substrate (Braissant et al., 2012). Some enzymes (e.g., phosphatase, peptidase) can also be inhibited when oxygen is absent (Freixa et al., 2016), which could explain the low enzyme activity at 50 cm depth (Figure 4). The microbial community at 93 cm depth was the least effective in degrading the organic compounds present in the R2A medium, as shown by the low heat release (Table 1). Contrastingly, the microbial community at 93 cm depth was immediately active. The microbial community present at 93 cm depth seemed adapted to limited resources (Braissant et al., 2012), with a slow metabolism but the ability to quickly degrade the substrate when that is amended. Although sodium and chloride are conservative elements that are not involved in microbial metabolic and/or diagenetic processes, they are proxies for determining potential contamination or mixing of porewater. For example, Cl⁻ may be used as a tracer because of its conservative behavior in most diagenetic reactions, making it useful for observations of mixing between different sources of water (Martin, 1999). In BXN-B and BXN-SC the sodium and chloride concentrations increased gently with depth to a depth of 65 centimeters (Figure 2). In BXN-

LC (Figure 3), both elements peaked at 0.5 meters before decreasing down to a depth of 2.5-3 meters and increasing down to the bottom of the core. These data indicate moderate salinity variations and no major freshwater intrusion (Martin et al., 2004).

The impact of microbial activity on early diagenesis

River water is a major source of Fe and Mn oxides to the surface sediment. Such oxides can be recycled and dissolved within months in surface estuarine sediments (De Chanvalon et al., 2016). Within sediments, redox boundaries can be characterized by the (re)precipitation of manganese and iron mineral phases (Burdige, 1993). In BXN-B and BXN-SC, Fe(II) has a greater concentration than Fe(III) between 6 and 50 cm (Figure 2), which is indicative of reducing conditions in the porewater. The depletion of dissolved iron and manganese between 20 and 25 cm in BXN-B and BXN-SC could be related to an active redox interface, associated with the precipitation of metals (Figure 2). A second zone of Mn and Fe depletion at 35 cm depth could reflect a second zone of precipitation (Burdige, 1993). Similar concentration trends were observed in other estuaries (Widerlund & Ingri, 1996; Oldham et al., 2019). In BXN-SC, XRF measurements (Figure S5) revealed an enrichment in sulfur and metals (Al, Fe, Mn) in the sediment between 15 and 37 cm depth.

Manganese oxides (or oxyhydroxides) typically referred as MnO_x (x>1), and the predominant Mn oxides found in estuarine sediments are todorokite or vernadite (Burdige, 1993). Mn oxides are amorphous and are often found as coatings on sedimentary particles (e.g., sand grains; Burdige, 1993). Manganese oxidation can occur through abiotic mechanisms such as reactions with dissolved O_2 or photochemically but can also result from reactions with bacterially produced extracellular super-oxidants (Learman et al., 2011). Oldham et al. (2019) found that most of the dissolved Mn pool in estuarine porewater was in the form of Mn(III)-organic ligands complexes. At circumneutral pH, Manganese oxides reduction does not lead to sulfide

precipitation, in contrast to reduction of Fe which does precipitate as iron sulfide complexes (Luther et al., 2018). Dissolved Mn tends to diffuse upward and reoxidize, leading to the precipitation of new oxides or carbonates, or can be released to estuarine water column (Burdige, 1993; Audry et al., 2006; De Chanvalon et al., 2016). Abiotic reduction of manganese oxides may also explain the variation of the Mn observed in the uppermost meter of the core (Figures 2 and 3). The manganese and iron cycles are tightly coupled and Fe(II) can be abiotically oxidized by manganese oxides through the reaction: $MnO_2 + 2Fe^{2+} + 2H_2O = Mn^{2+} + 2FeOOH + 2 H^+$ (Kappler et al, 2021). However, the correlation between dissolved iron and manganese in BXN-B and BXN-LC (r=0.72; Figure 6) and in BXN-LC (r= 0.68; Figure 7) indicates that this phenomenon is probably not prevalent in the sediments studied here. Below 50-60 cm, dissolved Mn concentrations remained close to zero, indicating a potential stability of Mn-bearing mineral phases. This was confirmed by the distinctive Mn peaks measured by XRF in BXN-LC at ca. 1 m, 2.9 m or 4.6 m (Figure S5).

In contrast to the depth profile of Mn, dissolved Fe(III) concentrations showed an increase with depth. The reduction of Fe^{2+} and Fe^{3+} ions in solution leads to the precipitation of iron hydroxides, oxyhydroxides or oxides (FeOOH) according to environmental conditions, e.g, pH and Eh (Cudennec & Lecerf, 2006). Iron oxides found in estuarine or marine sediment are generally metastable and poorly crystallized (Burdige, 1993). Ferrihydrite (Fe₂O₃.9H₂O or Fe(OH)₃) in sediments is a metastable phase that can transform to goethite (FeO(OH)) or hematite (Fe₂O₃). Hematite is favored at neutral conditions (Cudennec & Lecerf, 2006) and experimentally, in a ferrihydrite solution at ambient temperature (24°C), the maximum of hematite precipitation occurred between pH 7 and 8 (Schwertmann & Murad, 1983). In spite of microbial activity, pH of porewater in estuarine environments generally remains stable with a value of 7 to 8 (El Ghobary, 1983; Stumm & Morgan, 1996; Kraal et al., 2013; Fiket et al., 2019; Figure 2). Calculation of saturation indices using PhreeQc indicated that BXN-B and

BXN-SC porewaters were largely undersaturated with respect to ferrihydrite, slightly undersaturated or close to equilibrium with goethite, and locally supersaturated for hematite, especially between depths of 0 to 18 cm (Table 2). In BXN-LC, porewaters were supersaturated for iron oxides, slightly supersaturated for ferrihydrite, and supersaturated for hematite (SI up to 20.62; Table 3). These results were in agreement with our observations indicating preferential hematite precipitation at circumneutral pH. However, even if iron oxides should precipitate in the Gironde sediments, their actual form remains poorly known. Well-crystallized iron oxides were not found using XRD. Using transmission electron microscopy, Virolle (2019) observed Fe-rich nanoparticles associated with detrital clay coats using. It should be noted that some of the porewater samples from BXN-LC displayed an orange color, which could indicate the presence of a colloidal Fe(III) phase or of dissolved organic-Fe(III) complexes. Iron-reducing bacteria, such as Shewanella sp., are able to destabilize Fe(III) oxy(hydroxides), possibly in order to make Fe(III) bioavailable (Beckler et al., 2015). Fe(III) forms complexes with organic ligands, among which bacterial siderophores and larger organic molecules such as EPS (Norman et al., 2015; Kappler et al., 2021). Sedimentary EPS have a strong affinity for some metals, including Ca, Mg, Fe (e.g., Braissant et al., 2007; Norman et al., 2015; Pace et al., 2018). Bacterial and algal EPS complexation with Fe enhances iron solubility (Norman et al., 2015). Chan et al. (2011) documented the production of Fe-rich extracellular organic stalks by Feoxidizing bacteria. The stalks were made of carboxyl-rich polysaccharides, which could play a role in preventing cell encrustation by Fe minerals. Gironde sedimentary EPS are rich in acidic sites, predominantly carboxyl groups, and as such have a strong potential to bind metals (Duteil et al., submitted). The reduction of sulfates by SRB leads to an increase of sulfides, which can potentially interact with iron or manganese oxides according to the following reactions (Canfield et al., 1993):

 $H_2S + 4H^+ + 2FeOOH = S^0 + 2Fe^{2+} + 4H_2O$

and

$$H_2S + 2H^+ + MnO_2 = Mn^{2+} + S^0 + 2H_20.$$

Sulfide may also interact with dissolved Fe(II) to produce metastable iron sulfide phases such as mackinawite:

 $Fe^{2+} + HS^{-} = FeS + H^{+}$ (Jorgensen & Nelson, 2004).

Or stable phases such as pyrite:

 $FeS + H_2S = FeS_2 + H_2$ (Kraal et al. 2013)

Both types of iron sulfides were found in eutrophic and organic-rich salt marsh and estuarine sediments (Sorensen & Jorgensen, 1987; Luther et al., 1992; Kraal et al., 2013). In this study, we did not identify poorly crystalline iron sulfides (e.g., mackinawite, greigite), but the the presence of solid iron and sulfur documented with XRF between 15 and 37 cm (Figure S5) could indicate the precipitation of such iron sulfides. This observation was confirmed with PhreeQc modeling, which demonstrated that porewater was undersaturated with respect to mackinawite above 10 cm depth, and supersaturated deeper into the sediment column (Table 2). A shift in SI was also observed at 10 cm for pyrite, which was slightly supersaturated above 10 cm depth and largely supersaturated down to at least 65 cm depth. No major pyrite precipitation was observed in this zone despite this supersaturation. Pyrite accounts for less than 0.5% of the sediment volume in tidal sand bars from the Gironde estuary, and for less than 0.7%in the Ravenglass Estuary (UK; Griffiths et al., 2018). In tidal sands, pyrite is mostly found as framboids within detrital clay coats (Virolle et al., 2020). The precipitation rate of pyrite in natural sediments is usually much lower than the rate predicted by kinetic equations, and the dominant phases are metastable iron sulfides (Kraal et al., 2013). Fe(II) precipitation can be inhibited by dissolved organic matter (DOM), which forms complexes with Fe(II) (Kraal et al. 2013; Beckler et al., 2015). In the iron-rich Satilla river (USA), Beckler et al. (2015) proposed a scenario where Fe(III) is reduced to Fe(II) by iron-reducing bacteria. Fe (II) is chelated by DOM and these Fe(II)-DOM complexes are subsequently oxidized by Fe(III) (oxy)hydroxides, to generate soluble DOM-Fe(III) complexes in the porewater. These complexes could be in turn be reduced by iron-reducing bacteria, making Fe(III) bioavailable. Beckler et al. (2015) postulate that this reduction could result from a ligand exchange reaction, where the ligands (including EPS) are generated by iron oxidizing bacteria. An excess in organic ligands could explain the maintenance of iron(III) in soluble state (Oldham et al., 2019). Our study found significant concentrations of preserved EPS in the same core, including some horizons with concentrations similar to the surface biofilm (e.g., 4.33 m; Figure 5; Duteil et al., submitted). These EPS could form a dissolved complex with Fe(III), explaining the high Fe(III) concentrations measured in BXN-LC (Figure 3).

A possible conceptual geomicrobiological model

We propose a scenario describing the potential interactions between microbial metabolisms, exopolymeric substances and the early diagenetic fate of metals in the sediments of the Gironde Estuary (Figure 8). In this figure, the size of the diatom biofilm has been deliberately exaggerated to visualize its impact on the environment at the surface of the sediment. The assumed evolution of the concentration of the major dissolved elements in the water above the surface of the sediments is drawn in dotted line. Microbial metabolic reactions are indicated by as numbers and diagenetic processes as letters. Diatoms perform oxygenic photosynthesis in the surface biofilm (*reaction 1*), during which they excrete large amounts of EPS. These EPS can form complexes with clay and sand particles, and are thus partially integrated within the sediment (Duteil et al., 2020; Duteil et al., submitted). EPS constitute one of the main sources of organic carbon for heterotrophs deeper in the sediment column (e.g., Braissant et al., 2007; Decho & Guttierez, 2017). Oxygen is consumed through aerobic respiration (*reaction 2*), which is likely the dominant microbial metabolism in the uppermost centimeters of the sediment.

Aerobic respiration seems highly efficient in mineralization organic matter, considering the decrease of the amount of EPS observed, for example between the sediment surface and a depth of 6 cm (Figure 5). In this zone, the low concentrations of dissolved Mn and Fe indicate that these metals could be present as Fe(III) and Mn(IV) (oxy)hydroxides. Once most of the oxygen is consumed, nitrate become the main electron donor if available, and nitrate reduction (reaction 5) becomes the main pathway for organic matter mineralization. Calorimetry showed that the microbial community at 6 cm was highly reactive and presumably degraded organic carbon. The correlation of nitrate and dissolved manganese peaks at 10 cm depth could indicate nitrification (reaction 3), where nitrate production may result in manganese oxide dissolution (Hulth et al., 1999; Bartlett et al., 2008). Sulfate reduction (reaction 4) becomes the predominant metabolism below 10 cm, resulting in consumption of organic carbon consumption (including EPS, but mainly of LMWOC - mainly EPS degradation products) and in the production of dissolved sulfides to at least 60 cm depth. Some metal (e.g., Mn, Fe, Al; Figure S5) and sulfur in the porewater showed a relative increase in concentration indicating the potential for metal precipitation. Centimeter-thick horizons of maximum high rates of sulfate reduction (e.g., 25 cm, 35 cm) coincide with a decrease in dissolved Fe and Mn, that could indicate precipitation of these metals. The consumption of organic carbon by (an)aerobic heterotrophs could liberate the metals from metal-ligand complexes, leading to their precipitation (e.g., Dupraz et al., 2004; Braissant et al., 2007). The reduction of iron that could result from this would provide energy for iron-reducing bacteria, and the locally existing reducing conditions could then allow for the precipitation of Fe(II) as iron sulfides (e.g., mackinawite, *reaction B*, rarely pyrite) and the reduction of manganese oxides (*reaction C*). Nitrate and sulfate reduction could continue at a slower rate deeper in the sediment (at 1.5 and 3 m deep), each time resulting in local enrichment of metals in the sediment, supported by reactions such as Mn and Fe reduction (reactions 6 and 7). The increase in sulfate concentration at depth in the absence of oxygen may be due to dissolution of the metastable iron sulfide phase (*reaction D*) followed by sulfide oxidation. No dissolved Mn was measured below a depth of 1 meter, and dissolved Fe(III) progressively increased with depth down to the bottom of the core. Iron oxides seem limited in concentration despite porewater supersaturation around six meters depth. At this depth, iron could possibly be maintained in solution through the dissolution of iron sulfides and the complexation with organic ligands (Oldham et al., 2019) as long as it is not consumed through Fe(III) reduction by bacteria. EPS can be preserved in significant concentrations in some sedimentary horizons (e.g., 4.33 m; Figure 4; Duteil et al., submitted). These EPS contain mainly negatively charged groups, with the potential to bind metals including iron or calcium which subsequently could enhance Fe solubility. Overall, the biogeochemical cycles of C, S, N, Fe or Mn in the Bordeaux Nord point bar seemed driven by a combination of metabolic reactions.



Figure 8: Schematic conceptual model of the potential relationships between geochemical gradients, microbial activity, exopolymeric substances, and their impact on early diagenesis in estuarine sediments. (A; top panel) To the left, sedimentary description of BXN-B and BXN-SC. The size of the diatom biofilm at the surface is deliberately exaggerated. The profiles show the evolution of porewater composition (ions, oxygen and pH), microbial and enzymatic activities and EPS concentration. To the right, the potential depth range of metabolic reactions, as well as of chemical/mineral precipitation, are indicated by color bars. (B; bottom panel) To the left, description of the main geomicrobiological zones in the sediment related to the main metabolisms. To the right, geomicrobiological model showing potential interactions between microbial metabolisms and mineral precipitation/dissolution. Metabolic reactions are referenced by numbers, while precipitation reactions are referenced by letters. The color is the same for one compound in the two panels e.g., purple for nitrates.

Conclusion

We characterized porewater and sediment chemistry, microbial and enzyme activities, and properties of exopolymeric substances as a function of depth in three cores sampled in a point bar of the Gironde estuary. The concentration of major ions in porewater (Mn²⁺, Mg²⁺, Ca²⁺, Fe²⁺, Fe³⁺, HS⁻, SO₄²⁻, NO₃⁻, Cl⁻, Na⁺) showed significant fluctuations that could be related to biogeochemical reactions organized as a function of depth. This sequence partly followed the classical suite of reactions following the decreasing potential free energy yield of successive electron acceptors (i.e., oxygen, nitrate, Mn and Fe oxides, sulfate), but our results showed that some reactions were likely coupled or at least concomitant. For example, coexistent of denitrification and sulfate reduction around 10 cm depth. Measurements of metabolic and enzymatic activities sometimes confirmed or in other cases allowed to hypothesize the potential role of microorganisms in the various reactions. Most metabolic reactions were active in the uppermost meter of the sediment: (i) oxygenic photosynthesis was performed in the diatom biofilm at the surface of the sediment; (ii) aerobic respiration was predominant down to approximately 5 cm; (iii) a first reduction of Mn oxides was possibly coupled with nitrification (or sulfate reduction) between 5 and 10 cm, as indicated by the concomitant increase in dissolved Mn and nitrates; (iv) nitrates were subsequently reduced between 10 and 15 cm; (v) sulfate reduction was active at several depth intervals, but the main sulfate reduction zone occurred between 8 and 20 cm; (vi) the reduction and dissolution of iron and manganese oxides occurred in the zone of sulfate reduction, and was particularly active at 20 and 30 cm.

While metabolic activity decreased between the surface biofilm and the zone of aerobic respiration, it increased markedly in the zone of anaerobic respiration, between 10 and 30 cm. In contrast, the enzymatic activity peaked in the zone of aerobic respiration and was relatively constant and low in the anoxic part of the sediment column, revealing the typically observed sequence of different strategies for energy acquisition and organic carbon degradation among

heterotrophs. Exopolymeric substances showed a strong decrease between the surface biofilm and 50 cm, which could result from increased heterotrophic degradation. Some deeper horizons showed EPS in concentrations and with properties similar to those observed in the surface biofilm. These EPS were potentially preserved following rapid sedimentation events (e.g., burial by river flood deposits).

We documented enrichments for some metals (e.g., Fe, Mn, Ca) and sulfur in the zones of sulfate reduction. EPS could have played a significant role in diagenesis by complexing with metals throughout the sediment column. The increase in dissolved Fe(III) observed from the top to the bottom of the six-meter-long core could be explained by the formation of Fe(III)-ligand complexes (including with EPS and smaller, low-molecular weight organic molecules), maintaining iron in solution. The degradation of metal-EPS complexes by heterotrophs could have supported the precipitation of metals, for example iron sulfides, in zones of active sulfate reduction. Several deeper sedimentary horizons showed potential sulfate and nitrate reduction coupled to Fe depletion (e.g., at approximately 1.5 m, 3 m), which were associated with metal enrichments in the sediment. These results allowed us to propose a geomicrobiological conceptual model, which was more complex than the widely used classical stratification model of microbial metabolisms.

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References

- Aller, R.C., and Rude, P.D., 1988, Complete oxidation of solid phase sulfides by manganese and bacteria in anoxic marine sediments: Geochimica et Cosmochimica Acta, v. 52, p. 751–765, doi:10.1016/0016-7037(88)90335-3.
- Audry, S., Blanc, G., and Schäfer, J., 2005, The impact of sulphide oxidation on dissolved metal (Cd, Zn, Cu, Cr, Co, Ni, U) inputs into the Lot–Garonne fluvial system (France): Applied geochemistry, v. 20, p. 919–931.
- Audry, S., Blanc, G., Schäfer, J., Chaillou, G., and Robert, S., 2006, Early diagenesis of trace metals (Cd, Cu, Co, Ni, U, Mo, and V) in the freshwater reaches of a macrotidal estuary: Geochimica et Cosmochimica Acta, v. 70, p. 2264–2282.
- Bartlett, R., Mortimer, R.J.G., and Morris, K., 2008, Anoxic nitrification: Evidence from Humber
 Estuary sediments (UK): Chemical Geology, v. 250, p. 29–39, doi: 10.1016/j.chemgeo.2008.02.001.
- Battersby, N., Stewart, D., and Sharma, A., 1984, Effect of xanthan on the growth of sulphate-reduction bacteria in marine sediments., in 2. Colloque International de Bacteriologie Marine, Brest (France), 1-5.
- Battin, T.J., 1997, Assessment of fluorescein diacetate hydrolysis as a measure of total esterase activity in natural stream sediment biofilms: Science of the Total Environment, v. 198, p. 51–60.
- Baumgartner, L.K., Reid, R.P., Dupraz, C., Decho, A.W., Buckley, D.H., Spear, J.R., Przekop, K.M., and Visscher, P.T., 2006, Sulfate reducing bacteria in microbial mats: changing paradigms, new discoveries: Sedimentary Geology, v. 185, p. 131–145.
- Beck, M., Reckhardt, A., Amelsberg, J., Bartholomä, A., Brumsack, H.-J., Cypionka, H., Dittmar, T.,
 Engelen, B., Greskowiak, J., and Hillebrand, H., 2017, The drivers of biogeochemistry in beach
 ecosystems: a cross-shore transect from the dunes to the low-water line: Marine Chemistry, v.
 190, p. 35–50.
- Beckler, J.S., Jones, M.E., and Taillefert, M., 2015, The origin, composition, and reactivity of dissolved iron (III) complexes in coastal organic-and iron-rich sediments: Geochimica et Cosmochimica Acta, v. 152, p. 72–88.
- Bohórquez, J., McGenity, T.J., Papaspyrou, S., García-Robledo, E., Corzo, A., and Underwood, G.J.C.,
 2017, Different Types of Diatom-Derived Extracellular Polymeric Substances Drive Changes in Heterotrophic Bacterial Communities from Intertidal Sediments: Frontiers in Microbiology,
 v. 8, p. 245, doi:10.3389/fmicb.2017.00245.
- Braissant, O., Astasov-Frauenhoffer, M., Waltimo, T., and Bonkat, G., 2020, A review of methods to determine viability, vitality, and metabolic rates in microbiology: Frontiers in Microbiology, p. 2726.

Braissant, O., Bindschedler, S., Daniels, A.U., Verrecchia, E.P., and Cailleau, G., 2012, Microbiological

activities in moonmilk monitored using isothermal microcalorimetry (cave of Vers chez le Brandt, Neuchatel, Switzerland): Journal of Cave and Karst Studies, v. 74, p. 116–126.

- Braissant, O., Bonkat, G., Wirz, D., and Bachmann, A., 2013, Microbial growth and isothermal microcalorimetry: growth models and their application to microcalorimetric data: Thermochimica Acta, v. 555, p. 64–71.
- Braissant, O., Decho, A.W., Dupraz, C., Glunk, C., Przekop, K.M., and Visscher, P.T., 2007,
 Exopolymeric substances of sulfate-reducing bacteria: Interactions with calcium at alkaline pH and implication for formation of carbonate minerals: Geobiology, v. 5, p. 401–411, doi:10.1111/j.1472-4669.2007.00117.x.
- Braissant, O., Decho, A.W., Przekop, K.M., Gallagher, K.L., Glunk, C., Dupraz, C., and Visscher, P.T., 2009, Characteristics and turnover of exopolymeric substances in a hypersaline microbial mat: FEMS Microbiology Ecology, v. 67, p. 293–307, doi:10.1111/j.1574-6941.2008.00614.x.
- Burdige, D.J., 2011, Temperature dependence of organic matter remineralization in deeply-buried marine sediments: Earth and Planetary Science Letters, v. 311, p. 396–410.
- Burdige, D.J., 1993, The biogeochemistry of manganese and iron reduction in marine sediments: Earth Science Reviews, v. 35, p. 249–284.
- Burdige, D.J., and Gieskes, J.M., 1983, A pore water/solid phase diagenetic model for manganese in marine sediments: American Journal of Science, v. 283.
- Burdige, D.J., and Nealson, K.H., 1985, Microbial Manganese Reduction by Enrichment Cultures from Coastal Marine Sediments: Applied and Environmental Microbiology, v. 50, p. 491–497, doi:10.1128/aem.50.2.491-497.1985.
- Burdige, D.J., and Zheng, S., 1998, The biogeochemical cycling of dissolved organic nitrogen in estuarine sediments: Limnology and Oceanography, v. 43, p. 1796–1813.
- Canfield, D.E., Thamdrup, B., and Hansen, J.W., 1993, The anaerobic degradation of organic matter in Danish coastal sediments: iron reduction, manganese reduction, and sulfate reduction: Geochimica et Cosmochimica Acta, v. 57, p. 3867–3883.
- Chan, C.S., Fakra, S.C., Emerson, D., Fleming, E.J., and Edwards, K.J., 2011, Lithotrophic iron oxidizing bacteria produce organic stalks to control mineral growth: implications for biosignature formation: The ISME journal, v. 5, p. 717–727.
- de Chanvalon, A.T., Mouret, A., Knoery, J., Geslin, E., Peron, O., and Metzger, E., 2016, Manganese, iron and phosphorus cycling in an estuarine mudflat, Loire, France: Journal of Sea Research, v. 118, p. 92–102.
- Chatterjee, M., Silva Filho, E.V., Sarkar, S.K., Sella, S.M., Bhattacharya, A., Satpathy, K.K., Prasad, M.V.R., Chakraborty, S., and Bhattacharya, B.D., 2007, Distribution and possible source of trace elements in the sediment cores of a tropical macrotidal estuary and their ecotoxicological significance: Environment International, v. 33, p. 346–356.

Chen, J.-J., Erler, D.V., Wells, N.S., Huang, J., Welsh, D.T., and Eyre, B.D., 2020, Denitrification,

Chapter 4

anammox, and dissimilatory nitrate reduction to ammonium across a mosaic of estuarine benthic habitats, p. 17.

- Chiffoleau, J.-F., Cossa, D., Auger, D., and Truquet, I., 1994, Trace metal distribution, partition and fluxes in the Seine estuary (France) in low discharge regime: Marine Chemistry, v. 47, p. 145–158.
- Cudennec, Y., and Lecerf, A., 2006, The transformation of ferrihydrite into goethite or hematite, revisited: Journal of solid state chemistry, v. 179, p. 716–722.
- De Winder, B., Staats, N., Stal, L.J., and Paterson, D.M., 1999, Carbohydrate secretion by phototrophic communities in tidal sediments: Journal of Sea Research, v. 42, p. 131–146.
- Decho, A.W., 2000, Microbial biofilms in intertidal systems: an overview: Continental shelf research, v. 20, p. 1257–1273.
- Decho, A.W., and Gutierrez, T., 2017, Microbial extracellular polymeric substances (EPSs) in ocean systems: Frontiers in microbiology, v. 8, p. 922.
- Dong, L.F., Thornton, D.C.O., Nedwell, D.B., and Underwood, G.J.C., 2000, Denitrification in sediments of the River Colne estuary, England: Marine Ecology Progress Series, v. 203, p. 109– 122, doi:10.3354/meps203109.
- Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P. t, and Smith, F., 1956, Colorimetric method for determination of sugars and related substances: Analytical chemistry, v. 28, p. 350–356.
- Dupraz, C., Reid, R.P., Braissant, O., Decho, A.W., Norman, R.S., and Visscher, P.T., 2009, Processes of carbonate precipitation in modern microbial mats: Earth-Science Reviews, v. 96, p. 141–162.
- Dupraz, C., and Visscher, P.T., 2005, Microbial lithification in marine stromatolites and hypersaline mats: Trends in Microbiology, v. 13, p. 429–438, doi: 10.1016/j.tim.2005.07.008.
- Dupraz, C., Visscher, P.T., Baumgartner, L.K., and Reid, R.P., 2004, Microbe–mineral interactions: early carbonate precipitation in a hypersaline lake (Eleuthera Island, Bahamas): Sedimentology, v. 51, p. 745–765.
- El Ghobary, H., 1983, Interstitial water chemistry of the inner continental shelf sediments off the Gironde Estuary: Estuarine, Coastal and Shelf Science, v. 16, p. 639–650.
- Fernandes, S.O., Javanaud, C., Michotey, V.D., Guasco, S., Anschutz, P., and Bonin, P., 2016, Coupling of bacterial nitrification with denitrification and anammox supports N removal in intertidal sediments (Arcachon Bay, France): Estuarine, Coastal and Shelf Science, v. 179, p. 39–50, doi: 10.1016/j.ecss.2015.10.009.
- Fiket, Ž., Fiket, T., Ivanić, M., Mikac, N., and Kniewald, G., 2019, Pore water geochemistry and diagenesis of estuary sediments—an example of the Zrmanja River estuary (Adriatic coast, Croatia): Journal of Soils and Sediments, v. 19, p. 2048–2060.
- Freixa, A., Rubol, S., Carles-Brangarí, A., Fernàndez-Garcia, D., Butturini, A., Sanchez-Vila, X., and

Romaní, A.M., 2016, The effects of sediment depth and oxygen concentration on the use of organic matter: An experimental study using an infiltration sediment tank: Science of the total environment, v. 540, p. 20–31.

- Gil, M.M., Brandão, T.R.S., and Silva, C.L.M., 2006, A modified Gompertz model to predict microbial inactivation under time-varying temperature conditions: Journal of Food Engineering, v. 76, p. 89–94, doi: 10.1016/j.jfoodeng.2005.05.017.
- Glud, R.N., Ramsing, N.B., and Revsbech, N.P., 1992, Photosynthesis and photosynthesis-coupled respiration in natural niofilms quantified with oxygen microsensors: Journal of Phycology, v. 28, p. 51–60.
- Green, V.S., Stott, D.E., and Diack, M., 2006, Assay for fluorescein diacetate hydrolytic activity: optimization for soil samples: Soil Biology and Biochemistry, v. 38, p. 693–701.
- Griffiths, J., Worden, R.H., Wooldridge, L.J., Utley, J.E., and Duller, R.A., 2018, Detrital clay coats, clay minerals, and pyrite: a modern shallow-core analogue for ancient and deeply buried estuarine sandstones: Journal of Sedimentary Research, v. 88, p. 1205–1237.
- Hensen, C., Zabel, M., and Schulz, H.N., 2006, Benthic cycling of oxygen, nitrogen and phosphorus, in Marine Geochemistry, Springer, p. 207–240.
- Hong, Y., Wu, J., Wilson, S., and Song, B., 2019, Vertical stratification of sediment microbial communities along geochemical gradients of a subterranean estuary located at the Gloucester Beach of Virginia, United States: Frontiers in microbiology, v. 9, p. 3343.
- Hulth, S., Aller, R.C., and Gilbert, F., 1999, Coupled anoxic nitrification/manganese reduction in marine sediments: Geochimica et Cosmochimica Acta, v. 63, p. 49–66, doi:10.1016/S0016-7037(98)00285-3.
- Jenkins, M.C., and Kemp, W.M., 1984, The coupling of nitrification and denitrification in two estuarine sediments1,2: Limnology and Oceanography, v. 29, p. 609–619, doi:10.4319/lo.1984.29.3.0609.
- Jiang, L., Zheng, Y., Peng, X., Zhou, H., Zhang, C., Xiao, X., and Wang, F., 2009, Vertical distribution and diversity of sulfate-reducing prokaryotes in the Pearl River estuarine sediments, Southern China: FEMS microbiology ecology, v. 70, p. 249–262.
- Jørgensen, B.B., and Nelson, D.C., 2004, Sulfide oxidation in marine sediments: geochemistry meets Microbiology.
- Kappler, A., Bryce, C., Mansor, M., Lueder, U., Byrne, J.M., and Swanner, E.D., 2021, An evolving view on biogeochemical cycling of iron: Nature Reviews Microbiology, v. 19, p. 360–374.
- Klinkhammer, G.P., 1980, Early diagenesis in sediments from the eastern equatorial Pacific, II. Pore water metal results: Earth and Planetary Science Letters, v. 49, p. 81–101.
- Konhauser, K.O., 2009, Introduction to geomicrobiology: John Wiley & Sons.
- Kraal, P., Burton, E.D., and Bush, R.T., 2013, Iron monosulfide accumulation and pyrite formation in eutrophic estuarine sediments: Geochimica et Cosmochimica Acta, v. 122, p. 75–88.

- Kraepiel, A.M., Chiffoleau, J.-F., Martin, J.-M., and Morel, F.M., 1997, Geochemistry of trace metals in the Gironde estuary: Geochimica et Cosmochimica Acta, v. 61, p. 1421–1436.
- Kristjansson, J.K., Schönheit, P., and Thauer, R.K., 1982, Different Ks values for hydrogen of methanogenic bacteria and sulfate reducing bacteria: an explanation for the apparent inhibition of methanogenesis by sulfate: Archives of microbiology, v. 131, p. 278–282.
- Learman, D.R., Voelker, B.M., Vazquez-Rodriguez, A.I., and Hansel, C.M., 2011, Formation of manganese oxides by bacterially generated superoxide: Nature Geoscience, v. 4, p. 95–98.
- Liu, J., Yang, H., Zhao, M., and Zhang, X.-H., 2014, Spatial distribution patterns of benthic microbial communities along the Pearl Estuary, China: Systematic and Applied Microbiology, v. 37, p. 578–589, doi: 10.1016/j.syapm.2014.10.005.
- Lowry, O., Rosebrough, N., Farr, A., and Randall, R., 1951, Protein measurement with the Folin phenol reagent: J biol Chem, v. 193, p. 265–275.
- Luther, G.W., Kostka, J.E., Church, T.M., Sulzberger, B., and Stumm, W., 1992, Seasonal iron cycling in the salt-marsh sedimentary environment: the importance of ligand complexes with Fe(II) and Fe(III) in the dissolution of Fe(III) minerals and pyrite, respectively: Marine Chemistry, v. 40, p. 81–103, doi:10.1016/0304-4203(92)90049-G.
- Luther, G.W., Thibault de Chanvalon, A., Oldham, V.E., Estes, E.R., Tebo, B.M., and Madison, A.S., 2018, Reduction of manganese oxides: thermodynamic, kinetic and mechanistic considerations for one-versus two-electron transfer steps: Aquatic Geochemistry, v. 24, p. 257–277.
- Marin-Carbonne, J., Rollion-Bard, C., Bekker, A., Rouxel, O., Agangi, A., Cavalazzi, B., Wohlgemuth Ueberwasser, C.C., Hofmann, A., and McKeegan, K.D., 2014, Coupled Fe and S isotope variations in pyrite nodules from Archean shale: Earth and Planetary Science Letters, v. 392, p. 67–79.
- Martin, W.R., 2018, Chemical processes in estuarine sediments: Elements of Physical Oceanography: A derivative of the Encyclopedia of Ocean Sciences, p. 445.
- Martin, J.B., 1999, Nonconservative behavior of Br-/Cl- ratios during alteration of volcaniclastic sediments: Geochimica et Cosmochimica Acta, v. 63, p. 383–391.
- Martin, J.B., Cable, J.E., Swarzenski, P.W., and Lindenberg, M.K., 2004, Enhanced Submarine Ground Water Discharge from Mixing of Pore Water and Estuarine Water: Ground Water, v. 42, p. 1000–1010, doi:10.1111/j.1745-6584.2004.tb02639.x.
- McAllister, S.M., Barnett, J.M., Heiss, J.W., Findlay, A.J., MacDonald, D.J., Dow, C.L., Luther III, G.W., Michael, H.A., and Chan, C.S., 2015, Dynamic hydrologic and biogeochemical processes drive microbially enhanced iron and sulfur cycling within the intertidal mixing zone of a beach aquifer: Limnology and Oceanography, v. 60, p. 329–345.
- Megonigal, J.P., Hines, M.E., and Visscher, P.T., 2004, Anaerobic metabolism: linkages to trace gases: Biogeochemistry.
- Meiggs, D., and Taillefert, M., 2011, The effect of riverine discharge on biogeochemical processes in

estuarine sediments: Limnology and Oceanography, v. 56, p. 1797-1810.

- Migeon, S., Weber, O., Faugeres, J.-C., and Saint-Paul, J., 1998, SCOPIX: a new X-ray imaging system for core analysis: Geo-Marine Letters, v. 18, p. 251–255.
- Mortimer, R.J., Harris, S.J., Krom, M.D., Freitag, T.E., Prosser, J.I., Barnes, J., Anschutz, P., Hayes, P.J., and Davies, I.M., 2004, Anoxic nitrification in marine sediments: Marine Ecology Progress Series, v. 276, p. 37–52.
- Norman, L., Worms, I.A., Angles, E., Bowie, A.R., Nichols, C.M., Pham, A.N., Slaveykova, V.I., Townsend, A.T., Waite, T.D., and Hassler, C.S., 2015, The role of bacterial and algal e xopolymeric substances in iron chemistry: Marine Chemistry, v. 173, p. 148–161.
- Oldham, V.E., Siebecker, M.G., Jones, M.R., Mucci, A., Tebo, B.M., and Luther, G.W., 2019, The Speciation and Mobility of Mn and Fe in Estuarine Sediments: Aquatic Geochemistry, v. 25, p. 3–26, doi:10.1007/s10498-019-09351-0.
- O'Sullivan, L.A., Sass, A.M., Webster, G., Fry, J.C., Parkes, R.J., and Weightman, A.J., 2013, Contrasting relationships between biogeochemistry and prokaryotic diversity depth profiles along an estuarine sediment gradient: FEMS Microbiology Ecology, v. 85, p. 143–157, doi:10.1111/1574-6941.12106.
- Pace, A., Bourillot, R., Bouton, A., Vennin, E., Braissant, O., Dupraz, C., Duteil, T., Bundeleva, I., Patrier, P., and Galaup, S., 2018, Formation of stromatolite lamina at the interface of oxygenic– anoxygenic photosynthesis: Geobiology, v. 16, p. 378–398.
- Passow, U., and Alldredge, A.L., 1995, A dye-binding assay for the spectrophotometric measurement of transparent exopolymer particles (TEP): Limnology and Oceanography, v. 40, p. 1326–1335.
- Pereira, F., 2017, Manganese-Tolerant Bacteria from the Estuarine Environment and Their Importance in Bioremediation of Contaminated Estuarine Sites, in Naik, M.M. and Dubey, S.K. eds., Marine Pollution and Microbial Remediation, Singapore, Springer, p. 153–175, doi:10.1007/978-981-10-1044-6_10.
- Pitzer, K.S., 1979, Theory, ion interaction approach: Activity coefficients in electrolyte solutions, v. 1, p. 157–208.
- Relexans, J.C., 1996, Measurement of the respiratory electron transport system (ETS) activity in marine sediments: state-of-the-art and interpretation. I. Methodology and review of literature data: Marine Ecology Progress Series, v. 136, p. 277–287.
- Revsbech, N.P., Madsen, B., and Jørgensen, B.B., 1986, Oxygen production and consumption in sediments determined at high spatial resolution by computer simulation of oxygen microelectrode data: Limnol. Oceanogr, v. 31, p. 293–304.
- Robert, S., Blanc, G., Schäfer, J., Lavaux, G., and Abril, G., 2004, Metal mobilization in the Gironde Estuary (France): the role of the soft mud layer in the maximum turbidity zone: Marine Chemistry, v. 87, p. 1–13, doi:10.1016/S0304-4203(03)00088-4.
- Sagemann, J., Skowronek, F., Dahmke, A., and Schulz, H.D., 1996, Pore-water response on seasonal

environmental changes in intertidal sediments of the Weser Estuary, Germany: Environmental Geology, v. 27, p. 362–369.

- Schäfer, J., Coynel, A., and Blanc, G., 2022, Impact of metallurgy tailings in a major European fluvial estuarine system: Trajectories and resilience over seven decades: Science of The Total Environment, v. 805, p. 150195.
- Schwertmann, U., and Murad, E., 1983, Effect of pH on the formation of goethite and hematite from ferrihydrite: Clays and Clay Minerals, v. 31, p. 277–284.
- Sela-Adler, M., Ronen, Z., Herut, B., Antler, G., Vigderovich, H., Eckert, W., and Sivan, O., 2017, Co existence of methanogenesis and sulfate reduction with common substrates in sulfate-rich estuarine sediments: Frontiers in microbiology, v. 8, p. 766.
- Sørensen, J., and Jørgensen, B.B., 1987, Early diagenesis in sediments from Danish coastal waters: microbial activity and Mn-Fe-S geochemistry: Geochimica et Cosmochimica Acta, v. 51, p. 1583–1590.
- Sottolichio, A., Hurther, D., Gratiot, N., and Bretel, P., 2011, Acoustic turbulence measurements of near-bed suspended sediment dynamics in highly turbid waters of a macrotidal estuary: Continental Shelf Research, v. 31, p. S36–S49.
- de Souza Machado, A.A., Spencer, K., Kloas, W., Toffolon, M., and Zarfl, C., 2016, Metal fate and effects in estuaries: a review and conceptual model for better understanding of toxicity: Science of the Total Environment, v. 541, p. 268–281.
- Stecko, J.P., and Bendell-Young, L.I., 2000, Contrasting the geochemistry of suspended particulate matter and deposited sediments within an estuary: Applied Geochemistry, v. 15, p. 753–775.
- Stumm, W., and Morgan, J.J., 1996, An Introduction Emphasizing Chemical Equilibria in Natural Waters, p. 2.
- Sundby, B., Anderson, L.G., Hall, P.O., Iverfeldt, Å., van der Loeff, M.M.R., and Westerlund, S.F., 1986, The effect of oxygen on release and uptake of cobalt, manganese, iron and phosphate at the sediment-water interface: Geochimica et Cosmochimica Acta, v. 50, p. 1281–1288.
- Team, Rc., 2016, R: A language and environment for statistical computing [Computer software manual]: Vienna, Austria.
- Thamdrup, B., Fossing, H., and Jørgensen, B.B., 1994, Manganese, iron and sulfur cycling in a coastal marine sediment, Aarhus Bay, Denmark: Geochimica et Cosmochimica Acta, v. 58, p. 5115–5129.
- Thode-Andersen, S., and Jørgensen, B.B., 1989, Sulfate reduction and the formation of 35S-labeled FeS, FeS2, and S0 in coastal marine sediments: Limnology and Oceanography, v. 34, p. 793–806.
- Thornton, D.C.O., Dong, L.F., Underwood, G.J.C., and Nedwell, D.B., 2002, Factors affecting microphytobenthic biomass, species composition and production in the Colne Estuary (UK): Aquatic Microbial Ecology, v. 27, p. 285–300, doi:10.3354/ame027285.
- Tugel, J.B., Hines, M.E., and Jones, G.E., 1986, Microbial Iron Reduction by Enrichment Cultures

Isolated from Estuarine Sediments: Applied and Environmental Microbiology, v. 52, p. 1167–1172, doi:10.1128/aem.52.5.1167-1172.1986.

- Underwood, G., and Kromkamp, J., 1999, Primary Production by Phytoplankton and Microphytobenthos in Estuaries: Advances in Ecological Research, Vol 29, v. 29, p. 93–153, doi:10.1016/S0065-2504(08)60192-0.
- Underwood, G.J., and Paterson, D.M., 2003, The importance of extracellular carbohydrate productionby marine epipelic diatoms: Advances in botanical research, v. 40, p. 183–240.
- Virolle, M., 2019, Origine et prédiction spatio-temporelle des tapissages argileux dans les reservoirs silicoclastiques - Apports de la comparaison entre des réservoirs enfouis (Permien et Crétacé) et un analogue actuel (estuaire de la Gironde) [These de doctorat]: Université Paris-Saclay (ComUE), http://www.theses.fr/2019SACLS190 (accessed June 2022).
- Virolle, M., Brigaud, B., Bourillot, R., Féniès, H., Portier, E., Duteil, T., Nouet, J., Patrier, P., and Beaufort, D., 2019, Detrital clay grain coats in estuarine clastic deposits: Origin and spatial distribution within a modern sedimentary system, the Gironde Estuary (south-west France): Sedimentology, v. 66, p. 859–894.
- Virolle, M., Fenies, H., Brigaud, B., Bourillot, R., Portier, E., Patrier, P., Beaufort, D., Jalon-Rojas, I., Derriennic, H., and Miska, S., 2020, Facies associations, detrital clay grain coats and mineralogical characterization of the Gironde estuary tidal bars: a modern analogue for deeply buried estuarine sandstone reservoirs: Marine and Petroleum Geology, v. 114, p. 104225.
- Visscher, P.T., Beukema, J., and van Gemerden, H., 1991, In situ characterization of sediments: measurements of oxygen and sulfide profiles with a novel combined needle electrode: Limnology and oceanography, v. 36, p. 1476–1480.
- Visscher, P.T., Prins, R.A., and van Gemerden, H., 1992, Rates of sulfate reduction and thiosulfate consumption in a marine microbial mat: FEMS Microbiology Letters, v. 86, p. 283–294, doi:10.1111/j.1574-6968.1992.tb04820.x.
- Visscher, P.T., Reid, R.P., and Bebout, B.M., 2000, Microscale observations of sulfate reduction: Correlation of microbial activity with lithified micritic laminae in modern marine stromatolites: Geology, v. 28, p. 919–922, doi:10.1130/0091-7613(2000)28<919:MOOSRC>2.0.CO;2.
- Visscher, P.T., and Stolz, J.F., 2005, Microbial mats as bioreactors: populations, processes, and products, in Geobiology: Objectives, Concepts, Perspectives, Elsevier, p. 87–100.
- Wadsö, I., 2009, Characterization of microbial activity in soil by use of isothermal microcalorimetry: Journal of thermal analysis and calorimetry, v. 95, p. 843–850.
- Webster, G., Rinna, J., Roussel, E.G., Fry, J.C., Weightman, A.J., and Parkes, R.J., 2010, Prokaryotic functional diversity in different biogeochemical depth zones in tidal sediments of the Severn Estuary, UK, revealed by stable-isotope probing: FEMS microbiology ecology, v. 72, p. 179– 197.
- Widerlund, A., and Ingri, J., 1996, Redox cycling of iron and manganese in sediments of the Kalix River

230 Chapter 4

estuary, Northern Sweden: Aquatic Geochemistry, v. 2, p. 185–201, doi:10.1007/BF00121631. Wilkin, R.T., and Barnes, H.L., 1997, Pyrite formation in an anoxic estuarine basin: American Journal of Science, v. 297, p. 620–650, doi:10.2475/ajs.297.6.620.

Supplementary material



Figure S1: FDA and TTC assays observed on microbiological preparations from the surface biofilm. (A) FDA assay. Green fluorescence indicates zones of active FDA hydrolysis. In this diatom cell, the enzymatic activity is located close to the nucleus and in the lipid vesicles. Chloroplasts have a red autofluorescence due to the presence of chlorophyll. (B) TTC assay. The polarizing microscope image shows organelles (mainly mitochondria) stained in red by formazan.



Figure S2: Left part, PCA correlation circle plot of variables of the microbial activity and EPS physico-chemical properties in BXN-B and BXN-SC. Right part, correlation matrix of Pearson's r coefficients between depth, EPS and microbial activity variables for the 18 samples. Green boxes indicate a p-value ≤ 0.05 .



Figure S3: Plot of saturation indices calculated with PhreeQc for BXN-B and BXN-SC.



Figure S4: Plot of saturation indices calculated with PhreeQc for BXN-LC..



Figure S5: Plot of selected elements (S, Al, Mn, Fe) measured by core-scale XRF on BXN-B and BXN-SC.

Impact of microbial activity



Figure S6: Plot of selected elements (S, Al, Mn, Fe) measured by core-scale XRF on BXN-LC.
BNX- BOX &															Micr elect	o- rod				
BXN-SC	Dionex														es					
	NT	std	17	std	3.4	std	G	std	C	std	NO	std	CO 2-	std		std	C -	std		std
Depth	Na	dev	K	dev	Mg mg/	dev	Ca	dev	CI	dev	NO ₃	dev	SU 4 ^{2*}	dev	O_2	dev	S_2^-	dev	рН	dev
cm	mg/L	L IIIg/	mg/L	mg/L	L Ing/	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	μM	μM	μM	μM	μM	μM
2	11.33	0.57	2.16	0.11	7.37	0.37	31.99	1.60	17.8	0.89	0	0	8.39	0.42	4.65	2.22	0.0	0.00	8.00	0.03
4	10.76	0.54	2.49	0.12	6.91	0.35	35.79	1.79	16.7	0.83	0	0	7.73	0.39	11	3.56	1.0	0.00	7.93	0.02
6	10.56	0.53	1.93	0.10	6.57	0.33	27.42	1.37	17.3	0.86	0.11	0.01	4.88	0.24	0.75	0.96	6.0	2.00	8.03	0.04
8	4.16	0.21	1.51	0.08	2.56	0.13	20.99	1.05	9.9	0.49	6.10	0.30	8.39	0.42	6.75	3.40	26.8	4.90	8.15	0.04
10	23.57	1.18	2.69	0.13	9.56	0.48	35.21	1.76	24.7	1.23	1.83	0.09	9.24	0.46	5	2.58	73.3	13.48	8.18	0.01
12	33.01	1.65	3.42	0.17	9.46	0.47	26.02	1.30	32.8	1.64	2.84	0.14	7.54	0.38	6.25	2.06	109.8	19.28	8.22	0.03
16	49.90	2.49	3.62	0.18	7.86	0.39	19.33	0.97	55.9	2.79	0.05	0.00	3.26	0.16	0.5	1.00	171.0	10.59	8.22	0.03
18	60.63	3.03	3.38	0.17	6.74	0.34	18.93	0.95	74.1	3.70	0.01	0.00	0.00	0.00	0.25	0.50	199.5	9.63	8.23	0.01
20	63.20	3.16	3.77	0.19	5.68	0.28	16.39	0.82	77.0	3.85	0	0	2.00	0.10	0	0.00	239.8	7.51	8.23	0.03
22	81.28	4.06	4.17	0.21	6.85	0.34	25.10	1.25	90.0	4.50	0	0	0.00	0.00	0.5	1.00	189.0	19.21	8.25	0.01
24	81.57	4.08	4.08	0.20	5.50	0.28	18.87	0.94	98.3	4.91	0	0	0.71	0.04	0.25	0.50	157.3	13.39	8.23	0.01
26	91.31	4.57	3.15	0.16	5.89	0.29	22.87	1.14	102.0	5.10	0	0	0.13	0.01	0	0.00	NA	NA	8.20	0.02
30	88.56	4.43	4.52	0.23	3.98	0.20	15.24	0.76	86.9	4.34	0.06	0.00	7.80	0.39	0.25	0.50	152.3	4.04	8.19	0.01
35	97.18	4.86	7.22	0.36	4.89	0.24	22.28	1.11	109.9	5.50	0	0	0.24	0.01	0	0.00	159.7	7.37	8.25	0.01
40	62.93	3.15	2.83	0.14	2.28	0.11	8.87	0.44	72.5	3.62	0	0	5.90	0.30	0	0.00	163.3	26.50	8.21	0.02
50	104.62	5.23	3.14	0.16	5.80	0.29	17.38	0.87	116.1	5.80	0	0	0.02	0.00	0	0.00	129.0	3.16	8.22	0.02
60	109.00	5.45	3.51	0.18	7.08	0.35	19.23	0.96	126.9	6.34	0	0	0.00	0.00	0	0.00	165.5	3.42	8.18	0.03
65	116.37	5.82	3.84	0.19	6.63	0.33	17.86	0.89	138.5	6.93	0	0	0.10	0.01	0	0.00	NA	NA	8.15	0.03

	LCD												Colorimetric			
	ІСР	atd		atd		ata		atd		atd		atd	assays			
Denth	Ca	stu dev	Fe	stu dev	Mn	stu dev	Μσ	stu dev	Ph	stu dev	Zn	stu dev	TTC	std dev	FDA	std dev
Depen	Cu	ucv	10	ucv	11111	ucv		acr	1.0	uev	211	ucv	mol TTC /h/g	mol TTC /h/g	mol FDA/h/g	mg FDA/h/g
cm	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	μg/L	μg/L	μg/L	μg/L	sed	sed	sed	sed
2	19	7	0	0	5	2	0.045	0.014	0	0	80	24	1.58E-04	1.60E-05	2.93E-05	6.42E-07
4	17	6	0.00	0	5.3	2.1	0.15	0.005	0	0	110	33	3.73E-05	4.16E-06	3.33E-05	5.75E-07
6	16	6	0.00	0.001	4.9	2	0.29	0.009	0.1	0.04	120	36	8.06E-05	3.79E-05	3.14E-05	1.39E-06
8	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A
10	19	7	0.00	0	7.5	3	0.28	0.008	0	0	76	23	1.36E-04	5.88E-05	1.91E-05	1.08E-06
12	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A
16	14	5	0.04	0.015	6.4	2.6	0.3	0.009	0	0	87	26	1.71E-04	3.68E-05	1.87E-05	8.11E-07
18	11	4	0.15	0.053	4.6	1.8	0.4	0.001	0.1	0.04	73	22	N.A	N.A	N.A	N.A
20	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	1.84E-04	2.76E-05	1.89E-05	6.24E-07
22	11	4	0.02	0.008	4	1.6	0.32	0.096	0	0	190	57	N.A	N.A	N.A	N.A
24	5.4	1.9	0.00	0.001	2.1	0.8	0.014	0.004	0	0	95	28	N.A	N.A	N.A	N.A
26	13	5	0.14	0.049	4.3	1.7	0.49	0.15	0.5	0.13	93	28	1.91E-04	2.06E-06	1.90E-05	1.20E-06
30	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	1.75E-04	2.19E-05	1.69E-05	1.03E-07
35	11	4	0.00	0.002	4.4	1.8	0.3	0.09	0.5	0.13	45	14	6.81E-05	4.68E-06	1.19E-05	1.80E-06
40	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	2.87E-05	5.66E-06	1.84E-05	3.25E-06
50	12	4	0.07	0.023	4.9	2	0.46	0.014	0	0	50	15	7.30E-05	4.88E-06	1.76E-05	6.32E-07
60	12	4	0.01	0.004	5.3	2.1	0.24	0.007	0	0	95	29	2.02E-05	9.16E-07	1.71E-05	7.74E-07
65	2.2	0.8	0.00	0.001	0.93	0.4	0.068	0.002	0	0	0	0	2.29E-05	7.56E-07	1.82E-05	2.93E-07

Table S1: Data use in this study for the BXN-BOX and BXN-LC.

BXN-LC	Dionex													
Depth	Na	std dev	K	std dev	Mg	std dev	Ca	std dev	Cl	std dev	NO3	std dev	SO 4 ²⁻	std dev
m	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
0.06	39.64	1.98	4.44	0.22	9.71	0.49	36.09	1.80	53.56	2.68	1.01	0.05	0.54	2.72E-02
0.5	86.14	4.31	4.95	0.25	15.57	0.78	37.67	1.88	190.97	9.55	1.39	0.07	0.13	6.39E-03
0.93	46.40	2.32	6.80	0.34	14.37	0.72	23.58	1.18	102.69	5.13	0.29	0.01	0.15	7.52E-03
1.3	43.49	2.17	5.95	0.30	20.47	1.02	30.00	1.50	94.44	4.72	0.87	0.04	0.53	2.64E-02
1.63	39.19	1.96	6.60	0.33	19.36	0.97	42.74	2.14	80.96	4.05	0.00	0.00	1.03	5.17E-02
2.16	29.29	1.46	6.39	0.32	20.24	1.01	19.57	0.98	70.30	3.52	0.01	0.00	0.01	2.85E-04
2.59	27.14	1.36	7.07	0.35	22.21	1.11	19.24	0.96	62.74	3.14	0.00	0.00	0.00	0.00E+00
2.8	28.31	1.42	7.94	0.40	21.09	1.05	20.66	1.03	59.00	2.95	0.14	0.01	0.29	1.44E-02
3.25	36.12	1.81	8.75	0.44	28.70	1.43	15.79	0.79	58.82	2.94	0.17	0.01	0.55	2.77E-02
3.68	39.99	2.00	7.18	0.36	29.98	1.50	15.96	0.80	58.46	2.92	0	0	0	0
4.12	55.35	2.77	6.22	0.31	30.61	1.53	11.81	0.59	63.96	3.20	0	0	0	0
4.33	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A	N.A
4.76	77.11	3.86	6.88	0.34	31.30	1.57	13.10	0.66	75.42	3.77	0	0	0	0
5.2	85.84	4.29	6.45	0.32	26.75	1.34	14.78	0.74	89.00	4.45	0	0	0	0
5.64	99.04	4.95	7.74	0.39	31.75	1.59	13.68	0.68	96.78	4.84	0	0	0	0
6.08	99.46	4.97	5.56	0.28	27.57	1.38	11.04	0.55	113.58	5.68	0	0	0	0

											Colorimetric]				
	ICP								-		assays					
		std														
Depth	Ca	dev	Mn	dev	Mg	dev	Pb	dev	Zn	dev	TTC	std dev	FDA	std dev	Fe II	Fe III
											mol TTC/h/g	mol	mol FDA/h/g	mol FDA/h/g		
m	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	μg/L	μg/L	μg/L	μg/L	sed	TTC/h/g sed	sed	sed	mg/L	mg/L
0.06	22	8	7.1	2.8	1.4	0.4	0.2	0.06	83	25	5.86E-05	6.57E-06	1.74E-05	7.16E-07	0.23	0.14
0.5	32	11	10	4	1.2	0.4	0.2	0.06	190	57	2.19E-05	5.87E-06	1.25E-05	1.36E-06	0.94	0.67
0.93	25	9	11	4	0.04	0.0	0.2	0.06	34	10	5.97E-05	1.61E-05	1.35E-05	8.94E-08	0.06	0.62
1.3	16	6	13	5	0.01	0.0	0.2	0.06	0	0	1.56E-04	2.96E-05	1.87E-05	1.26E-06	0.22	1.32
1.63	18	6	12	5	0.00	0.0	0.3	0.08	19	6	2.77E-05	4.00E-07	1.45E-05	6.18E-06	0	0
2.16	16	6	15	6	0.02	0.0	0	0	0	0	7.32E-05	3.29E-05	1.49E-05	8.71E-06	0.22	1.84
2.59	13	5	16	6	0.01	0.0	0	0	0	0	4.46E-05	8.97E-06	1.42E-05	1.31E-05	0.29	1.76
2.8	13	5	13	5	0.00	0.0	0.1	0.04	0	0	1.33E-04	1.63E-05	1.12E-05	3.41E-06	0	0.53
3.25	11	4	19	9	0.00	0.0	0	0	0	0	6.41E-05	9.62E-06	1.93E-05	4.04E-06	0	1.35
3.68	11	4	20	8	0.00	0.0	0	0	0	0	7.22E-05	6.03E-05	2.05E-05	5.99E-06	0	0.97
4.12	10	4	21	8	0.00	0.0	0.1	0.04	0	0	2.56E-05	2.03E-05	1.74E-05	1.57E-07	N.A	N.A
4.33	0.66	0.2	0.66	0.3	0.00	0.0	0.2	0.06	0	0	1.73E-05	3.67E-06	1.96E-05	3.37E-06	N.A	N.A
4.76	12	4	21	8	0.01	0.0	0.1	0.04	0	0	1.79E-05	1.80E-05	8.43E-06	3.57E-07	0.20	1.54
5.2	9.5	3.3	18	7	0.00	0.0	0	0	0	0	3.33E-05	1.63E-05	1.22E-05	8.15E-06	N.A	N.A
5.64	12	4	23	9	0.01	0.0	0.2	0.06	0	0	4.20E-05	2.01E-05	1.77E-05	1.14E-06	0	0.43
6.08	13	5	22	9	0.10	0.0	0.1	0.04	0	0	1.10E-05	1.52E-06	1.77E-05	1.14E-06	0	2.39

Table S2: Data use in this study for the BXN-LC.



Chapter 5: Role of diatom biofilm Exopolymeric Substances on clay coat formation in estuaries



Table of contents

Chapte	r 5: Role of diatom biofilm Exopolymeric Substances on clay coat for	rmation in estuaries
		241
5.1	Clay coats in the Bordeaux North Point Bar	
5.1	.1 Origin of detrital clay coats in modern sands	
5.1	.2 Quantification of clay coats with depth	
5.2	Experimental formation of clay-coated sand grains using diatom b	iofilm exopolymers
		249
5.3	Specific EPS-clay mineral interactions	
Refe	ences	
Anne	Х	

5.1 Clay coats in the Bordeaux North Point Bar

5.1.1 Origin of detrital clay coats in modern sands

The presence of clay minerals in modern sands and ancient sandstones has long been questioned. Indeed, the primary deposition processes tend to lead to the physical separation of clay-sized and sand-sized fractions during deposition due to due to differences in physical behavior (Van Ledden, 2003). On estuarine sand bars, mud can be deposited in alternance with sands in the form of mud drapes, principally during high and low tide slack waters (Fenies & Tastet, 1998). The erosion of the intertidal zone and of the supratidal marshes also supplies mud pebbles of variable size (mm to dm), which can be incorporated into the sand. In modern estuaries, significant proportions of sand grains are clay-coated (10% to 35% of sands in the Gironde estuary, Virolle et al., 2019; 50% to 87% in the Ravenglass Estuary, Worden et al., 2020). Recent studies have discussed the origin of such detrital clay coats (Schepetkina et al., 2016; Wooldridge et al., 2017; Griffiths et al., 2018). The most commonly cited physical process is deep infiltration of muddy water as a result of groundwater fluctuations or tidal pumping (in tidally dominated environments) which can result in clay minerals transfer in the sand shortly after deposition (Worden & Morad, 1999; Needham et al., 2004; Worden et al., 2006). This process has been reproduced experimentally by Matlack et al. (1989; Figure 5.1A and 5.1B). However, no structure related to water infiltration were observed in the Gironde estuary (Virolle et al., 2020). The ingestion of sand and mud by worms is another mechanism that could lead to the aggregation of clay in sandy deposits (Mcllroy et al., 2003; Needham et al., 2004). Experimental ingestion of sand by arenicolous worms (Arenicola marina sp.) has shown that bioturbation can lead to the establishment of clay coatings via a sticky mucus inside the worm intestine (Needham et al., 2005; Figure 5.1C and 5.1D). The excreted sand-size grains are also coated with a layer of mucus that sticks the clay coat to the sand grain. Bioturbation is scarce to absent from the Bordeaux Nord point bar, as well as from the other sand bars, implying that clay coats are probably not formed through bioturbation in these sedimentary bodies (Virolle et al., 2019). On the Bordeaux Nord point bar, the scarce bioturbation is explained by the brackish salinity, comprised on average between 1 and 2 g.L-1. It is indeed in this salinity range that the diversity and density benthic organisms are at their lowest (La Croix et al., 2015; Dashtgard & La Croix, 2015).



Figure 5.1: (A) and (B) SEM images from Matlack et al. (1989), (C) and (D) BSEM images from Needham et al. (2005). (A) Grain surface showing chlorite particles forming coat on the sand grain. (B) Bridge of chlorite formed between two coated quartz grains. (C) Image of a sand grain from within a fecal cast with coating of clay minerals with variability in clay-rim thickness. (D) Image of a zoom on the edge of a sand grain from a fecal cast highlighting the very fine-grained nature of the grain coating.

Schepetkina et al. (2016) hypothesized a role for diatoms and their extracellular polymeric substance (EPS) in the aggregation of minerals in estuaries. Wooldridge et al. (2017) showed a spatial correlation between the abundance chlorophyll a (a proxy for biofilm coverage) and the proportion of detrital clay coats. However, no experimental proof of the role EPS in the formation of detrital clay coats existed to date.

5.1.2 Quantification of clay coat with depth

Thin sections were prepared from BXN-LC sediments following the protocol described in the material and methods (Chapter 2). Three thin sections could not be done (1.3, 5.2 and 6.08m). The relative % surface area was calculated through point counting for five classes of grains (clean sand, coated sand, silt and clay particles, other grains and porosity) with the Jmicrovision software (Henry, 2020), as described in Chapter 2. Thin section scans are available in the annex of this chapter. An example is given in Figure 5.2.



Figure 5.2: (A) Scan of a thin section (depth: 93 cm) with the twelve areas selected for point counting. (B) Polarizing microscope image of Zone 2 showing coated quartz (Qz), micas (Mi), clean quartz at the border of a silt-clay drape. (C) Polarizing microscope image of Zone 3 showing sand grains (quartz and micas) linked together by detrital clay coats forming bridges. (D) Polarizing microscope image of Zone 4 showing coated and non-coated sands, clean quartz and possibly a dark volcanic lithoclast (Ot).

The average composition of BXN-LC sediments is: 2% of clean sand, 13% coated sand, 44% of silt and clay particles, 2% of other grains and 21% of porosity. The percentage after subtraction of the porosity are: 26% of clean quartz, 16% of coated quartz, 55% of silt and clay particles and 3% of other grains. These results are in the range of 10% to 35% of coated grains calculated by Virolle et al. (2019) in the Gironde estuary, but are low compared to the proportions calculated by Wooldridge et al. (2017) in the Ravenglass estuary. Figure 5.3 shows the evolution of the proportions of the five categories in function of depth. As expected, the samples with the highest proportions sand grains also have a high amount of coated sands (e.g., 4.33 or 4.76 m depth). However, two samples with high silt-clay contents also have a high proportion of coated sands (0.93 and 3.68 m depth). Sand-rich facies have a high porosity (ca. 30% on average), on the contrary to fine-grained particles rich facies (silt and clay; ca. 12% on average).



Figure 5.3: Evolution of sedimentary facies, grain size and proportions of grains and porosity with depth along the BXN Long Core. First column to the left: sedimentary description. (1) X-Ray image (SCOPIX); (2) core photographs; (3) sketch; (4) facies; (5) sedimentary log, and (6) sedimentary sequences. Sample depths are written in red. Second column: median grain size (D50; yellow dots). Third column: particle size class percentage: clay (brown), silt (grey), and sand (yellow). Fourth column: Thin section counting. Non-coated sands (yellow), coated sands (orange), silt and clay (grey), other grains (black) and porosity (blue). *NB: there is an error in the quantification of the sample at 163.5 cm, which will be checked after the submission of this thesis.*

5.2 Experimental formation of clay-coated sand grains using diatom biofilm exopolymers

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Résumé en français:

Dans les environnements sédimentaires, l'argile et le sable sont séparés par des processus hydrodynamiques. Pourtant, les clay-coats, de fines enveloppes d'argile recouvrant les grains de sable, sont abondantes dans les dépôts sédimentaires côtiers modernes et anciens. Nous présentons ici des expériences de laboratoire dans lesquelles nous avons produit des grains de quartz recouverts d'argile similaires à ceux observés dans les sables estuariens modernes et anciens. Ces coats détritiques ont été produits à température ambiante en mélangeant des substances exopolymériques (EPS) extraites de biofilms à diatomées intertidaux avec des matériaux de référence : minéraux argileux et quartz. L'imagerie des mélanges sédiments-EPS à l'aide de la cryo-microscopie électronique à balayage et de la microscopie à force atomique a démontré que les EPS forment des ponts organiques entre l'argile et le quartz. Les propriétés physicochimiques des EPS ont été caractérisées indépendamment par des tests colorimétriques et par de la spectroscopie infrarouge à transformée de Fourier.

Les résultats ont indiqué que plusieurs composants des EPS (par exemple, les protéines ou les polysaccharides) avaient le potentiel de se complexer avec le quartz et l'argile. Nos résultats fournissent de nouvelles perspectives sur l'importance des biofilms dans l'agrégation des particules d'argile détritiques et du sable. Les clay-coats détritiques provenant d'anciens grès estuariens présentent des similitudes texturales avec les complexes argile-EPS expérimentaux et pourraient être considérées comme des biosignatures du développement de biofilms et de la production d'EPS dans des environnements passés.

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Experimental formation of clay-coated sand grains using diatom biofilm exopolymers

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ABSTRACT

In sedimentary environments, clay and sand are segregated by hydrodynamic processes. Yet, clay coats, thin clay envelopes lining sand grains, are abundant in modern and ancient coastal sedimentary deposits. Here, we present laboratory experiments in which we produced clay-coated quartz sands similar to those observed in modern and ancient estuarine sands. These coats were produced at ambient temperature by mixing exopolymeric substances (EPS) derived from intertidal diatom biofilms with clay minerals and quartz reference materials. The imaging of sediment-EPS mixes using cryo-scanning electron microscopy and atomic force microscopy demonstrated that EPS form organic bridges between clay and quartz. The physicochemical properties of the EPS were characterized independently through wet chemical assays and Fourier transform infrared spectroscopy. The results indicated that several EPS components (e.g., proteins, polysaccharides) had a potential to complex with quartz and clay. Our findings provide novel insights in the importance of biofilms in the aggregation of clay and sand. Detrital coats from ancient estuarine sandstones show textural similarities to the experimental clay-EPS complexes and could be considered as biosignatures of biofilm development and EPS production in past environments.

INTRODUCTION

Biofilms derived from benthic diatoms cover the surface of intertidal and shallow subtidal coastal and estuarine sediments (Smith and Underwood, 1998). Diatoms are photoautotrophic organisms, which excrete copious amounts of exopolymeric substances (EPS; Underwood and Kromkamp, 1999). EPS provide diatom mobility at the sediment surface, protect against desiccation, and control the diffusion of nutrients, metabolites, and communication molecules between cells (Decho and Gutierrez, 2017). Diatom EPS are composed of 40%-90% polysaccharides, but they also include proteins, nucleic acids, and lipids (Flemming and Wingender, 2010). The charged functional groups and the neutral oxygen groups form the basis for EPS sorptive and adhesive properties, concentrating organic compounds, cations, and minerals (Decho, 1990). For example, the negatively charged groups in EPS (e.g., carboxyls) can form bidentate bridges with minerals (e.g., clay minerals, oxides) in the presence of divalent cations (Kleber et al., 2007; Ghashoghchi et al., 2017). Consequently, the production of EPS has a profound effect on the cohesiveness of fine-grained (<63 µm) intertidal sediments (Stal, 2010) and can strongly affect sand transport and bed-form morphology (Malarkey et al., 2015). In this paper, clay refers to clay minerals, unless otherwise mentioned.

In estuarine and coastal environments, fine particles (i.e., clay- and silt-grade sedidifferences in hydrodynamic behavior (van Ledden, 2003). Yet, detrital clay aggregates are found on sand grains in modern estuaries (Dowey et al., 2017; Wooldridge et al., 2017; Virolle et al., 2019a). Such aggregates, called detrital clay coats, are regarded as a potential source material for diagenetic clay rims (mainly chlorite). Clay coats have been documented in a number of ancient coastal sandstones, where they allow the preservation of reservoir properties by limiting quartz overgrowth (Dowey et al., 2012; Worden et al., 2020). Several studies have questioned the processes of adhesion between sand and silt/clay-grade particles in coastal sediments. Based on (1) clay coat texture, (2) identification of organic compounds in clay coats, and (3) spatial correlation of chlorophyll a and the proportion of detrital clay coats, Wooldridge et al. (2017) proposed a critical role for photosynthetic biofilm-EPS in clay coatings. In the Gironde Estuary, southwest France, Virolle et al. (2019a) observed abundant EPS linking clay- and sand-grade sediments in (1) intertidal, low-energy muds covered by biofilms, and (2) mobile high-energy tidal sand bars. The combined results of these studies suggested a prominent role for EPS in the aggregation of clay- and sand-grade sediments, but to date no experimental evidence supported this hypothesis.

ments) tend to segregate from sands due to

In this study, we mixed EPS extracted from diatom biofilms sampled in the Gironde Estuary



Figure 1. (A) Location of sampling sites in the Gironde Estuary, southwest France: Plassac channel tidal bar and Bordeaux Nord point bar. (B) Field of dunes in a high-energy tidal sand bar of the Plassac site. Arrow indicates interdune depressions where muddy sediments accumulate at low-tide slack water. (C) Diatom biofilm in the intertidal zone on the Bordeaux Nord point bar. (D) Cryo–scanning electron microscope (cryo-SEM) image of sediment from intertidal domain (Fig. 1C) showing diatom and clay particles attached to exopolymeric substances (EPS). (E) Cryo-SEM image of sediment from sand dunes (Fig. 1B) showing clay-EPS complex coating quartz sand grain.

with clay- and sand-grade sediments at ambient temperature in controlled laboratory conditions. The texture of experimental clay-coated sand grains was imaged using high-resolution microscopy and compared to natural samples. Additional analyses of physicochemical EPS properties were used to support a conceptual model for the EPS-quartz-clay interactions, filling the gap of knowledge in this widespread sedimentological process.

MATERIALS AND METHODS

Field campaigns were held on the Plassac tidal bar in May 2017 and on the Bordeaux Nord point bar in September 2017 (both west of Bordeaux; Figs. 1A–1C). Two types of samples were taken: (1) biofilms were scraped off the sediment surface (Fig. 1C) and then used for cryo–scanning electron microscope (cryo–SEM) fixation and EPS extraction, purification, and characterization; and (2) sandy sediments were sampled from trenches inside dunes (Fig. 1B) and used for cryofixation. The samples were immediately



Figure 2. Fourier transform infrared spectroscopy spectra of purified exopolymeric substances recovered from Gironde (southwest France) diatom biofilm. Assignments of vibrational bands are given in Table S3 (see footnote 1).



Figure 3. Results of exopolymeric substances (EPS)-clay-sand mixing experiments. (A) Scanning electron microscope (SEM) image of clay-EPS coats forming bridges between sand grains. (B) Cryo-SEM image showing the surface of a quartz grain covered with a mix of EPS and clay (cf. Figs. 1D and 1E). (C) Cryo-SEM view showing clay EPS complex enveloping a quartz grain. (D) Atomic force microscopy image of a quartz grain covered with clay-EPS complex. (E) SEM image of a sand grain from the Sego Formation (Upper Cretaceous; Colorado, USA) showing detrital coat with clay platelets following an alveolar fabric, which is a common feature in modern clay-EPS complexes. (F) SEM image of estuarine clay-coated quartz grain from the Petrel Basin (Permian; Australia). Relicts of detrital coat on the quartz grain are covered by a diagenetic coat made of honeycomb chlorite and fibrous illite.

stored in the dark at 4 °C. EPS were extracted as described by Pace et al. (2018). Liquid EPS solutions were kept at 4 °C.

Field samples were cryogenically fixed by high-pressure freezing (HPF) and stored in liquid nitrogen (-196 °C) until observation under cryo-SEM with a Gemini SEM 300 (Zeiss).

Fourier transform infrared spectroscopy (FTIR) analyses of the EPS were performed on a Frontier (Perkin Elmer) Spectrometer in attenuated total reflection (ATR) mode. One drop of EPS solution was placed on the diamond ATR element and evaporated. The composition of EPS was further assessed using three colorimetric assays: phenol-sulfuric acid assay and Alcian Blue assay as described by Braissant et al. (2009), and Pierce-modified Lowry assay (ThermoFisher Scientific).

Four clay reference minerals (Clay Minerals Society; Table S1 in the Supplemental Material¹) were mechanically crushed. Fontainebleau

sand (Sibelco, France) was used as reference for quartz. Organic matter was removed from quartz and clay reference minerals by washing (30% H₂O₂, 24 h). The reference material powders were mixed in the same proportion as present in the Gironde sediments (Virolle et al., 2019a; see also Table S1) and added to deionized water to obtain a 10 g L-1 clay suspension. EPS solution (245 µL) was mixed with Gironde-equivalent clay suspension (105 µL) and sand. The suspension was left for 72 h to react, without stirring, at ambient temperature (22 °C). Cryo-SEM samples were prepared and observed in the same conditions as the natural samples. The EPS-sediment mixtures were also observed using a Phenom X Pro SEM. For atomic force microscopy (AFM), a drop of mixture of clay- and sand-grade sediments and EPS was deposited on a glass plate and air dried. Observations were carried out on a Dimension 3100 AF microscope (Bruker Corporation).

CLAY COATS IN THE GIRONDE ESTUARY AND IN LABORATORY EXPERIMENTS

Diatom biofilms covered the low-energy, muddy intertidal zones of the sandbars (Fig. 1B), producing EPS at the sediment surface (Fig. 1C). FTIR and colorimetric assays confirmed the presence of polysaccharides and proteins, but also the presence of nucleic acids and lipids in the EPS (Fig. 2; Braissant et al., 2007). EPS were composed of an organic alveolar network surrounding the diatom frustules (Fig. 1D). Clay platelets were abundant within EPS, with their orientation aligning with the EPS framework (Figs. 1E and 1D). On the high-energy intertidal sand dunes (Fig. 1B), the clay-EPS complexes partially to entirely coated the quartz silt and sand grains (Fig. 1D).

Clay-EPS coats were reproduced in our laboratory experiments (Fig. 3). Quartz sand grains were covered by a continuous or discontinuous clay-EPS organo-mineral coating (Fig. 3A). Cryo-SEM images revealed that the clay particles were attached to the three-dimensional alveolar EPS network (Figs. 3B and 3C). AFM images showed that EPS incorporated clay platelets and almost completely covered the surface of sand grains (Fig. 3D). The laboratory-produced coats were comparable to the coats observed in Gironde sediments (Figs. 1D and 1E). Cryo-SEM, AFM images (Fig. S1), and FTIR spectra (Fig. S2) all support potential interactions of each of the four clay mineral standards with EPS.

CLAY COAT FORMATION

Based on our observations, we propose a conceptual model for clay coat formation (Fig. 4). Diatoms secrete abundant EPS, which form the biofilm on the intertidal mudflats in estuaries. Clay-EPS complexes are likely formed at the

¹Supplemental Material. AFM and cryo-SEM images of clay-EPS experiments, FTIR spectra of clay-EPS experiments, physico-chemical properties of standard clay minerals, potential mechanisms of interaction between EPS and mineral surfaces, and a list of of FTIR absorption bands in Figure 2. Please visit https://doi.org/10.1130/GEOL.S.12456320 to access the supplemental material, and contact editing@geosociety.org with any questions.



Figure 4. Multiscale model of exopolymeric substances (EPS)-mineral interactions and formation of clay coats in estuarine sediments; ltwl-low-tide water level; htwl-high-tide water level. (A) Sedimentary cross section from the supratidal to intertidal domain of an estuarine tidal bar. Biofilms mostly develop on muddy substrates in the intertidal domain. (B) Biofilm-clay interface results in formation of clay-EPS complexes in the intertidal zone. (C) Transport by tidal current, and deposition of clay-EPS complexes in interdune ponds initiate the formation of clay-EPS coats (at the surface or in macropores close to the surface). (D) Detail of fabric of clay-coated grains. (E) Potential molecular-scale mechanisms explaining clay-EPS adhesion and formation of clay coats.

biofilm-clay interface on low-energy mudflats (Figs. 4A and 4B). Underwood and Kromkamp (1999) estimated that between 30% and 50% of the EPS produced in intertidal biofilms could be resuspended and transported by tidal currents during a semidiurnal cycle. The presence of EPS and clay-EPS complexes in the water column likely enhances the flocculation of suspended particles (Eisma et al., 1991). Complexes settle on sand bars during high-tide and low-tide slack water (Figs. 4A and 4C). Interfaces between clay-EPS complexes and sand could be formed in, e.g., interdune ponds (Fig. 1B), either at the sediment surface or through percolation in the macropores (Figs. 1C, 4A, and 4C). This process could explain the formation of clay-coated grains (Figs. 1D, 1E, and 4D), which cover 10%–35% of intertidal sands in the Gironde Estuary (Virolle et al., 2019a) and 50%–87% in the Ravenglass Estuary (UK; Wooldridge et al., 2017; Worden et al., 2020). Production of EPS by diatoms attached to sand grains could also result in clay coat formation (Wooldridge et al., 2017), although diatoms are scarce in Gironde sands (Virolle et al., 2019a). Estuarine clay coats are resistant to abrasion, which implies they could be exported and preserved in open-marine environments (Verhagen et al., 2020).

Clay coats result from complex processes. EPS can interact with two areas of the clay platelet: the basal surface (external and interlayer surface) and the particle edge sites (Fig. 4E; Bergaya and Lagaly, 2013). The four different clay minerals used in our experiments possessed a siloxane surface (Bergaya and Lagaly, 2013). The siloxane-surface oxygen could form weak hydrogen bonds with water molecules. The resulting hydrophobic surface would promote the adsorption of EPS hydrophobic molecules (Kleber et al., 2007), such as uncharged protein moieties (Fig. 4E). The negative charge formed by substitutions in clay minerals (e.g., montmorillonite or chlorite) may attract positively charged EPS groups (e.g., amino groups; Fig. 2) through electrostatic bonds (Fig. 4E). Alternatively, the cations sorbed to the surface may bridge anionic groups through cation bridging with EPS (Fig. 4E; Kleber et al., 2007; Ghashoghchi et al., 2017). The strong hydration enthalpy of cations compensating the substitutions can also convert the hydrophobic siloxane surface into a hydrophilic surface (Bergaya and Lagaly, 2013). The solvation shell surrounding the cations can attract EPS moieties through a dipole-dipole interaction with water (Fig. 4E; Kleber et al., 2007). Hydroxyl groups located at the edge of the four clay minerals in our study (Table S1; Bergaya and Lagaly, 2013) can also be involved in sorption of negatively charged groups in EPS (e.g., carboxylic acids; Figs. 2 and 4E). Similarly, interactions between clay-EPS complexes and the surface of quartz sands could be the result of hydrophobic interactions between, e.g., proteins and the neutral siloxane surface, as well as electrostatic bonds formed between charged groups of EPS and silanol groups (Fig. 4E; Table S2; Tang et al., 2015).

CLAY COATS AS BIOSIGNATURES

Biofilms can produce microbially induced sedimentary structures (MISS; Westall et al., 2006), which are generally concentrated on low-energy muddy and sandy substrates of tidal flats (Noffke, 2009). Conversely, MISS and other biosignatures (e.g., lipid biomarkers) are considered to be scarce and generally overlooked within high-energy sandstones. The experimental production of clay coats (Fig. 3) indicated a critical role for diatom biofilm EPS in their formation. Consequently, the presence of clay coats in sandstones may be viewed as a biosignature of biofilm development.

The oldest pennate benthic diatoms are dated as Late Cretaceous. Some of their first representatives could have excreted EPS through an apical pore field (Sims et al., 2006). Proteinaceous compounds of EPS can form strong bonds with clay mineral surfaces that are stable over extended periods of time (Kleber et al., 2007). Prolonged heating (85 °C, 7 d) degrades proteins but increases the binding of their remnants to clay mineral surfaces (Craig and Collins, 2002). Consequently, the organo-mineral structures formed by EPS and clay have a potential for preservation in the sedimentary record. Detrital clay coats similar to modern and laboratory examples developed in the Late Cretaceous, i.e., close to the emergence of biofilm-forming diatoms (Fig. 3E), but also in the Permian, i.e., predating the appearance of diatoms (Fig. 3F; Virolle et al., 2019b), and older estuarine sandstones (Dowey et al., 2012). This raises the question of pre-Cretaceous biofilm communities and their production of EPS. Phylogenetic analyses show that low-salinity environments, including estuaries, could have hosted photosynthetic eukaryotes ~1.9 b.y. ago (Sánchez-Baracaldo et al., 2017), although the fossil record of this group starts ~1.05 b.y. ago in coastal settings (Gibson et al., 2018). Some modern red and green microalga produce EPS (e.g., polysaccharides; Xiao and Zheng, 2016), which implies that these groups could have played a role in clay coat formation before the emergence of diatoms.

Some studies have documented clay-coated sandstones in the Precambrian, including Paleoarchean estuarine deposits (Moodies Group, ca. 3.2 Ga; Heubeck et al., 2016). EPS-like structures associated with detrital minerals have been identified in 3.4–3.3-b.y.-old intertidal microbial mats from the Barberton greenstone belt (Westall et al., 2006). The ability of prokaryotes to produce EPS that trap detrital particles appeared early in the sedimentary record and seems to be a common characteristic of different groups of biofilm-forming microorganisms through time (Tice and Lowe, 2004).

Some lipid biomarkers characteristic of EPSsecreting organisms (Brocks and Banfield, 2009) can also survive thermal degradation through the oil window (>60 °C; Holba et al., 1998). Given the affinity of clay minerals for organic molecules, clay-rich lithologies are specifically targeted in the search for such biomarkers on Earth, but also on Mars (e.g., Ehlmann et al., 2008). Our study shows that clay-coated sands could also be a primary target in such investigations.

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REFERENCES CITED

- Bergaya, F., and Lagaly, G., eds., 2013, Handbook of Clay Science, Volume 5: Amsterdam, Elsevier, 1752 p.
- Braissant, O., Decho, A.W., Dupraz, C., Glunk, C., Przekop, K.M., and Visscher, P.T., 2007, Exopolymeric substances of sulfate-reducing bacteria: Interactions with calcium at alkaline pH and implication for formation of carbonate minerals: Geobiology, v. 5, p. 401–411, https://doi .org/10.1111/j.1472-4669.2007.00117.x.
- Braissant, O., Decho, A.W., Przekop, K.M., Gallagher, K.L., Glunk, C., Dupraz, C., and Visscher, P.T., 2009, Characteristics and turnover of exopolymeric substances in a hypersaline microbial mat: FEMS Microbiology Ecology, v. 67, p. 293–307, https://doi.org/10.1111/j.1574-6941.2008.00614.x.
- Brocks, J.J., and Banfield, J., 2009, Unravelling ancient microbial history with community proteogenomics and lipid geochemistry: Nature Reviews: Microbiology, v. 7, p. 601–609, https:// doi.org/10.1038/nrmicro2167.
- Craig, O.E., and Collins, M.J., 2002, The removal of protein from mineral surfaces: Implications for residue analysis of archaeological materials: Journal of Archaeological Science, v. 29, p. 1077– 1082, https://doi.org/10.1006/jasc.2001.0757.
- Decho, A.W., 1990, Microbial exopolymer secretions in ocean environments: Their role(s) in food webs and marine processes: Oceanography and Marine Biology, v. 28, p. 9–16.
- Decho, A.W., and Gutierrez, T., 2017, Microbial extracellular polymeric substances (EPSs) in ocean systems: Frontiers in Microbiology, v. 8, p. 922, https://doi.org/10.3389/fmicb.2017.00922.
- Dowey, P.J., Hodgson, D.M., and Worden, R.H., 2012, Pre-requisites, processes, and prediction of chlorite grain coatings in petroleum reservoirs: A review of subsurface examples: Marine and Petroleum Geology, v. 32, p. 63–75, https://doi .org/10.1016/j.marpetgeo.2011.11.007.
- Dowey, P.J., Worden, R.H., Utley, J., and Hodgson, D.M., 2017, Sedimentary controls on modern sand grain coat formation: Sedimentary Geology, v. 353, p. 46–63, https://doi.org/10.1016/ j.sedgeo.2017.03.001.
- Ehlmann, B.L., Mustard, J.F., Fassett, C.I., Schon, S.C., Head, J.W., III, Des Marais, D.J., Grant, J.A., and Murchie, S.L., 2008, Clay minerals in delta deposits and organic preservation potential on Mars: Nature Geoscience, v. 1, p. 355, https:// doi.org/10.1038/ngeo207.
- Eisma, D., Bernard, P., Cadée, G.C., Ittekkot, V., Kalf, J., Laane, R., Martin, J.M., Mook, W.G., Van Put, A., and Schuhmacher, T., 1991, Suspended-matter particle size in some west-European estuaries: Part II. A review on floc formation and break-up: Netherlands Journal of Sea Research, v. 28, p. 215–220, https://doi .org/10.1016/0077-7579(91)90018-V.
- Flemming, H.-C., and Wingender, J., 2010, The biofilm matrix: Nature Reviews: Microbiology, v. 8, p. 623, https://doi.org/10.1038/nrmicro2415.
- Ghashoghchi, R.A., Hosseini, M.R., and Ahmadi, A., 2017, Effects of microbial cells and their associated extracellular polymeric substances

on the bio-flocculation of kaolin and quartz: Applied Clay Science, v. 138, p. 81–88, https://doi.org/10.1016/j.clay.2017.01.002.

- Gibson, T.M., Shih, P.M., Cumming, V.M., Fischer, W.W., Crockford, P.W., Hodgskiss, M.S., Wörndle, S., Creaser, R.A., Rainbird, R.H., and Skulski, T.M., 2018, Precise age of *Bangiomorpha pubescens* dates the origin of eukaryotic photosynthesis: Geology, v. 46, p. 135–138, https://doi .org/10.1130/G39829.1.
- Heubeck, C., Biasing, S., Grund, M., Drabon, N., Homann, M., and Nabhan, S., 2016, Geological constraints on Archean (3.22 Ga) coastal-zone processes from the Dycedale syncline, Barberton greenstone belt: South African Journal of Geology, v. 119, p. 495–518, https://doi.org/10.2113/ gssajg.119.3.495.
- Holba, A.G., Dzou, L.I.P., Masterson, W.D., Hughes, W.B., Huizinga, B.J., Singletary, M.S., Moldowan, J.M., Mello, M.R., and Tegelaar, E., 1998, Application of 24-norcholestanes for constraining source age of petroleum: Organic Geochemistry, v. 29, p. 1269–1283, https://doi .org/10.1016/S0146-6380(98)00184-3.
- Kleber, M., Sollins, P., and Sutton, R., 2007, A conceptual model of organo-mineral interactions in soils: Self-assembly of organic molecular fragments into zonal structures on mineral surfaces: Biogeochemistry, v. 85, p. 9–24, https://doi.org/10.1007/ s10533-007-9103-5.
- Malarkey, J., Baas, J.H., Hope, J.A., Aspden, R.J., Parsons, D.R., Peakall, J., Paterson, D.M., Schindler, R.J., Ye, L., and Lichtman, I.D., 2015, The pervasive role of biological cohesion in bedform development: Nature Communications, v. 6, p. 6257, https://doi.org/10.1038/ncomms7257.
- Noffke, N., 2009, The criteria for the biogenicity of microbially induced sedimentary structures (MISS) in Archean and younger, sandy deposits: Earth-Science Reviews, v. 96, p. 173–180, https://doi.org/10.1016/j.earscirev.2008.08.002.
- Pace, A., et al., 2018, Formation of stromatolite lamina at the interface of oxygenic-anoxygenic photo-

synthesis: Geobiology, v. 16, p. 378–398, https:// doi.org/10.1111/gbi.12281.

- Sánchez-Baracaldo, P., Raven, J.A., Pisani, D., and Knoll, A.H., 2017, Early photosynthetic eukaryotes inhabited low-salinity habitats: Proceedings of the National Academy of Sciences of the United States of America, v. 114, p. E7737–E7745, https://doi.org/10.1073/pnas.1620089114.
- Sims, P.A., Mann, D.G., and Medlin, L.K., 2006, Evolution of the diatoms: Insights from fossil, biological and molecular data: Phycologia, v. 45, p. 361–402, https://doi.org/10.2216/05-22.1.
- Smith, D.J., and Underwood, G.J., 1998, Exopolymer production by intertidal epipelic diatoms: Limnology and Oceanography, v. 43, p. 1578–1591, https://doi.org/10.4319/lo.1998.43.7.1578.
- Stal, L.J., 2010, Microphytobenthos as a biogeomorphological force in intertidal sediment stabilization: Ecological Engineering, v. 36, p. 236–245, https://doi.org/10.1016/j.ecoleng.2008.12.032.
- Tang, C., Zhu, J., Li, Z., Zhu, R., Zhou, Q., Wei, J., He, H., and Tao, Q., 2015, Surface chemistry and reactivity of SiO₂ polymorphs: A comparative study on α-quartz and α-cristobalite: Applied Surface Science, v. 355, p. 1161–1167, https:// doi.org/10.1016/j.apsusc.2015.07.214.
- Tice, M.M., and Lowe, D.R., 2004, Photosynthetic microbial mats in the 3,416-Myr-old ocean: Nature, v. 431, p. 549–552, https://doi .org/10.1038/nature02888.
- Underwood, G.J.C., and Kromkamp, J.C., 1999, Primary production by phytoplankton and microphytobenthos in estuaries: Estuaries, v. 29, p. 93–153, https://doi.org/10.1016/S0065-2504(08)60192-0.
- van Ledden, M., 2003, Sand-Mud Segregation in Estuaries and Tidal Basins [Ph.D. thesis]: Sydney, Australia, University of Technology Sydney, 221 p.
- Verhagen, I.T., Crisóstomo-Figueroa, A., Utley, J.E., and Worden, R.H., 2020, Abrasion of detrital grain–coating clays during sediment transport: Implications for diagenetic clay coats: Sedimentary Geology, v. 403 p. 105653, https://doi .org/10.1016/j.sedgeo.2020.105653.

- Virolle, M., Brigaud, B., Bourillot, R., Féniès, H., Portier, E., Duteil, T., Nouet, J., Patrier, P., and Beaufort, D., 2019a, Detrital clay grain coats in estuarine clastic deposits: Origin and spatial distribution within a modern sedimentary system, the Gironde Estuary (south-west France): Sedimentology, v. 66, p. 859–894, https://doi .org/10.1111/sed.1250.
- Virolle, M., Brigaud, B., Luby, S., Portier, E., Féniès, H., Bourillot, R., Patrier, P., and Beaufort, D., 2019b, Influence of sedimentation and detrital clay grain coats on chloritized sandstone reservoir qualities: Insights from comparisons between ancient tidal heterolithic sandstones and a modern estuarine system: Marine and Petroleum Geology, v. 107, p. 163–184, https://doi.org/10.1016/ j.marpetgeo.2019.05.010.
- Westall, F., De Ronde, C.E., Southam, G., Grassineau, N., Colas, M., Cockell, C., and Lammer, H., 2006, Implications of a 3.472–3.333 Gyrold subaerial microbial mat from the Barberton greenstone belt, South Africa, for the UV environmental conditions on the early Earth: Philosophical Transactions of the Royal Society of London: Series B, Biological Sciences, v. 361, p. 1857– 1876, https://doi.org/10.1098/rstb.2006.1896.
- Wooldridge, L.J., Worden, R.H., Griffiths, J., Thompson, A., and Chung, P., 2017, Biofilm origin of clay-coated sand grains: Geology, v. 45, p. 875– 878, https://doi.org/10.1130/G39161.1.
- Worden, R.H., Griffiths, J., Wooldridge, L.J., Utley, J.E.P., Lawan, A.Y., Muhammed, D.D., Simon, N., and Armitage, P.J., 2020, Chlorite in sandstones: Earth-Science Reviews, v. 204 p. 103105, https://doi.org/10.1016/ j.earscirev.2020.103105.
- Xiao, R., and Zheng, Y., 2016, Overview of microalgal extracellular polymeric substances (EPS) and their applications: Biotechnology Advances, v. 34, p. 1225–1244, https://doi.org/10.1016/ j.biotechadv.2016.08.004.

Printed in USA

Duteil, T., et al., 2020, Experimental formation of clay-coated sand grains using diatom biofilm exopolymers: Geology, v. 48, https://doi.org/10.1130/G47418.1

Name	Chemical	Chemical composition	Mineral	CEC	Surface area	Proportion in the
	formula	(oxides; %Wt; from literature)	type	meq/100g	m²/g (from	Gironde eq.
				(measured)	literature)	solution (10g/L)
Kaolinite	Al2Si2O5	SiO2: 44.2, Al2O3: 39.7, TiO2:	1:1	2	10,05 (1)	7.4%
(KGa-1B)	(OH)4	1.39, Fe ₂ O ₃ : 0.13, FeO: 0.08,				
		MnO: 0.002, MgO: 0.03, Na ₂ O:				
		0.013, K2O: 0.05, F: 0.013,				
		P2O5: 0.034 (3)				
Illite	(K,H3O)(Al,Mg,	SiO2: 49.3, Al2O3: 24.25, TiO2:	1:2	4	105 (1)	57.3%
(Imt-2)	Fe) 2	0.55, Fe ₂ O ₃ : 7.32, FeO: 0.55,				
2.20 m	(Si,Al)4O10	MnO: 0.03, MgO: 2.56, CaO:				
	[(OH)2,(H2O)]	$0.43, K_2O: 7.83, P_2O_5: 0.08$ (3)				
Ca-rich	(Na,Ca)0,3	SiO2: 60.4, Al2O3: 17.6, TiO2:	1:2	114	97,42 (2)	27.8%
Montmo-	(Al,Mg)2	0.24, Fe ₂ O ₃ : 1.42, FeO: 0.08,	6045334 mm312	6.6 F.C. 1994		A CONTRACTOR AND A
rillonite	Si4O10	MnO: 0.099, MgO: 6.46, CaO:				
(Saz-2)	$(OH)_2$	2.82, Na ₂ O: 0.063, K ₂ O: 0.19,				
(042 2)		F:0.287, P2O5: 0.020 (3)				
Ripidolite	(Fe,Mg,Al)6	SiO2: 26.0, Al2O3: 20.0, TiO2:	1:2	0	2,5 (4)	11.1%
(Cca-2)	(Si,Al)4	0.476, Fe ₂ O ₃ : 26.6, FeO: 20.8,				
	O10(OH)8	MnO: 0.1, MgO: 17.2, CaO:				
		0.25, Na ₂ O:<0.1, K ₂ O: <0.1,				
		P2O5: 0.02 (3)				

Table S1. Properties of clay mineral standards (The Clay Minerals Society; Chantilly, USA) used in clay-sand-EPS experiments.

Mechanism	Organic functional groups
Cation exchange	Amino
Cations bridging	Carboxylate, sulfate, alcoholic OH, carbonyl, amines
Water bridging	Carboxylate, sulfate, alcoholic OH, carbonyl, amines
Hydrogen bonding	Amino, carboxyl, alcoholic OH, carbonyl
Van der Waals interaction	Uncharged organic unit/moiety

Table S2. Potential mechanisms of interaction between EPS and mineral surfaces.

Wavenumber (cm ⁻¹)	Vibrational modes
3000-4000	O-H/N-H vibrations
2800-3000	Symmetric and antisymmetric stretching of C-H from
	CH_2 and CH_3 groups
1733	Stretching of C=O in protonated carboxylic acid group
1636	Stretching of C=O in amide (amide I)
1540	N-H bending and C-N stretching in amide (amide II)
1450	CH₂ scissoring
1373	Symmetric stretching of COO ⁻
1247	Vibrations of -COOH and C-O-C group in esters
1210	Stretching of P=O bond in phosphate
950-1150	Asymmetric and symmetric stretching of PO ₂ ⁻ ; vibrations
	of C-OH and C-C bonds in polysaccharides and alcohols

Table S3. FTIR Absorption bands of Gironde diatom biofilm EPS.



Figure S1. Experiments of interactions between EPS and the four species of standard clay minerals (Table S1). For each experiment, we prepared a solution of 245 μ L of EPS solution and 105 μ L of 10g/L clay suspension. (A) SEM image of the kaolinite (KGa-1b)-EPS experiment. The EPS matrix forms a complex with kaolinite platelets. (B) SEM image of the montmorillonite (Saz-2)-EPS experiment. The clay platelet is covered by EPS. (C) SEM image of ripidolite (Cca-2)-EPS experiment. An organo-mineral complexes is formed between EPS fibers and ripidolite platelets. (D) AFM image of the illite (Imt-2)-EPS experiment. Clay platelets are embedded within the EPS organic matrix. A close-up view shows the cleavage plane of the illite crystal.



Figure S2. FTIR spectra of EPS and clay standard minerals experiments. See the caption of Fig. S2 and main text for detailed about experimental procedures.

References:

(1) Kahr, G., and Madsen, F.T., 1995, Determination of the cation exchange capacity and the surface area of bentonite, illite and kaolinite by methylene blue adsorption: Applied Clay Science, v. 9, p. 327–336.

(2) Lorenz, P.M., Meier, L., and Kahr, G., 1999, Determination of the cation exchange capacity (CEC) of clay minerals using the complexes of copper (II) ion with triethylenetetramine and tetraethylenepentamine: Clays and clay minerals, v. 47, p. 386–388.

(3) Mermut, A.R., and Cano, A.F., 2001, Baseline studies of the clay minerals society source clays: chemical analyses of major elements: Clays and Clay Minerals, v. 49, p. 381–386.

(4) Sondi, I., Stubičar, M., and Pravdić, V., 1997, Surface properties of ripidolite and beidellite clays modified by high-energy ball milling: Colloids and Surfaces A: Physicochemical and Engineering Aspects, v. 127, p. 141–149.

5.3 Specific EPS-clay mineral interactions

We tested independently the interactions between EPS and each of the four clay mineral species: montmorillonite, chlorite, illite and kaolinite. Detailed information about clay minerals characteristics are available in the introduction of this thesis (Chapter 1). EPS were extracted from the biofilm with the same protocol as specified above (part 5.2). 105 μ L of clay mineral solution (10 g.L-1) were mixed with 245 μ L of EPS solution (1 g.L-1), and left for reaction during 72h at ambient temperature (22°C). The results of these experiments were characterized with FTIR, AFM and cryo-MEB acquisitions. FTIR spectra are presented in Figure S2 above. AFM and cryo-SEM imaging of the experiments are presented in Figure 5.4 (EPS-chlorite), Figure 5.5 (EPS-illite), Figure 5.6 (EPS-kaolinite) and Figure 5.7 (EPS-montmorillonite). EDX profiles were also acquired (Figure 5.8 for EPS-montmorillonite).

On FTIR spectra, the interactions between clay minerals and EPS can be potentially expressed by peak shifts, in particular amide I (from 1620 to 1640 cm-1) and carbohydrate C-O (from 1025 to 1110 cm-1). However, the shift of amide I could also be explained by the angular deformation of water from EPS or clay minerals (Chukanov & Chervonnyi, 2016). The four experiments showed EPS fibers attached to clay platelets, which are often embedded in the organic matrix (Figures 5.4 to 5.7). EPS seem to adhere to both the basal surface and the edge of clay crystals. AFM images also show clay particles partially to totally embedded within EPS (Figures 5.4 to 5.7). The difference between a "clean" clay surface and a clay surface covered with EPS can be seen in figure 5.7E and 5.7F.

These observations show that the four clay mineral species can form complexes with EPS. However, these results do not allow to hierarchize their affinity for EPS, or to determine the relative strength of EPS-clay bonds. The EPS from the Gironde biofilms possess reactive functional groups: carboxyls, sulfates, amino-groups which could be responsible for part of the observed interactions with clay minerals. EPS are highly complex mixes of macromolecules, and our knowledge of these molecules is still too limites. In order to better characterize the potential mineral-specific interactions, interaction experiments could be done using model EPS of known compositions (e.g., dextran, xanthan) and compared to experiments with natural EPS.



Figure 5.4: AFM and cryo-SEM images of the ripidolite-EPS interaction experiment. (A) EPS matrix covered with clay platelets. (B) EPS fibers embedding ripidolite particles. (C) and (D) AFM images in planar and 3D view showing a ripidolite platelet coated by the EPS matrix.



Figure 5.5: AFM and cryo-SEM images of the illite- EPS interaction experiment. (A) EPS matrix covered with illite platelets. (B) Zoom on the EPS matrix. EPS fibers form an organic network between illite crystlas. (C) Illite platelet attached to the EPS. (D) Zoom on the EPS matrix showing EPS fibers interacting with clay particles. (E) and (F) AFM images in planar and 3D view of an illite platelet coated by EPS matrix. The basal cleavage plane is visible.



Figure 5.6: AFM and cryo-SEM images of the kaolinite-EPS interaction experiment. (A) A clay platelet is trapped in EPS. (B) Zoom showing the EPS fibers interacting with the crystal surface. (C) Kaolinite particle maintained by EPS fiber. (D) Large kaolinite particle in the EPS matrix. (E) and (F) AFM images in planar and 3D view of kaolinite platelets coated by the EPS matrix.



Figure 5.7: AFM and cryo-SEM images of the montmorillonite-EPS interaction experiment. (A) Clay platelet trapped in the EPS matrix. (B) Zoom on the clay particle showing the clay sheets. EPS adhere to the basal surface, but also to the edge of the crystal. (C) EPS fibers trapping montmorillonite particles. (D) Zoom on the EPS matrix showing EPS fiber interacting with clay crystals. (E) AFM image, 3D view of a montmorillonite platelet coated by EPS matrix. (F) Zoom on (E) showing the clay crystal largely covered by the alveolar EPS.



Figure 5.8: Cryo-SEM images with associated EDX profiles of the montmorillonite-EPS interaction experiment. (A) Three spectra were acquired at different locations on the montmorillonite crystal. The spectra show peaks of sodium, aluminium, silicon, nitrogen and oxygen. (B) Montmorillonite embedded in EPS. The three spectra show peaks of aluminium, silicon, nitrogen and oxygen.

References

- Chukanov, N.V., and Chervonnyi, A.D., 2016, Infrared spectroscopy of minerals and related compounds: Springer.
- Dashtgard, S.E., and La Croix, A.D., 2015, Sedimentological trends across the tidal-fluvial transition, Fraser River, Canada: a review and some broader implications: Developments in Sedimentology, v. 68, p. 111–126.
- Fenies, H., and Tastet, J.-P., 1998, Facies and architecture of an estuarine tidal bar (the Trompeloup bar, Gironde Estuary, SW France): Marine Geology, v. 150, p. 149–169.
- Griffiths, J., Worden, R.H., Wooldridge, L.J., Utley, J.E., and Duller, R.A., 2018, Detrital clay coats, clay minerals, and pyrite: a modern shallow-core analogue for ancient and deeply buried estuarine sandstones: Journal of Sedimentary Research, v. 88, p. 1205–1237.
- Henry, A., 2020, Etude sedimentologique et geochimique des corps sedimentaires de l'estuaire de la Gironde. Stage de troisième année d'école ingénieur à l'ENSEGID, Bordeaux INP.
- La Croix, A.D., Dashtgard, S.E., Gingras, M.K., Hauck, T.E., and MacEachern, J.A., 2015, Bioturbation trends across the freshwater to brackish-water transition in rivers: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 440, p. 66–77.
- van Ledden, M., 2003, Sand-mud segregation in estuaries and tidal basins: University of Technology Sydney.
- Matlack, K.S., Houseknecht, D.W., and Applin, K.R., 1989, Emplacement of clay into sand by infiltration: Journal of Sedimentary Research, v. 59, p. 77–87.
- McIlroy, D., Worden, R.H., and Needham, S.J., 2003, Faeces, clay minerals and reservoir potential: Journal of the Geological Society, v. 160, p. 489–493.
- Needham, S.J., Worden, R.H., and Mcilroy, D., 2004, Animal-sediment interactions: the effect of ingestion and excretion by worms on mineralogy: Biogeosciences, v. 1, p. 113–121.
- Needham, S.J., Worden, R.H., and McIlroy, D., 2005, Experimental production of clay rims by macrobiotic sediment ingestion and excretion processes: Journal of Sedimentary Research, v. 75, p. 1028–1037.
- Virolle, M., Brigaud, B., Bourillot, R., Féniès, H., Portier, E., Duteil, T., Nouet, J., Patrier, P., and Beaufort, D., 2019, Detrital clay grain coats in estuarine clastic deposits: Origin and spatial distribution within a modern sedimentary system, the Gironde Estuary (south-west France): Sedimentology, v. 66, p. 859–894.
- Virolle, M., Fenies, H., Brigaud, B., Bourillot, R., Portier, E., Patrier, P., Beaufort, D., Jalon-Rojas, I., Derriennic, H., and Miska, S., 2020, Facies associations, detrital clay grain coats and mineralogical characterization of the Gironde estuary tidal bars: a modern analogue for deeply buried estuarine sandstone reservoirs: Marine and Petroleum Geology, v. 114, p. 104225.

Wooldridge, L.J., Worden, R.H., Griffiths, J., Thompson, A., and Chung, P., 2017, Biofilm origin of

clay-coated sand grains: Geology, v. 45, p. 875-878, doi:10.1130/G39161.1.

- Worden, R.H., Griffiths, J., Wooldridge, L.J., Utley, J.E.P., Lawan, A.Y., Muhammed, D.D., Simon,N., and Armitage, P.J., 2020, Chlorite in sandstones: Earth-Science Reviews, v. 204, p. 103105.
- Worden, R.H., and Morad, S., 1999, Clay minerals in sandstones: controls on formation, distribution and evolution: Clay mineral cements in sandstones, p. 1–41.
- Worden, R.H., Needham, S.J., and Cuadros, J., 2006, The worm gut; a natural clay mineral factory and a possible cause of diagenetic grain coats in sandstones: Journal of Geochemical Exploration, v. 89, p. 428–431.

Annex



Figure 5.10: Thin section scans of Bordeaux North Long Core. For each thin section, 10 areas were randomly determined in order to perform the count. these areas are highlighted by black frames on the figure.



Chapter 6: Discussion and way forward


Table of contents

Chapter 6: D	Discussion and way forward	271
6.1 Pro of sedimen	oduction, composition and preservation of Exopolymeric Substances: a comparis ntary environments	on 274
6.1.1	Structure and functioning of hypersaline microbial mats	274
6.1.2	Composition of EPS in open marine and hypersaline microbial mats	277
6.1.3 sedimen	Comparison of EPS concentrations and physico-chemical properties in various ntary environments	277
6.1.4	Sediment-EPS interactions: aggregation, attachment, trapping and binding	284
6.1.5	Evolution of EPS with depth in the sediment	285
6.2 EP	S in the sediment to rock transition and their potential preservation	289
6.2.1. diagenes	EPS Relationships between EPS cycling, carbonate precipitation and early sis in hypersaline microbialites	289
6.2.2.	Role of EPS in silicate precipitation	290
6.2.3.	A potential role for EPS in the diagenesis of estuarine sandstones	292
6.2.4.	EPS-mineral interactions as biosignatures of past microbial activity	296
6.3 Per	rspectives	298
6.3.1	Experiments on EPS from diatom cultures	298
6.3.2 diagenes	Interactions between EPS, clay minerals and metals, and evolution through sis	302
Reference	S	303

6.1 Production, composition and preservation of Exopolymeric Substances: a comparison of sedimentary environments

Our study of exopolymeric substances in the Gironde estuary brings new knowledge in the understanding of role of EPS in sedimentation and early diagenesis in estuaries. Our results can also be compared with other sedimentary environments where EPS have been studied. In the first part of this chapter, we will discuss the production, composition and preservation of EPS in various sedimentary systems (e.g., lakes, lagoons, rivers). The second part of the discussion will focus on the potential roles of EPS in the surface to burial diagenetic transition. We will especially discuss EPS-mineral interactions, the biosignatures that could result from these interactions, and the potential preservation of such biosignatures in the sedimentary record.

6.1.1 Structure and functioning of hypersaline microbial mats

Another large set of studies have been carried out on the EPS of microbial mats developing in hypersaline lakes or lagoons. Microbial mats can be defined as benthic, layered organosedimentary biofilms that exhibit tightly coupled element cycles. Microbial mats can reach several centimeters in thickness, and their photosynthetic communities are often dominated by cyanobacteria (e.g., Dupraz et al., 2009), in contrast with estuarine biofilms, which are usually millimeter thick and dominated by diatoms (e.g., Underwood & Paterson, 1993; Decho, 2000). When the microbial mat is mineralized and lithified, it is called a microbialite. Microbialites have two mechanisms of accretion: (i) trapping and binding of detrital grains and (ii) precipitation of minerals: mostly carbonates, but also silicates, oxides or sulfides (Burne & Moore, 1987; Dupraz et al., 2009). Seven main groups of microorganisms compose hypersaline microbial mats (Van Gemerden, 1993; Visscher & Stolz, 2005; Dupraz et al., 2009): (i) photolitho-autotrophs (mainly cyanobacteria), (ii) anoxygenic phototrophs (purple and green sulfur bacteria), (iii) aerobic (chemoorgano-) heterotrophs, (iv) fermenters, (v) anaerobic heterotrophs (sulfate reducing bacteria or SRB), (vi) sulfide oxidizing bacteria (SOB) and (vii) methanogens. Within the mat, some microorganisms secrete EPS which have similar functions as in estuarine biofilms, e.g., adhesion to the substrate and physical cohesion of the microbial mat (Decho & Kawaguchi, 1999; Decho, 2000), protection of the community against various environmental stresses (e.g., UV, desiccation; Costerton & Stoodley, 2003). EPS and low

molecular weight organic carbon (LMWOC) are the main source of organic carbon for chemorganoheterotrophs, fermenters and anaerobic heterotrophic bacteria (Stal, 2003).

The development and mineralization hypersaline can be summed up in five steps (Figure 6.1; Dupraz et al., 2011; Pace et al., 2018). The upper layer of the mat receives more light and is dominated by cyanobacteria (Des Marais, 1995; Stal, 2000). Cyanobacteria are considered as the most efficient EPS producers through oxygenic photosynthesis (Decho et al., 2005). Oxygen is subsequently consumed by aerobic heterotrophs, constituting an anoxic zone at the bottom of the mat. This anoxic zone is colonized by anaerobic heterotrophs. In hypersaline mat, abundant SRB degrade EPS and reduce sulfates, resulting in the production of sulfides (Visscher et al., 1992; Visscher & Stolz, 2005). A layer of purple sulfur bacteria then develops at the bottom of the mat, as they oxidize sulfide through anoxygenic photosynthesis (Visscher & Stolz, 2005; Figure 6.1). The layering of microbial mats is primarily controlled by the quantity and quality of light, the production of organic carbon by phototrophs and by geochemical gradients (e.g., oxygen, sulfides) produced by the main metabolic reactions.

276 Chapter 6



Figure 6.1: Pictures and conceptual model of microbial mat development and initial mineralization steps from Pace et al. (2018). (A) and (B) Pictures of the microbial mat from Cayo Coco lagoon (Cuba). (i) Initial development of coccoid and filamentous cyanobacteria fixing the CO₂ to form biomass and producing O₂ through oxygenic photosynthesis. (ii) Through aerobic respiration, aerobic heterotrophic bacteria degrade the organic carbon produced by cyanobacteria. The oxygen consumption results in a steeper O₂ gradient and the formation of an anoxic zone below the active cyanobacteria layer. The elevated pH associated with increased cyanobacteria activity in the upper millimeters of the mat leads to the nucleation of the Mg-Si phase in the exopolymeric substances (EPS) and around coccoid cyanobacteria clusters. (iii) Anaerobic heterotrophic bacteria (SRB) develop both in the oxic and anoxic zones, but are more abundant in the top of the anoxic zone. SRB produce sulfide and compounds and consume EPS. Their activity can locally increase pH, leading to an additional precipitation of Mg-Si phase. (iv) Purple sulfur bacteria develop in the anoxic zone, where they predominantly perform anoxygenic photosynthesis. The combined activity of cyanobacteria, purple sulfur bacterium (PSB), and SRB at the green and red laminae interface creates a pH maximum. This, combined with a radical change in EPS properties, leads to the formation of a high-magnesium calcite (HMC) lamina (i.e., the white lamina) at the oxygenic-anoxygenic photosynthetic interface. (v) HMC precipitates mostly at the oxygenic-anoxygenic photosynthetic interface but also in the green and red lamina at spots of heterotrophic degradation of EPS.

6.1.2 Composition of EPS in open marine and hypersaline microbial mats

EPS from microbial mats are predominantly composed of polysaccharides, uronic acids, proteins, with lower quantities of other other organic (e.g., nucleic acids, lipids) and inorganic compounds (e.g., sulfates; Decho, 1990). Most studies of hypersaline mat EPS consisted in bulk characterizations of the main types of molecules (e.g., phenol sulfuric and Alcian Blue assays). Kawaguchi & Decho (2002a) extracted EPS from cultures of the cyanobacterium Schizothrix sp. isolated from open marine stromatolites (Highborne Cay; Bahamas). They showed that EPS were mainly composed of large acidic polysaccharides with a predominant size around 300 kD, with a small fraction of larger sugars (500 kD). In another study, the same authors determined the monosaccharide composition of the cyanobacteria Synechocystis sp. and Oscillatoria sp. Monosaccharides consisted predominantly in glucose (42.5%), galactose (28%), xylose (10.4%), with low proportions of arabinose, rhamnose and fucose (Kawaguchi & Decho, 2002b). Aspartic and glutamic acids made up to 30% of the amino acids composing the proteins from the same EPS, which also included alanine, leucine, isoleucine and phenylalanine. Aspartic and glutamic acids are known to carry carboxylic acid reactive groups. In EPS, polysaccharides and proteins possess functional groups that are able to bind large quantities of metals (e.g., carboxyls, hydroxyls, sulfates, phosphoryl; Braissant et al., 2009). EPS produced in cultures of bacteria isolated from microbial mats generally have a high affinity for calcium and other metals (Perry et al., 2005). For example, cyanobacterial EPS are able to bind metal ions such as cadmium, manganese, copper, lead, mercury and calcium to various functional groups that were present in sugars and amino acids constituting the EPS (e.g., Somers & Brown, 1978; Mohamed, 2001; Mehta & Gaur, 2005). Braissant et al. (2007) measured calcium binding capacities comprised between 120 and 150 mg_{Ca}.g⁻¹_{EPS} in EPS from SRB isolated from Bahamian hypersaline mats. EPS directly extracted from hypersaline mats also very high affinity for calcium (386 mg_{Ca}. g^{-1}_{EPS}) and magnesium (50 mg_{Mg}. g^{-1}_{EPS}).

6.1.3 Comparison of EPS concentrations and physico-chemical properties in various sedimentary environments

In this section, we synthesized some of the studies which characterized the quantity and composition of EPS in various sedimentary environments: (i) rivers, (ii) estuarines and coastal

mudflats, (iii) open marine environment; (iv) hypersaline lakes and lagoons. The concentrations are compared in Figure 6.2.

Concentrations of EPS in river, estuarine and mudflat biofilms and associated sediments. An important work has been done in the 1990's and 2000's to characterize the properties and evolution of EPS quantity and quality in biofilms from intertidal estuarine and coastal zones in United Kingdom (e.g., Underwood et al., 1995; Taylor et al., 1999; De Brouwer & Stal, 2001; Table 6.1). The comparison between these studies showed a great variability in term of colloidal or bound EPS concentrations: for example, Taylor et al. (1999) measured up to 3.5 mg_{EPS}.g_{sediment}⁻¹ (DW) in EPS from the Eden estuary, while Underwood et al. (1995) measured *ca*. 100 to 300 μ g_{EPS}.g_{sediment}⁻¹ (DW) in EPS from the Blackwater estuary (Table 6.1; Figure 6.2). One key question is to determine whether this variability comes from real differences in EPS productivity or from different sampling or analytical procedures.

Differences related to analytical procedures. EPS extraction could partly explain some differences. Detailed information about EPS extraction methods are given in chapter 2 material and methods. Physical extraction (e.g., sonication) coupled with precipitation in alcohol yields EPS concentrations comprised in the order of tens to hundreds of micrograms of EPS per grams of sediment (DW). In contrast, chemical extraction (e.g. cation exchange resins, EDTA) without alcohol precipitation are often associated with concentration in the order of 1-10 mg.g⁻¹ (DW). The precipitation in alcohol favors high molecular weight over low molecular weight EPS (Underwood et al., 1995), which could also explain a part the differences in the quantity of extracted EPS. This shows the importance of taking into account extraction methods when comparing quantities of EPS from different studies. The techniques used for EPS quantification may also differ between studies. The phenol sulfuric assay is widely used to estimate the concentration of polysaccharides in EPS (Braissant et al., 2020). However, the standards are not always the same: while most studies have used glucose (M=180.156 g.mol⁻¹), xanthan (M=933,746 g.mol⁻¹) is closer to the large polysaccharides present in natural EPS (this study). As xanthan is composed of five hexoses, we multiplied the concentrations measured in xanthan equivalent by 5 in Figure 6.2. Two different assays are used for protein quantification, the Lowry and Bradford assays. The Bradford assay will lead in overestimated protein concentrations for samples rich in arginine, while the Lowry assay can interact with carbohydrates or EDTA (Olson & Markwell, 2007).

Discussion and way forward

Study site	Sampling sediment	Depth	Extraction method	Quantification	Dominant EPS-	Bound EPS	Colloidal EPS	Proteins	Ref
		sampled		method	secreting organism				
Texel island, Netherland	Tidal sandy beach	10 mm	Hot water (30°C) dilution and alcohol precipitation for colloidal part, EDTA and alcohol precipitation for bound	Gas chromatography	Benthic diatoms and cyanobacteria	1.5-2.5 mg.g ⁻¹	0.2-6 mg.g ⁻¹	N.A	De Winder et al., 1999 (1)
Severn estuary, UK	Intertidal mudflat	5 mm	Saline water dilution and alcohol precipitation	Phenol sulfuric assay	Benthic diatoms	N.A	119-137 μg.g ⁻¹ DW sed (glucose eq.)	N.A	Underwood et al., 1995 (2)
Severn estuary, UK	Intertidal mudflat	5 mm	EDTA and alcohol precipitation	Phenol sulfuric assay	Benthic diatoms	N.A	136-295 μg.g ⁻¹ DW sed (glucose eq.)	N.A	Underwood et al., 1995 (2)
Blackwater estuary, UK	Lower saltmarsh	5 mm	Saline water dilution and alcohol precipitation	Phenol sulfuric assay	Benthic diatoms	N.A	1.64 mg.g ⁻¹ DW sed (glucose eq.)	N.A	Underwood et al., 1995 (2)
Blackwater estuary, UK	Lower saltmarsh	5 mm	EDTA and alcohol precipitation	Phenol sulfuric assay	Benthic diatoms	N.A	4.4 mg.g ⁻¹ DW sed (glucose eq.)	N.A	Underwood et al., 1995 (2)
Westerschelde estuary, Netherland	Intertidal mudflat	1 mm	DI water dilution and centrifugation for colloidal, 0.1 M EDTA and centrifugation for bound	Phenol sulfuric assay	Benthic diatoms	450-950 μg.g ⁻ ¹ DW sed	150-300 μg.g ⁻¹ DW sed (glucose eq.)	N.A	De Brouwer & Stal., 2001 (3)
Eden estuary, UK	Intertidal mudflat	1 cm	DI water (20°C) dilution and alcohol precipitation for colloidal EPS Residual sediments from colloidal extraction for bound EPS	Phenol sulfuric assay	Benthic diatoms and cyanobacteria	20-40 mg.g ⁻¹ DW sed	1-3.5 mg.g ⁻¹ DW sed (glucose eq.)	N.A	Taylor et al., 1999 (4)
Humber estuary, UK	Intertidal mudflat	5 mm	Saline water (20°C) dilution and centrifugation	Phenol sulfuric assay	Benthic diatoms	N.A	20-130 µg.g ⁻¹ DW sed (glucose eq.)	N.A	Paterson et al., 2000 (5)
Humber and Eden estuaries, UK	Intertidal mudflats	30 cm	DI water dilution and centrifugation for colloidal EPS, sediment for bound EPS	Phenol sulfuric assay	Benthic diatoms	7.2-23 mg.g ⁻¹ DW sed	0.6-2 mg.g ⁻¹ DW sed (glucose eq.)	N.A	Taylor & Paterson, 1998 (6)
Marennes-Oléron Bay, France	Intertidal mudflat	1 cm	Saline (artificial sea water) water dilution, centrifugation and alcohol precipitation for colloidal EPS, Dowex resin and alcohol precipitation for bound EPS	Phenol sulfuric assay Bradford assay for proteins	Benthic diatoms	6-23 μg.g ⁻¹ DW sed	4-21 μg.g ⁻¹ DW sed (glucose eq.)	0-2 μg.g ⁻¹ DW sed	Orvain et al., 2014 (7)
Colne estuary, UK	Intertidal mudflat	2 mm	25‰ saline solution dilution and alcohol precipitation	Phenol sulfuric assay	Benthic diatoms	N.A	99.7-220 μg. cm ⁻² (glucose eq.)	N.A	Bellinger et al., 2005 (8)
Severn estuary, UK	Intertidal mudflat	2 mm	25‰ saline solution dilution and alcohol precipitation for colloidal	Phenol sulfuric assay	Benthic diatoms	N.A	3-3.1 mg.g ⁻¹ DW sed (glucose eq.)	N.A	Yallop et al., 2000 (9)
Gironde estuary, France	Intertidal mudflat	6 m	DI water dilution, sonication, centrifugation, ethanol precipitation and dialyze	Phenol sulfuric assay Lowry assay for proteins	Benthic diatoms	N.A	8-16 μg.g ⁻¹ DW sed (xanthan eq.)	0.6-37 μg.g ⁻¹ DW sed	This study (10)
Chausey archipelago, France	Tidal flats	2 mm	DI water dilution and centrifugation	Phenol sulfuric assay Lowry assay for proteins	Benthic diatoms	N.A	1-4 mg.cm ⁻³ (glucose eq.)	2-6 mg.cm ⁻³	Passarelli et al., 2012 (11)
Neckar River, Germany	River sediments	5 mm	DI water dilution, dower resin and centrifugation for colloidal, dower resin and centrifugation for bound from sediment	Phenol sulfuric assay Lowry or Bradford assay for proteins	Benthic diatoms	1.6-3.6 mg.g ⁻¹ DW sed	0.2-0.5 mg.g ⁻¹ DW sed (glucose eq.)	0.9-10.2 mg.g ⁻¹ DW sed	Gerbersdorf et al., 2009 (12)
Elbe River, Germany	River sediments	5mm	DI water dilution, dower resin and centrifugation for colloidal, dower resin and centrifugation for bound from sediment	Phenol sulfuric assay Lowry or Bradford assay for proteins	Benthic diatoms	1.3-6.7 mg.g ⁻¹ DW sed	0.4-3.4 mg.g ⁻¹ DW sed (glucose eq.)	2-7.4 mg.g ⁻¹ DW sed	Gerbersdorf et al., 2009 (12)

Study site	Sampling sediment	Depth sampled	Extraction methods	Quantification method	Main organism secreting EPS	Bound EPS	Colloidal EPS	Protein	Ref
Highborne Cay, Bahamas	Shallow subtidal stromatolite	2 cm	Hot water (40°C) dilution with 4 mM EDTA, centrifugation and alcohol precipitation	Phenol sulfuric assay	Cyanobacteria and heterotrophic bacteria	N.A	0.1-0.7 mg.g ⁻¹ DW sed (glucose eq.)	N.A	Decho et al., 2005 (a)
Cayo Coco hypersaline Lagoon, Cuba	Microbial mat	10 mm	DI water dilution with 1 mM EDTA, centrifugation, alcohol precipitation and dialyze	Phenol sulfuric assay Micro-bicinchoninic (BCA) assay for proteins	Cyanobacteria and heterotrophic bacteria	N.A	0.7-50 μg.g ⁻¹ WW sed (xanthan eq.), 13 to 128 μg.g ⁻¹ WW (glucose eq.)	28-172 μg.g ⁻¹ DW sed	Pace et al., 2018 (b)
Salt Pan hypersaline lake, Bahamas	Microbial mats	4 cm	DI water dilution with 100 mM EDTA, centrifugation, alcohol precipitation and dialyse	Phenol sulfuric assay	Cyanobacteria and heterotrophic bacteria	N.A	2-10 mg.g ⁻¹ DW sed (xanthan eq.)	N.A	Braissant et al., 2009 (c)
Rangiroa and Tetiaroa atolls, brackish ponds, French Polynesia	Microbial mats	50 cm	Hot NaCl water (20 g·L ⁻¹ , 80°C) dilution, centrifugation, alcohol precipitation and dialyze	Orcinol-sulfuric acid for neutral sugars and Lowry assay for proteins	Cyanobacteria and heterotrophic bacteria	N.A	25-40 mg.g ⁻¹ DW sed	4-13 mg.g ⁻¹ DW sed	Che et al., 2001 (d)
Salins-de-Giraud hypersaline lake/salina, France	Microbial mats	10 cm	DI water or saline water (10%) dilution, centrifugation, alcohol precipitation and dialyze	Phenol sulfuric assay	Cyanobacteria and heterotrophic bacteria	N.A	9.7-32 mg. g ⁻¹ DW sed (glucose eq.)	N.A	Klock et al., 2007 (e)
Salins-de-Giraud hypersaline lake/salina, France	Microbial mats	10 cm	DI water or saline water (10%) with EDTA (4 mM) dilution, centrifugation, alcohol precipitation and dialyze	Phenol sulfuric assay	Cyanobacteria and heterotrophic bacteria	N.A	15-43 mg. g ⁻¹ DW sed (glucose eq.)	N.A	Klock et al., 2007 (e)

Table 6.2: Comparison of EPS concentrations in various studies on brackish to hypersaline microbial mats. The extraction methods and the techniques used to estimate the concentrations are also indicated.

Table 6.1: Page (279) above. Comparison of EPS concentrations in various studies on river, estuarine or mudflat biofilms. The extraction methods and the techniques used to estimate the concentrations are also indicated.



Figure 6.2: Range of colloidal EPS concentrations measured in biofilms, microbial mats and sediments from different depositional environments: (i) estuaries (brown), (ii) rivers (green) and (iii) brackish to hypersaline mats (blue). Detailed about extraction protocols and measurement methods are available in tables 6.1 and 6.2. Mudflat biofilm EPS concentration is in brown, river biofilms in green and microbial mats in blue. Numbers refer to studies on the table 6.1 and letter to studies in the table 6.2. *Pace et al. (2018) is expressed in $\mu g.g$ sediment⁻¹ WW.

Differences related to environmental factors. Differences in EPS concentrations can be related to the hydrodynamics of the studied site. Protected bay are for example less subject to erosion than open estuaries (e.g., Deloffre et al., 2007). In inner estuaries affected by river floods, the biofilm can undergo significant erosion and transport (e.g., Duteil et al., submitted, chapter 3). Large, opened estuaries can also be subject to waves generated by strong winds during storms. Such waves erode and transport diatoms and EPS, as observed in the Severn estuary (UK; Underwood & Paterson, 1993). Grazing and bioturbation by e.g., worms or mollusks, can also increase sediment and biofilm erodibility (e.g., Widdows & Brinsley, 2002).

Concentrations of EPS in brackish to hypersaline microbial mats. In microbial mats, EPS concentrations are also highly variable (Table 6.2; Figure 6.2). Most studies extracted the colloidal fraction of EPS, and only one study measured the amount of proteins using the BCA assay (Pace et al., 2018). Most studies used EDTA-extraction, which gives the highest yields (Klock et al., 2007). The lowest polysaccharide amounts were measured by Pace et al. (2018) in hypersaline lakes from Cuba, between 20 and 150 μ g_{EPS}.g_{sediment}⁻¹ WW. However, the concentrations in this study are expressed in EPS per grams of wet sediment, which could largely underestimate the concentrations. Che et al. (2004) measured concentrations up to 40 mg.g_{sediment}⁻¹ DW, but EPS were extracted in hot water, leading to possible overestimations. Other studies measured concentrations comprised between hundreds of μ g_{EPS}.g_{sediment}⁻¹ DW (Decho et al., 2005; Highborne Cay, Bahamas) and several tens of mg_{EPS}.g_{sediment}⁻¹ DW (Braissant et al., 2009; Salt Pan, Bahamas).

EPS in mats versus biofilms: different approaches or real difference in productivity? The majority of studies considered here characterized the polysaccharides in the colloidal fraction of EPS (for a detailed definition, see section 1.2.2.1), more rarely bound EPS or proteins. The wide use of the phenol-sulfuric assay facilitates comparisons for polysaccharides. Techniques to measure proteins in EPS are more diverse: Lowry, Bradford and BCA assays, and often adapted to the specificities of the EPS. These different approaches imply that care must be taken when comparing the concentrations between studies (Tables 6.1 and 6.2). Standardization methods could be developed by using the techniques listed above on a series of EPS of known compositions. Besides, some EPS components remain underexplored despite an established influence on the physico-chemical properties of these organic matrices, e.g., proteins, nucleic acids (Decho, 1990; Flemming & Wingender, 2001; Decho & Gutierrez, 2017). For example, proteins account for 10 to 15% of the EPS of the benthic diatom *Cylindrotheca closterium* (Schlekat et al., 1998), and for up to 60% of bacterial EPS (Decho & Lopez, 1993; Flemming et al., 1999). Among the studied sedimentary systems, lagoonal to microbial mats present the

highest average concentrations in colloidal polysaccharide (2.5 mg.g_{sediment}⁻¹ DW). Our results contrasts with the results of De Winder et al. (1999), who found 5 to 100 times more carbohydrates in diatom biofilms than in cyanobacterial mats. Environmental factors such as light quality and quantity (Han et al., 2014), nutrient limitation, high salinity (Paliwal et al., 2017) are known to increase or decrease the secretion of EPS. Hypersaline microbial mats are exposed to strong salinity changes (e.g., Bouton et al., 2016). For example, the halophile alga Dunaliella is able to increase the production of polysaccharides or amino acids in response to salt stress (Mishra et al., 2008). Intertidal diatom biofilms could also increase the production of proteins to increase carbon storage, or protect against desssiccation or increasing salinity The sedimentary dynamics could also play a major role in EPS concentrations. The sedimentation rates calculated for the Bordeaux Nord point bar are comprised between 20 and 40 mm.yr⁻¹ (Virolle et al., 2021). In the Cayo Coco hypersaline lagoon, they are comprised between 0.05 and 6 mm.yr⁻¹ (Pace et al., 2018). An intense detrital sedimentation could dilute EPS despite a reasonable productivity. In order to better understand the potential impact of environmental factors on EPS production, we plan to complete our dataset with water chemistry (e.g., salinity, pH, dissolved nutrients), sedimentation (sedimentation rates, current velocity) or climatic parameters (temperature, insolation). Microbial and enzymatic activities could also help estimating the EPS degradation potential in various types of sediments, but are rarely quantified.

We have also compared the monosaccharide composition of colloidal EPS in microbial mats and diatom biofilms (Table 6.3). In both cases, monosaccharides are mainly composed of glucose and also include lower quantities of galactose, xylose and rhamnose. In intertidal biofilms, the monosaccharides in colloidal and bound EPS change seasonally (Pierre et al., 2014). EPS produced in the summer are richer in glucose, and contain less structural sugars (xylose and mannose) and uronic acids. This seasonal increase in glucose could be a response to trophic need of EPS consumers (e.g., heterotrophic bacteria).

Monosaccharide	Diatom biofilm	Microbial mat
Glucose	32%	42.5%
Galactose	15.5%	28%
Xylose	33%	10.4%
Arabinose	0%	4.6%
Rhamnose	10.5%	6.5%
Fucose	0%	8%
Mannose	9%	0%

Table 6.3: Comparison of monosaccharide composition of colloidal EPS in diatom biofilm (Ems-Dollard estuary, Netherlands; data from Staats et al., 1999), and in a hypersaline microbial mat (Highborne Cay, Bahamas; data from Kawaguchi & Decho, 2002b). These are only two examples and fair amount of variation might be found in composition.

6.1.4 Sediment-EPS interactions: aggregation, attachment, trapping and binding

Interactions between EPS and sedimentary grains are common, but occur through contrasted mechanisms. In this thesis, we documented the role of diatom biofilm EPS in the aggregation of clay and silt- sized particles and sands, resulting in the formation of clay-coated sand grains (see chapter 5 and Duteil et al., 2020; Figure 6.3A and 6.3B). Our experiments have shown that the clay coating could form in the absence of active diatom or other microorganisms. Epipsammic (i.e., living in sands) diatoms, usually small forms, are able to secrete a mucilage in order to attach to sand grains in estuarine and coastal sands (e.g., Stal, 2003; Jewson et al., 2006), but also in carbonate microbialites (Winsborough, 2000). This mucilage is rich in proteins and glycoproteins and probably has a contrasted composition with epipelic biofilm EPS (Cooksey & Wigglesworth, 1995).

Cyanobacteria are rarely found on muddy substrates in estuaries or mudflats, possibly because of the rapid attenuation of light in this type of sediments or because they are less motile in fine sediments than diatoms (Stal, 2003). Trapping and binding of sands (e.g., bioclasts, quartz) is a common process in marine and hypersaline microbial mats. The principal growth process in open marine stromatolites from the Bahamas is through trapping of ooids by filamentous cyanobacteria (Reid et al., 2000), and this trapping could be achieved through the secretion of EPS (Paterson et al., 2008). Trapping of ooids and quartz was also documented in coccoid-

dominated microbial mats and microbialites in Great Salt Lake (Vennin et al., 2019). In nonlithified mats, these grains are fully embedded in EPS (Figure 6.3C and 6.3D; Duteil, 2017).



Figure 6.3: Cryo-SEM and SEM pictures showing interactions between detrital grains and EPS in two contrasted environments: estuarine diatom biofilms (A and B) and in Great Salt Lake hypersaline microbial mat (C and D). (A) Quartz sand grain and clay particle trapped in EPS matrix. (B) Zoom at the edge of the sand grain showing alveolar EPS sticking to the grain surface. (C) Detrital grains (carbonate or quartz) trapped in EPS matrix; (B) Zoom on the same grains showing that the EPS completely engulfs the grains. The last two pictures were taken during one of my internships (Duteil, 2017).

6.1.5 Evolution of EPS with depth in the sediment

In this sub-section, we will consider the colloidal EPS fraction. Depth EPS profiles were established in estuarine mudflat sediments (e.g., De Winder et al., 1999; Taylor et al., 1999; Paterson et al., 2000; De Brouwer & Stal, 2001; Duteil et al., submitted, chapter 3; Figure 6.4) or in hypersaline and open marine microbial mats (Decho et al., 2005; Braissant et al., 2009; Pace et al., 2018; Figure 6.5). Estuarine and fluviatile sediments show high concentrations of colloidal EPS at the surface or close to the surface, followed by a rapid decrease in the first millimeters to centimeters of the sediment (Figure 6.4). This general trend can be more complex

locally, with profiles showing an initial increase preceding a decrease, as documented for example in the Humble Estuary (Paterson et al., 2000). High concentrations of EPS are correlated with high concentrations of chlorophyll a and with an elevated diatom biomass (Paterson et al., 2000). Hypersaline and normal marine mats (Figure 6.5) either show an increase followed by a decrease in colloidal EPS concentrations in the first centimeter (e.g., Braissant et al. 2009), or successive trends of decreasing concentrations (e.g., Decho et al., 2005; Pace et al., 2018). Such trends could reflect repetitive processes of production and degradation of EPS during the growth of the microbial mat (Pace et al., 2018). Such process could result in the formation of lamination in stromatolites (Dupraz et al., 2013; Pace et al., 2018).

Beyond local specificities, most authors observe an overall decrease in colloidal EPS concentrations at depth ranging from millimeters to decimeters. Such decrease has been locally correlated to enzymatic activity (e.g., glucosidase and galactosidase), reflecting consumption of EPS by heterotrophic bacteria (Braissant et al., 2009). In estuaries, benthic heterotrophic bacterial production is tightly coupled to colloidal EPS secretion of diatoms (van Duyl et al. 1999). Degradation of EPS consists in a multistep process representing sequential degradation of different components, ranging from highly labile to relatively refractory (Nankai et al., 1999). The resultant removal of charged monomers or low molecular weight organic compounds (LMWOC) will result in a net loss of functional groups on EPS, and potentially reduce their potential interactions with metals (Braissant et al., 2009). De Winder et al. (1999) observed a reverse trend between water-extractable and EDTA-extractable EPS (called bound EPS in their study), with an increase with depth. Consequently, they considered bound EPS as a more refractory fraction susceptible to accumulate in the sediment. EPS were rarely extracted and analyzed in sediments deeper than a few decimeters (e.g., Gerbersdorf et al., 2009; Figure 6.4), but our study has shown that reactive EPS can be preserved several meters deep in estuarine sediments. Such preservation could be explained by (i) an incomplete microbial degradation of EPS; (ii) a protection of degradation through complexation of part of the EPS with mineral surfaces or (iii) a rapid burial following a sedimentation event (see Duteil et al., submitted, chapter 3, for a more in-depth discussion on the topic). Although diatoms and cyanobacteria are highly efficient and predominant EPS producers in most sedimentary systems (e.g., Stal, 2003), other groups of microorganisms are able to produce EPS. In hypersaline mats from Cuba, Pace et al. (2018) found concentrations of proteins six times higher in the purple sulfur bacteria-dominated layer than in the cyanobacteria layer. This result could reflect significant differences in the nature of EPS produced by oxygenic and anoxygenic phototrophs. Heterotrophic communities (e.g., sulfate-reducing bacteria) are also able to produce EPS, albeit at lower production rates than photosynthetic communities and often by consuming EPS already present in the sediment (Braissant et al., 2007). SRB could be active several meters deep in the Gironde sediments (Duteil et al., in prep., chapter 4) and could participate to the production of EPS in relatively deep sediments. Non-negligible amounts of reactive EPS could be preserved at depth in various sedimentary environments.



Figure 6.4: Examples of depth colloidal EPS profiles in sediments covered by biofilms. (A) Profiles to the left have been measured in mudflats from the Humber estuary (UK; Paterson et al., 2000) and the Westerschelde estuary (Netherlands; De Brouwer & Stal, 2001). (B) The profile to the right was measured in sedimentary cores from the river Elbe. Note the difference in scales between the two profiles, the first is in millimeters while the second is in centimeters.



Figure 6.5: Examples of depth-EPS profiles in sediments covered by microbial mats. (A) The profile to the left was measured in an open marine stromatolite from Highborne Cay (Bahamas; Decho et al., 2005), the profile in the middle in the lagoon of Cayo Coco (B; Cuba; Pace et al., 2018) and the profile to the right in Salt Pan, a hypersaline lake from the Bahamas (C; Braissant et al., 2009).

6.2 EPS in the sediment to rock transition and their potential preservation

6.2.1. EPS Relationships between EPS cycling, carbonate precipitation and early diagenesis in hypersaline microbialites

EPS have a strong affinity for divalent cations. The adsorption of metals to EPS can have advantages for the microbial community: (i) it regulates the flow of micronutrients (e.g. iron, zinc, copper) to the cells (Decho & Gutierrez, 2017); (ii) it protects cells against metal toxicity (Bhaskar & Bhosle, 2006; Duong et al., 2008). Freshly secreted EPS have a high capacity to chelate metals from the environment. Metals e.g., calcium, iron, can be loosely or strongly bound to EPS (Stal, 2003; Braissant et al., 2009; Pace et al., 2018). The same way, Pace et al. (2018) measured tightly bound Ca, Mg, Fe and Si in EPS from Cayo Coco hypersaline mats. Tightly bound calcium represented ca. 2% of EPS calcium binding capacity, and tightly bound magnesium ca. 5%. EPS are generally less abundant and reactive with increasing sediment depth, owing to degradation by heterotrophic bacteria using EPS as a source of carbon (Braissant et al., 2009; see above, section 6.1.5). Metals can be bound to surface biofilm EPS, but also inside the sediment through interactions between porewater and preserved EPS (Duteil et al., in prep., chapter 4). The subsequent microbial or physicochemical degradation of EPS is susceptible to release these metals, which become available for diagenetic reactions.

In hypersaline microbial mats, the heterotrophic degradation of LMWOC results in the release of Ca²⁺ and in the precipitation of CaCO₃, ultimately leading to the formation of a microbialite (e.g., Braissant et al., 2009; Figure 6.6). This process creates a microenvironment favorable to the precipitation of carbonates, mostly in the form of Mg-calcite and/or aragonite. In addition, microbial degradation of EPS produces also HCO3⁻, increases the alkalinity and reduces the quantity of cation-binding sites, inhibiting CaCO₃ precipitation (Braissant et al., 2009). The correlation between the sulfate reduction activity and the precipitated CaCO₃ implies a strong involvement of SRB in such mechanism (Visscher et al., 2000; Dupraz et al., 2004; Dupraz et al., 2013; Pace et al., 2016). In that case, the precipitation of carbonates is microbially-induced (Dupraz et al., 2009). Extrinsic or intrinsic supersaturation of EPS by calcium have been proposed as alternative mechanisms for carbonate precipitation (supersaturation of EPS binding sites...) in hypersaline and marine stromatolites (Dupraz et al., 2011). In the latter case, the precipitation is microbially-influenced. The precipitation of carbonate minerals occurs close to the mat surface and probably in a very short time (i.e., within months), resulting in the formation of carbonate lamina (Pace et al., 2018). The degradation of EPS increases further at depth resulting in increase carbonate precipitation and early diagenetic modifications (Dupraz et al., 2013). Dissolution of the initial aragonite

in interaction with degraded EPS in circumneutral porewater could result in early diagenetic dolomite precipitation, as observed in Great Salt Lake (Pace et al., 2016). EPS-metal interactions can result in the precipitation of other mineral phases. Metal-enriched sulfides aligned with mat stratification form during the earliest phase of diagenesis in hypersaline microbialites. This sulfide precipitation could result from an abiotic remobilization and concentration of iron in EPS during the early diagenesis (Sforna et al., 2017). EPS can serve as a substrate for mineral precipitation, even in the absence of metabolically active cells. Braissant et al. (2009) documented CaCO₃ precipitation within 5 days in purified microbial mat EPS placed under CO₂ and NH₃ atmosphere, without calcium addition. The same way, Bontognali et al. (2014a) precipitated Ca-Mg carbonates experimentally only in the presence of EPS by adding Ca²⁺ and Mg²⁺ in the medium.

6.2.2. Role of EPS in silicate precipitation

Due to their reactivity, EPS have not only interactions with metals, but can also be the substrate for silicate precipitation. Magnesium silicates (e.g., kerolite, stevensite, sepiolite) have been documented in EPS from hypersaline and alkaline mats and microbialites (Leveille et al., 2000; Burne et al., 2014, Zeyen et al., 2015, Pace et al., 2016, 2018; del Buey et al., 2021). In order to precipitate, these minerals require a high pH (>8.5) combined with elevated Mg and Si activities. In microbial mats, these conditions are met for example in the zone of oxygenic photosynthesis, where pH locally reaches 11. Diatom frustule dissolution in such alkaline porewater is a possible source for silica. Leveille et al. (2000) documented kerolite crystals forming tangential layers around cyanobacteria and covering EPS in microbial mats from basaltic cavities. In Great Salt Lake, Mg silicates covered the alveolar EPS network (Pace et al., 2016), while in Cayo Coco they formed patches within the EPS or encrusted coccoid cells (Pace et al., 2018). Mg silicate patches could also nucleate around SRB clusters (Pace et al., 2018), which are known to increase pH in their immediate environment (Decho, 2010).

Ueshima and Tazaki (2001) documented nontronite (Fe-smectite), whose layer followed the orientation of the EPS matrix in deep-sea sediments. In subsequent experiments, the same authors mixed a model-EPS solution (dextrin and pectin) with iron hydroxides (derived from ferro-siliceous groundwater), which resulted in the formation of Fe-rich layered silicates. They proposed that the accumulation of silica and iron in EPS could result in the precipitation of nontronite, with EPS serving as a template for layered-silicate nucleation. del Buey et al. (2021) proposed a relationship between the density of amino-groups in EPS and the growth of palygorskite crystals in microbial mats from hypersaline, playa lakes. As documented by Bontognali et al. (2014b) in experiments, the presence of certain organic acids (e.g., succinic acid) can enhance smectite precipitation. Succinic acid is common product of EPS degradation

by aerobic respirers. Pace et al. (2018) proposed that Mg-Si phase precipitation could result from Mg and Si scavenging by EPS, followed by site-specific heterotrophic degradation.



Figure 6.6: Conceptual model of microbially mediated CaCO₃ precipitation in the EPS matrix from hypersaline microbial mats, after Braissant et al., 2009. Step 1: Two types of functional groups (A, B) with different pK and Kd values are found on EPS. Step 2: calcium binds either loosely or tightly to functional groups A and B. Step 3: LMWOC form a bidentate complex with calcium on EPS. Step 4: microbial degradation activity consumes LMWOC (solid arrow), which is subsequently oxidized to bicarbonate (dashed arrow). Step 5: the EPS–Ca complex binds carbonate. CaCO₃ either remains linked to the EPS matrix (as EPS-A-CaCO₃) or exists forms pockets within the EPS matrix.

6.2.3. A potential role for EPS in the diagenesis of estuarine sandstones

Deeply buried (>2 km) estuarine sandstones are rich iron-rich diagenetic chlorite (e.g., Dowey et al., 2012; Worden et al., 2020). Fe-chlorite crystallizes as rims around sand grains, whereby inhibiting the development of quartz cements and preserving reservoir properties (Worden & Morad, 1999). Fe-chlorite precipitation could result from the transformation of intermediate mixed layer mineral series during burial diagenesis (Beaufort et al., 2015). These reactions depend on increasing temperature, pressure, and on the availability of some metal species such as iron or potassium (Virolle et al., 2019; Figure 6.7). First, the interaction between detrital aluminous silicates (e.g., kaolinite) and iron-rich minerals in reducing sedimentary fluids results in the precipitation of precursor clay minerals, such as berthierine (Virolle et al., 2020). Berthierine (Fe^{II},Fe^{III},Al)₃(Si,Al)₂O₅(OH)₄) has a chemical formula close to Fe-chlorite, but a different crystalline structure. Berthierine can be subsequently transformed into Fe-chlorite at a temperature range comprised between 40 and 120°C (Beaufort et al., 2015; Figure 6.7). Virolle et al. (2020) have identified berthierine in clay coatings within Early Cretaceous sandstones from the Paris Basin (France). Berthierine was absent at depths between 400 to 600 meters, while present at a depth of 900 meters. Their results indicate that berthierine precipitates at relatively low temperatures (<40°C), probably over long periods of times. The formation of berthierine necessitates a source of Fe^{2+} , which may derive from the reduction of iron, for example deriving from early diagenetic iron (oxy-)hydroxides, pyrite or siderite (Virolle et al., 2020).

	Burial stage	Origin of clay supply	Clay minerals							
1	Sedimentation	Detrital	Mica/Illite	Chlorite	Kaolinite			Dioctahedral smectite (Montmorillonite)		
Increasing burial and temperature	Shallow burial	Eogenetic			(+K ⁺) ↓ /S _(<u>R0)</u>	Authigeni _kaolinite	$+Fe^{2+}$ \downarrow C Berthierine	HFe ²⁺ ↓ Berthierine	(+K ⁺) ↓ I/S _ (R0)_	
	Deep burial (compaction)	Mesogenetic	•••••	•••••	↓ I/S (R1) ↓	↓ Kaolinite + Dickite ↓	↓ C/B ↓	↓ C/B ↓	↓ I/S (R1) ↓	
			Mica/Illite	Chlorite	Illite	Dickite	Fe-chlorite	Fe-chlorite	Illite	

Figure 6.7: Schematic illustration of possible evolutions of detrital clay minerals during burial diagenesis. I/S= Illite-smectite mixed layer; C/B= chlorite-berthierine mixed layer. After Virolle et al. (2019).

Iron (oxy-)hydroxides can nucleate on acidic polysaccharides, such as those produced by iron-oxidizing bacteria (Chan et al., 2011). Experiments and modelling have shown that alginates produced by *Shewanella* (an iron-reducing bacteria) decrease the nucleation barrier for hematite and ferrihydrite precipitation (Sand et al., 2020). Iron-EPS complexes could be a major source of iron for clay mineral diagenetic transformations. Penn et al. (2001) documented coatings composed of a mixture of Si- and Al-rich nanophases, smectite and goethite in an aquifer on the Atlantic coastal plain. Iron oxides occurred as nanoparticles within the Si and Al nanophases. Diatom frustules and putative remains of iron-oxidizing bacteria were observed in the same sediments. Microbially-mediated dissolution of detrital clay minerals (e.g., smectite) releasing iron and silica could constitute another source of iron in sandstone diagenesis (Vorhies & Gaines, 2009). The EPS matrix could also be involved in the precipitation of other iron-rich mineral phases such as iron-phosphates (Konhauser et al., 1994), ferrihydrite (Konhauser, 1998) or Fe-Al-silicates complexes (Konhauser, 1998).

In this study, we have shown the role of EPS in the formation of detrital clay coats in surface estuarine sediments (Duteil et al., 2020; chapter 5). Preliminary measurements show that Gironde EPS bind iron in surface biofilms. Detrital coats are composed of a mix of detrital clay minerals and highly reactive EPS, which is preserved at least six meters below the sediment surface (Duteil et al., submitted; chapter 3). Clay minerals were not significantly transformed in the first meters of the sediment (Virolle, 2019). We have also observed concentrations of dissolved Fe(III) increasing with depth in the studied sediment porewaters (Duteil et al., submitted; chapter 3). We have not determined yet if this iron was free or complexed to small organic molecules which could derive from EPS degradation. Based on our results and on comparisons from the literature, we propose a new hypothesis where iron is released in the sediment through microbial and thermal degradation of EPS (Figures 6.8 and 6.9).



Figure 6.8: Schematic illustration of the diagenetic evolution of clay coats, from detrital clay coatings formed in the sedimentary environment to iron rich chlorite coatings precipitated during deep burial. In red, the potential roles or signatures of EPS. Modified from Billault et al. (2003), Wooldridge et al. (2018) and Virolle (2019).



Figure 6.99: (A) Conceptual model of the interactions between EPS, iron and clay minerals during the diagenesis of estuarine sands, inspired by the models Dupraz & Visscher (2005) and Braissant et al. 2009 in hypersaline mats. (A.1) Fresh EPS possess functional groups (a, b) with different pK and Kd values for iron. (A.2) Iron binds more or less tightly to functional groups A and B, respectively. (A.3) LMW organic carbon and iron form a bidentate complex on EPS. (A.4) Microbial degradation activity removes both LMW organic carbon and iron from EPS leading to the precipitation of iron rich minerals. These minerals can be iron (oxy)hydroxides or sulfides depending on porewater redox conditions, and can be linked to the EPS matrix or exist freely in pockets within the EPS matrix. The dissolution minerals or the degradation of EPS-mineral complexes can be a source of iron for clay transformations during diagenesis. (B) Estuarine sediments and sandstones at increasing diagenetic stages (B.1 to B.3). The potential transformations depicted in (A) would occur between stage (B.1) and (B.2). (B.1) Modern sediment, Gironde Estuary from Virolle et al. (2019). (B.2) Moderately buried estuarine sandstone (0.5 km deep), Early Cretaceous, Paris Basin from Virolle et al. (2022). (B.3) Deeply buried sandstone (3.5 km deep), Permian, Petrel Basin (Australia) from Saïag et al. (2016).

6.2.4. EPS-mineral interactions as biosignatures of past microbial activity

Minerals precipitated in interaction with EPS are increasingly used as biosignatures of past microbial activity (e.g., Sforna et al., 2017; Pace et al., 2016, 2018, Visscher et al., 2020, Bourillot et al., 2020, Marin-Carbonne et al., 2022). Microbialites are among the best documented examples of EPS-related carbonate precipitation (see section 6.2.1; Figure 6.10). They are an obvious marker of past microbial activity, as well as arguably represent the oldest complex microbial ecosystems preserved on Earth, dating back to at least 3.43 Ga (e.g., Allwood et al., 2006). Biosignatures of EPS-mineral interactions are less documented in siliciclastic sedimentary systems. The physical properties of the gel-like cohesive matrix formed by EPS may produce macroscopical biosedimentary features called microbially induced sedimentary structures or MISS (Noffke, 2009). Some MISS are formed through interactions between biofilms and sedimentary particles, e.g., wavy crinkle lamina. These MISS are generally concentrated in the zone of biofilm stability, i.e., on low-energy muddy and sandy substrates of tidal flats or lagoons (Noffke, 2009), and have been documented in rocks as old as 3.3-3.4 Ga (Westall et al., 2006; Tice, 2009). The potential to preserve such biosignatures in high-energy tidal deposits is very low. Our results show that detrital clay coats are produced through the aggregation of EPS, fine-grained sediments (clay and silt-sized particles) and sands. EPS are mainly produced in biofilms located on intertidal mudflats and subsequently transported to high energy area such as estuarine point bars or tidal bars (Virolle et al., 2019; Duteil et al., 2020; Virolle et al., 2021). Therefore, detrital clay coats can be viewed as biosignatures of biofilm development and EPS production which can be preserved in highenergy sandstones (Figure 6.10). Detrital clay coats have been documented in many ancient sandstones, not only in estuarine or coastal settings, but also in desert and deep water environments (Dowey et al., 2012). This thesis calls for new investigations on these ancient sandstones, which could be a unique archive of the evolution of biofilm communities on Earth.



Figure 6.1010: Preservation of EPS-sediment interactions in the sedimentary record of two sedimentary environments. (A) Estuarine environment: during their development, diatom secrete EPS in order to attach to sediments (left). Detrital clay coats form through interactions between EPS and sediments (middle). Similar detrital clay coats could be biosignatures of EPS production in ancient estuarine sandstones, here Upper Cretaceous estuarine sandstones from the Sego Formation (right; Utah, USA). (B) Hypersaline lakes: microbial mats are rich in EPS mainly secreted by photoautotrophic communities (left; cyanobacteria (A-B) and Purple Sulfur Bacteria (C-D); from Pace et al. (2018). The degradation of EPS by heterotrophs results in carbonate precipitation (middle) and in the formation of microbialites which can be preserved in the sedimentary record (right; stromatolite from Great Salt Lake, USA).

6.3 Perspectives

6.3.1 Experiments on EPS from diatom cultures

In order to produce higher amounts of EPS for new experiments, we have cultivated diatoms extracted from Gironde biofilms (Figure 6.11). Cultures were prepared by N. Le Roy (postdoc), I. Betelu and J. Caumartin (engineering students) and myself. A drop of diatom biofilm is poured in an autoclaved Erlenmeyer flask filled with 50mL a mix of F/2 culture medium (Guillard & Ryther, 1962; Guillard, 1975; Annex 9 chapter 2) and artificial sea water (1g/L). The flasks were capped with a cotton plug and placed under natural light, other under artificial light. Cultures started December 7, 2020, and their evolution was monitored every four days through microscopic observations and colorimetric assays: TTC, FDA and Alcian Blue; see chapter 2 for details about the assays). Microscopic monitoring allowed to observe the evolution of the diatom community (e.g., genera, abundance), and potential contaminations. Colorimetric assays allowed to characterize the state of the biofilm, e.g., active cells (TTC) or production of EPS (Alcian Blue). In order to avoid contamination by green algae, a purification of the cultures was performed by successive transplantations following the Purification by Micropipette protocol described by Andersen (2005). Two cultures with the best characteristics (diatom dominance and abundant EPS secretion) were selected at each transplantation phase. A pellet consisting mainly of diatoms and EPS is sampled in the mother culture and then deposited in another flask to start a daughter culture. A 2 weeks growth phase was followed by a potential new transplantation (Figure 6.11).



Figure 6.1111: Method used for the laboratory culture of diatoms. At the beginning, a drop of biofilm is taken to be grown in F/2 medium under artificial light. Following the growth phase, a monitoring of the cultures is done through microscopic observations and colorimetric assays. The two best cultures are subsequently transplanted. After Betelu & Caumartin (2021).

Some of the cultures were contaminated by green algae (e.g., *Desmodesmus* sp.) and/or cyanobacteria (Figure 6.12), which appeared to be more competitive than diatoms and take precedence in the concerned samples. After one month of growth and three transplantations, two morphotypes of diatoms were dominant in the cultures. These diatoms were identified as *Navicula* sp. and *Achnanthes* or *Cocconeis* sp. The colorimetric assays confirmed that they were active and secreted a reactive EPS (Figure 6.13). After three months and six transplants, the selected cultures were mostly largely by diatoms, and the objective was to start EPS production. Unfortunately, this culture project had to stop partly because of restricted access to the lab during the Covid crisis. We had nevertheless considered subsequent experiments, which are described below.



Figure 6.1212: Culture contaminated by green algae. (A) Erlenmeyer flask showing a green colour following the development of green algae and/or cyanobacteria; (B) Unicellular green algae; (C) Filamentous cyanobacteria; (D) Association of diatoms, filamentous cyanobacteria and green algae; (E) Association of diatoms and green algae (including *Desmodesmus* sp.).

Influence of the sediment on EPS production. A subsequent experiment could be to grow diatoms on various types of substrata to test the influence of the sediment grain size, and mineralogy on EPS secretion. Diatoms could be cultured on sterilized sediment of different composition, e.g., Gironde mud, pure quartz, various clay minerals. EPS quantity and physicochemistry could be assessed at various times of the experiments by using rapid techniques such as colorimetric assays (alcian blue, phenol-sulfuric or Lowry), FTIR, or more in-depth characterizations such as proteomics. Chlorophyll-a could be used as a proxy to monitor the growth of diatoms (Andersen, 2005), and to standardize EPS measurements. The objective would be to test various hypothesis. For example, epipsammic diatoms secrete a specific mucilage to adhere to sand grains (Jewson et al., 2006) and bound EPS proteins could have a role in the pioneering stages of the biofilm (Ubertini et al., 2015). Besides, proteins are more reactive than polysaccharides towards quartz (Ghashoghchi et al., 2017). Therefore, we could expect an increase protein secretion or a change in protein composition in the presence of quartz. We could also test the influence of clay minerals with various characteristics, e.g. cation exchange capacity, specific surface area, on EPS secretion. Diatoms secrete EPS to attach to their substratum, but can they change the composition of secreted EPS in function of the surface reactivity?

Discussion and way forward



Figure 6.13: Diatom-rich culture characterized by a typical brown/golden color (A). (B, C, E) Culture rich in *Navicula* sp. (D, F, G) Culture rich in a smaller morphotype, possibly *Achnanthes* or *Cocconeis* sp. Acidic exopolymers are stained with Alcian Blue in (D) and (G).

6.3.2 Interactions between EPS, clay minerals and metals, and evolution through diagenesis

During this thesis, we have done preliminary measurements of the affinity of the Gironde diatom biofilm EPS for some metals: Ca, Mg, Zn. This cation binding capacity was measured using isothermal calorimetry (ITC) with Dr. Olivier Braissant at the University of Basel (Switzerland). ITC consists in measuring the adsorption enthalpy of a given metal on EPS by measuring the heat flow released through successive addition of the selected metal to EPS. Our objective would be to measure the affinity for metals in "fresh" and microbially degraded EPS, but also to measure the cation binding capacity for redox sensitive metals such as Fe and Mn. In order to evaluate the role of EPS in the early diagenesis of clay minerals and test the hypothesis discussed in section 6.2.3, we also propose to perform experimental diagenesis experiments involving EPS, clay minerals, metals and sand. Sediment burial conditions could be simulated using a high-pressure/high-temperature bioreactor developed at the Institut de Chimie de la Matière Condensée Bordeaux. The reactor allows to reach temperatures of 135°C and pressures up to 50 MPa, which are close to the conditions encountered by estuarine sandstones buried several kilometers deep in sedimentary basins. Such experiments could be set up on various mixes (e.g., with or without the addition of selected metals), and for various durations, from a few weeks to several months. The objective is to follow the potential degradation of EPS, the transformation of mineral and metal phases in various diagenetic conditions using measurements in situ (e.g., Raman probe) or ex situ (e.g., X-Ray diffraction, X-Ray absorption spectroscopy). We expect to document transfers of metal between EPS and mineral phases, and potential mineral transformations (e.g., nucleation of chlorite precursors). The next step would be to compare the mineralogical and EPS molecular diagenetic products in these experiments and in rocks from ancient estuaries. Finally, it also allows to demonstrate the chemical modifications of EPS and to establish targeted biomolecules that could be found in the sediment records. This would be a big step in understanding the impacts of diatom EPS on sedimentary mechanisms and in discussing whether they are biosignatures.

References

- Allwood, A.C., Walter, M.R., Kamber, B.S., Marshall, C.P., and Burch, I.W., 2006, Stromatolite reef from the Early Archaean era of Australia: Nature, v. 441, p. 714–718, doi:10.1038/nature04764.
- Andersen, 2005, Algal culturing techniques: Joaurnal of Phycology, v. 41, p. 906–908, doi:10.1111/j.1529-8817.2005.00114.x.
- Beaufort, D., Rigault, C., Billon, S., Billault, V., Inoue, A., Inoue, S., and Patrier, P., 2015, Chlorite and chloritization processes through mixed-layer mineral series in low-temperature geological systems – a review: Clay Minerals, v. 50, p. 497–523, doi:10.1180/claymin.2015.050.4.06.
- Bellinger, B., Abdullahi, A., Gretz, M., and Underwood, G., 2005, Biofilm polymers: relationship between carbohydrate biopolymers from estuarine mudflats and unialgal cultures of benthic diatoms: Aquatic Microbial Ecology, v. 38, p. 169–180, doi:10.3354/ame038169.
- Betelu, I., and Caumartin, J., 2021, Establishment and monitoring of diatom culture from the Gironde estuary: Stage de troisième année d'école ingénieur à l'ENSEGID, Bordeaux INP, p. 102.
- Bhaskar, P.V., and Bhosle, N.B., 2006, Bacterial extracellular polymeric substance (EPS): A carrier of heavy metals in the marine food-chain: Environment International, v. 32, p. 191–198, doi: 10.1016/j.envint.2005.08.010.
- Billault, V., Beaufort, D., Baronnet, A., and Lacharpagne, J.-C., 2003, A nanopetrographic and textural study of grain-coating chlorites in sandstone reservoirs: Clay Minerals, v. 38, p. 315–328.
- Bontognali, T.R., Martinez-Ruiz, F., McKenzie, J.A., Bahniuk, A., Anjos, S., and Vasconcelos, C., 2014a, Smectite synthesis at low temperature and neutral pH in the presence of succinic acid: Applied clay science, v. 101, p. 553–557.
- Bontognali, T.R.R., McKenzie, J.A., Warthmann, R.J., and Vasconcelos, C., 2014b, Microbially influenced formation of Mg-calcite and Ca-dolomite in the presence of exopolymeric substances produced by sulphate-reducing bacteria: Terra Nova, v. 26, p. 72–77, doi:10.1111/ter.12072.
- Bourillot, R., Vennin, E., Dupraz, C., Pace, A., Foubert, A., Rouchy, J.-M., Patrier, P., Blanc, P., Bernard, D., and Lesseur, J., 2020, The record of environmental and microbial signatures in ancient microbialites: The terminal carbonate complex from the Neogene basins of southeastern Spain: Minerals, v. 10, p. 276.
- Bouton, A., Vennin, E., Pace, A., Bourillot, R., Dupraz, C., Thomazo, C., Brayard, A., Désaubliaux, G., and Visscher, P.T., 2016, External controls on the distribution, fabrics and mineralization of modern microbial mats in a coastal hypersaline lagoon, Cayo Coco (Cuba): Sedimentology, v. 63, p. 972–1016.
- Braissant, O., Astasov-Frauenhoffer, M., Waltimo, T., and Bonkat, G., 2020, A review of methods to determine viability, vitality, and metabolic rates in microbiology: Frontiers in Microbiology, p. 2726.
- Braissant, O., Decho, A.W., Dupraz, C., Glunk, C., Przekop, K.M., and Visscher, P.T., 2007,

Exopolymeric substances of sulfate-reducing bacteria: interactions with calcium at alkaline pH and implication for formation of carbonate minerals: Geobiology, v. 5, p. 401–411.

- Braissant, O., Decho, A.W., Przekop, K.M., Gallagher, K.L., Glunk, C., Dupraz, C., and Visscher, P.T., 2009, Characteristics and turnover of exopolymeric substances in a hypersaline microbial mat:
 FEMS Microbiology Ecology, v. 67, p. 293–307, doi: 10.1111/j.1574-6941.2008.00614.x.
- de Brouwer, J., and Stal, L., 2001, Short-term dynamics in microphytobenthos distribution and associated extracellular carbohydrates in surface sediments of an intertidal mudflat: Marine Ecology Progress Series, v. 218, p. 33–44, doi:10.3354/meps218033.
- del Buey, P., Sanz-Montero, M.E., Braissant, O., Cabestrero, Ó., and Visscher, P.T., 2021, The role of microbial extracellular polymeric substances on formation of sulfate minerals and fibrous Mgclays: Chemical Geology, v. 581, p. 120403, doi: 10.1016/j.chemgeo.2021.120403.
- Burne, R.V., and Moore, L.S., 1987, Microbialites: organosedimentary deposits of benthic microbial communities: Palaios, p. 241–254.
- Burne, R.V., Moore, L.S., Christy, A.G., Troitzsch, U., King, P.L., Carnerup, A.M., and Hamilton, P.J., 2014, Stevensite in the modern thrombolites of Lake Clifton, Western Australia: A missing link in microbialite mineralization? Geology, v. 42, p. 575–578, doi:10.1130/G35484.1.
- Chan, C.S., Fakra, S.C., Emerson, D., Fleming, E.J., and Edwards, K.J., 2011, Lithotrophic iron oxidizing bacteria produce organic stalks to control mineral growth: implications for biosignature formation: The ISME journal, v. 5, p. 717–727.
- Cooksey, K.E., and Wigglesworth-Cooksey, B., 1995, Adhesion of bacteria and diatoms to surfaces in the sea: a review: Aquatic microbial ecology, v. 9, p. 87–96.
- Costerton, J.W., and Stoodley, P., 2003, A closer look: the complexities of dental biofilm: Dimensions of Dental Hygiene, v. 1, p. 36–37.
- Decho, A.W., 2000, Microbial biofilms in intertidal systems: an overview: Continental shelf research, v. 20, p. 1257–1273.
- Decho, A., 1990, Microbial exopolymer secretions in ocean environments: their role (s) in food webs and marine processes: Oceanogr. Mar. Biol. Annu. Rev., v. 28, p. 73–153.
- Decho, A.W., 2010, Overview of biopolymer-induced mineralization: what goes on in biofilms? Ecological Engineering, v. 36, p. 137–144.
- Decho, A.W., and Gutierrez, T., 2017, Microbial extracellular polymeric substances (EPSs) in ocean systems: Frontiers in microbiology, v. 8, p. 922.
- Decho, A.W., and Kawaguchi, T., 1999, Secretions Using N anoplast® Resin: BioTechniques, v. 27, p. 1246–1252.
- Decho, A.W., and Lopez, G.R., 1993, Exopolymer microenvironments of microbial flora: Multiple and interactive effects on trophic relationships: Limnology and Oceanography, v. 38, p. 1633–1645, doi:10.4319/lo.1993.38.8.1633.
- Decho, A.W., Visscher, P.T., and Reid, R.P., 2005, Production and cycling of natural microbial

exopolymers (EPS) within a marine stromatolite: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 219, p. 71–86, doi: 10.1016/j.palaeo.2004.10.015.

- Deloffre, J., Verney, R., Lafite, R., Lesueur, P., Lesourd, S., and Cundy, A.B., 2007, Sedimentation on intertidal mudflats in the lower part of macrotidal estuaries: sedimentation rhythms and their preservation: Marine Geology, v. 241, p. 19–32.
- Dowey, P.J., Hodgson, D.M., and Worden, R.H., 2012, Pre-requisites, processes, and prediction of chlorite grain coatings in petroleum reservoirs: a review of subsurface examples: Marine and Petroleum Geology, v. 32, p. 63–75.
- Duong, T.T., Morin, S., Herlory, O., Feurtet-Mazel, A., Coste, M., and Boudou, A., 2008, Seasonal effects of cadmium accumulation in periphytic diatom communities of freshwater biofilms: Aquatic Toxicology, v. 90, p. 19–28, doi: 10.1016/j.aquatox.2008.07.012.
- Dupraz, C., Fowler, A., Tobias, C., and Visscher, P.T., 2013, Stromatolitic knobs in Storr's Lake (San Salvador, Bahamas): a model system for formation and alteration of laminae: Geobiology, v. 11, p. 527–548, doi:10.1111/gbi.12063.
- Dupraz, C., Reid, R.P., Braissant, O., Decho, A.W., Norman, R.S., and Visscher, P.T., 2009, Processes of carbonate precipitation in modern microbial mats: Earth-Science Reviews, v. 96, p. 141–162, doi: 10.1016/j.earscirev.2008.10.005.
- Dupraz, C., Reid, R.P., Visscher, P.T., Reitner, J., and Thiel, V., 2011, Microbialites, modern: Encyclopedia of geobiology, p. 617–635.
- Dupraz, C., and Visscher, P.T., 2005, Microbial lithification in marine stromatolites and hypersaline mats: Trends in Microbiology, v. 13, p. 429–438, doi: 10.1016/j.tim.2005.07.008.
- Dupraz, C., Visscher, P.T., Baumgartner, L.K., and Reid, R.P., 2004, Microbe–mineral interactions: early carbonate precipitation in a hypersaline lake (Eleuthera Island, Bahamas): Sedimentology, v. 51, p. 745–765.
- Duteil, T., 2017, Interaction between Extracellular Polymeric Substances and minerals: comparison between two microbial sedimentary systems: Stage de troisième année d'école ingénieur à l'ENSEGID, Bordeaux INP.
- van Duyl, F., de Winder, B., Kop, A., and Wollenzien, U., 1999, Tidal coupling between carbohydrate concentrations and bacterial activities in diatom-inhabited intertidal mudflats: Marine Ecology Progress Series, v. 191, p. 19–32, doi:10.3354/meps191019.
- Flemming, R.G., Murphy, C.J., Abrams, G.A., Goodman, S.L., and Nealey, P.F., 1999, Effects of synthetic micro- and nano-structured surfaces on cell behavior: Biomaterials, v. 20, p. 573–588, doi:10.1016/S0142-9612(98)00209-9.
- Flemming, H.-C., and Wingender, J., 2001, Relevance of microbial extracellular polymeric substances (EPSs) - Part I: Structural and ecological aspects: Water Science and Technology, v. 43, p. 1–8, doi:10.2166/wst.2001.0326.
- Gerbersdorf, S.U., Westrich, B., and Paterson, D.M., 2009, Microbial Extracellular Polymeric

Substances (EPS) in Fresh Water Sediments: Microbial Ecology, v. 58, p. 334–349, doi:10.1007/s00248-009-9498-8.

- Ghashoghchi, R.A., Hosseini, M.R., and Ahmadi, A., 2017, Effects of microbial cells and their associated extracellular polymeric substances on the bio-flocculation of kaolin and quartz: Applied Clay Science, v. 138, p. 81–88.
- Guillard, R.R.L., 1975, Culture of Phytoplankton for Feeding Marine Invertebrates, in Smith, W.L. and Chanley, M.H. eds., Culture of Marine Invertebrate Animals, Boston, MA, Springer US, p. 29– 60, doi:10.1007/978-1-4615-8714-9 3.
- Guillard, R.R.L., and Ryther, J.H., 1962, Studies of marine planktonic diatoms: I. Cyclotella nana Hustedt, and Detonula confervacea (Cleve) Gran.: Canadian Journal of Microbiology, v. 8, p. 229–239, doi:10.1139/m62-029.
- Han, P., Sun, Y., Jia, S., Zhong, C., and Tan, Z., 2014, Effects of light wavelengths on extracellular and capsular polysaccharide production by Nostoc flagelliforme: Carbohydrate polymers, v. 105, p. 145–151.
- Jewson, D.H., Lowry, S.F., and Bowen, R., 2006, Co-existence and survival of diatoms on sand grains: european Journal of Phycology, v. 41, p. 131–146.
- Kawaguchi, T., and Decho, A.W., 2002a, A laboratory investigation of cyanobacterial extracellular polymeric secretions (EPS) in influencing CaCO3 polymorphism: Journal of Crystal Growth, v. 240, p. 230–235.
- Kawaguchi, T., and Decho, A.W., 2002b, Isolation and biochemical characterization of extracellular polymeric secretions (EPS) from modern soft marine stromatolites (Bahamas) and its inhibitory effect on CaCO3 precipitation: Preparative Biochemistry and Biotechnology, v. 32, p. 51–63.
- Klock, J.-H., Wieland, A., Seifert, R., and Michaelis, W., 2007, Extracellular polymeric substances (EPS) from cyanobacterial mats: characterisation and isolation method optimisation: Marine Biology, v. 152, p. 1077–1085, doi:10.1007/s00227-007-0754-5.
- Konhauser, K.O., 1998, Diversity of bacterial iron mineralization: Earth-Science Reviews, v. 43, p. 91– 121.
- Konhauser, K.O., Fyfe, W.S., Schultze-Lam, S., Ferris, F.G., and Beveridge, T.J., 1994, Iron phosphate precipitation by epilithic microbial biofilms in Arctic Canada: Canadian Journal of Earth Sciences, v. 31, p. 1320–1324, doi:10.1139/e94-114.
- Léveillé, R.J., Fyfe, W.S., and Longstaffe, F.J., 2000, Geomicrobiology of carbonate-silicate microbialites from Hawaiian basaltic sea caves: Chemical Geology, v. 169, p. 339–355, doi:10.1016/S0009-2541(00)00213-8.
- Marais, D.J.D., 1995, The biogeochemistry of hypersaline microbial mats: Advances in microbial ecology, p. 251–274.
- Marin-Carbonne, J. et al., 2022, Early precipitated micropyrite in microbialites: A time capsule of

microbial sulfur cycling: Geochemical Perspectives Letters, v. 21, p. 7–12, doi:10.7185/geochemlet.2209.

- Mehta, S.K., and Gaur, J.P., 2005, Use of algae for removing heavy metal ions from wastewater: progress and prospects: Critical reviews in biotechnology, v. 25, p. 113–152.
- Mishra, A., Mandoli, A., and Jha, B., 2008, Physiological characterization and stress-induced metabolic responses of Dunaliella salina isolated from salt pan: Journal of industrial microbiology and biotechnology, v. 35, p. 1093.
- Mohamed, Z.A., 2001, Removal of cadmium and manganese by a non-toxic strain of the freshwater cyanobacterium Gloeothece magna: Water Research, v. 35, p. 4405–4409.
- Nankai, H., Hashimoto, W., Miki, H., Kawai, S., and Murata, K., 1999, Microbial System for Polysaccharide Depolymerization: Enzymatic Route for Xanthan Depolymerization by Bacillus sp. Strain GL1: Applied and Environmental Microbiology, v. 65, p. 2520–2526.
- Noffke, N., 2009, The criteria for the biogeneicity of microbially induced sedimentary structures (MISS) in Archean and younger, sandy deposits: Earth-Science Reviews, v. 96, p. 173–180, doi: 10.1016/j.earscirev.2008.08.002.
- Olson, B.J., and Markwell, J., 2007, Assays for determination of protein concentration: Current Protocols in Pharmacology, v. 38, p. A. 3A. 1-A. 3A. 29.
- Orvain, F., De Crignis, M., Guizien, K., Lefebvre, S., Mallet, C., Takahashi, E., and Dupuy, C., 2014, Tidal and seasonal effects on the short-term temporal patterns of bacteria, microphytobenthos and exopolymers in natural intertidal biofilms (Brouage, France): Journal of Sea Research, v. 92, p. 6–18, doi: 10.1016/j.seares.2014.02.018.
- Pace, A. et al., 2018, Formation of stromatolite lamina at the interface of oxygenic–anoxygenic photosynthesis: Geobiology, v. 16, p. 378–398, doi:10.1111/gbi.12281.
- Pace, A., Bourillot, R., Bouton, A., Vennin, E., Galaup, S., Bundeleva, I., Patrier, P., Dupraz, C.,
 Thomazo, C., and Sansjofre, P., 2016, Microbial and diagenetic steps leading to the mineralisation of Great Salt Lake microbialites: Scientific Reports, v. 6, p. 1–12.
- Paliwal, C., Mitra, M., Bhayani, K., Bharadwaj, S.V.V., Ghosh, T., Dubey, S., and Mishra, S., 2017, Abiotic stresses as tools for metabolites in microalgae: Bioresource Technology, v. 244, p. 1216–1226, doi: 10.1016/j.biortech.2017.05.058.
- Passarelli, C., Meziane, T., Thiney, N., Boeuf, D., Jesus, B., Ruivo, M., Jeanthon, C., and Hubas, C., 2015, Seasonal variations of the composition of microbial biofilms in sandy tidal flats: Focus of fatty acids, pigments and exopolymers: Estuarine, Coastal and Shelf Science, v. 153, p. 29–37, doi: 10.1016/j.ecss.2014.11.013.
- Paterson, D.M., Aspden, R.J., Visscher, P.T., Consalvey, M., Andres, M.S., Decho, A.W., Stolz, J., and Reid, R.P., 2008, Light-dependant biostabilisation of sediments by stromatolite assemblages: PLoS One, v. 3, p. e3176.
- Paterson, D.M., Tolhurst, T.J., Kelly, J.A., and Honeywill, C., 2000, Variations in sediment properties,
Skeffling mudflat, Humber Estuary, UK: Continental Shelf Research, p. 24.

- Penn, R.L., Zhu, C., Xu, H., and Veblen, D.R., 2001, Iron oxide coatings on sand grains from the Atlantic coastal plain: High-resolution transmission electron microscopy characterization: Geology, v.
- 29, p. 843, doi:10.1130/0091-7613(2001)029<0843:IOCOSG>2.0.CO;2.
- Perry, T.D., Klepac-Ceraj, V., Zhang, X.V., McNamara, C.J., Polz, M.F., Martin, S.T., Berke, N., and Mitchell, R., 2005, Binding of harvested bacterial exopolymers to the surface of calcite: Environmental Science & Technology, v. 39, p. 8770–8775.
- Pierre, G., Zhao, J.-M., Orvain, F., Dupuy, C., Klein, G.L., Graber, M., and Maugard, T., 2014, Seasonal dynamics of extracellular polymeric substances (EPS) in surface sediments of a diatomdominated intertidal mudflat (Marennes–Oléron, France): Journal of sea research, v. 92, p. 26– 35.
- Reid, R.P., Visscher, P.T., Decho, A.W., Stolz, J.F., Bebout, B.M., Dupraz, C., Macintyre, I.G., Paerl, H.W., Pinckney, J.L., and Prufert-Bebout, L., 2000, The role of microbes in accretion, lamination and early lithification of modern marine stromatolites: Nature, v. 406, p. 989–992.
- Saïag, J., Brigaud, B., Portier, É., Desaubliaux, G., Bucherie, A., Miska, S., and Pagel, M., 2016,
 Sedimentological control on the diagenesis and reservoir quality of tidal sandstones of the Upper
 Cape Hay Formation (Permian, Bonaparte Basin, Australia): Marine and Petroleum Geology, v.
 77, p. 597–624, doi: 10.1016/j.marpetgeo.2016.07.002.
- Sand, K.K., Jelavić, S., Dobberschütz, S., Ashby, P.D., Marshall, M.J., Dideriksen, K., Stipp, S.L.S., Kerisit, S.N., Friddle, R.W., and DeYoreo, J.J., 2020, Mechanistic insight into biopolymer induced iron oxide mineralization through quantification of molecular bonding: Nanoscale Advances, v. 2, p. 3323–3333, doi:10.1039/D0NA00138D.
- Schlekat, C.E., Decho, A.W., and Chandler, G.T., 1998, Sorption of cadmium to bacterial extracellular polymeric sediment coatings under estuarine conditions: Environmental Toxicology and Chemistry, v. 17, p. 1867–1874, doi:10.1002/etc.5620170930.
- Sforna, M.C. et al., 2017, Patterns of metal distribution in hypersaline microbialites during early diagenesis: Implications for the fossil record: Geobiology, v. 15, p. 259–279, doi:10.1111/gbi.12218.
- Somers, G.F., and Brown, M., 1978, The affinity of trichomes of blue-green algae for calcium ions: Estuaries, p. 17–28.
- Staats, N., De Winder, B., STAL, L.J., and MUR, L.R., 1999, Isolation and characterization of extracellular polysaccharides from the epipelic diatoms Cylindrotheca closterium and Navicula salinarum: European Journal of Phycology, v. 34, p. 161–169.
- Stal, L.J., 2000, Cyanobacterial mats and stromatolites. The Ecology of Cyanobacteria: Their Diversity in Time and Space: Kluwer Academic Press, Dordrecht.
- Stal, L.J., 2003, Microphytobenthos, their extracellular polymeric substances, and the morphogenesis of intertidal sediments: Geomicrobiology Journal, v. 20, p. 463–478.

- Taylor, I.S., and Paterson, D.M., 1998, Microspatial Variation in Carbohydrate Concentrations with Depth in the Upper Millimetres of Intertidal Cohesive Sediments: Estuarine, Coastal and Shelf Science, v. 46, p. 359–370, doi:10.1006/ecss.1997.0288.
- Taylor, I.S., Paterson, D.M., and Mehlert, A., 1999, The quantitative variability and monosaccharide composition of sediment carbohydrates associated with intertidal diatom assemblages: Biogeochemistry, v. 45, p. 303–327, doi:10.1007/BF00993005.
- Tice, M.M., 2009, Environmental Controls on Photosynthetic Microbial Mat Distribution and Morphogenesis on a 3.42 Ga Clastic-Starved Platform: Astrobiology, v. 9, p. 989–1000, doi:10.1089/ast.2008.0330.
- Ubertini, M., Lefebvre, S., Rakotomalala, C., and Orvain, F., 2015, Impact of sediment grain-size and biofilm age on epipelic microphytobenthos resuspension: Journal of Experimental Marine Biology and Ecology, v. 467, p. 52–64, doi: 10.1016/j.jembe.2015.02.007.
- Ueshima, M., and Tazaki, K., 2001, Possible Role of Microbial Polysaccharides in Nontronite Formation: Clays and Clay Minerals, v. 49, p. 292–299, doi:10.1346/CCMN.2001.0490403.
- Underwood, G.J., and Paterson, D.M., 1993, Seasonal changes in diatom biomass, sediment stability and biogenic stabilization in the Severn Estuary: Journal of the Marine Biological Association of the United Kingdom, v. 73, p. 871–887.
- Underwood, G.J.C., Paterson, D.M., and Parkes, R.J., 1995, The measurement of microbial carbohydrate exopolymers from intertidal sediments: Limnology and Oceanography, v. 40, p. 1243–1253.
- Van Gemerden, H., 1993, Microbial mats: a joint venture: Marine Geology, v. 113, p. 3-25.
- Vennin, E., Bouton, A., Bourillot, R., Pace, A., Roche, A., Brayard, A., Thomazo, C., Virgone, A., Gaucher, E.C., and Desaubliaux, G., 2019, The lacustrine microbial carbonate factory of the successive Lake Bonneville and Great Salt Lake, Utah, USA: Sedimentology, v. 66, p. 165–204.
- Virolle, M., Brigaud, B., Bourillot, R., Féniès, H., Portier, E., Duteil, T., Nouet, J., Patrier, P., and Beaufort, D., 2019a, Detrital clay grain coats in estuarine clastic deposits: origin and spatial distribution within a modern sedimentary system, the Gironde Estuary (south-west France): Sedimentology, v. 66, p. 859–894, doi:10.1111/sed.12520.
- Virolle, M., Brigaud, B., Féniès, H., Bourillot, R., Portier, E., Patrier, P., Derriennic, H., and Beaufort, D., 2021, Preservation and distribution of detrital clay coats in a modern estuarine heterolithic point bar in the Gironde estuary (Bordeaux, France): Journal of Sedimentary Research, v. 91, p. 812–832, doi:10.2110/jsr.2020.146.
- Virolle, M., Brigaud, B., Luby, S., Portier, É., Féniès, H., Bourillot, R., Patrier, P., and Beaufort, D., 2019b, Influence of sedimentation and detrital clay grain coats on chloritized sandstone reservoir qualities: Insights from comparisons between ancient tidal heterolithic sandstones and a modern estuarine system: Marine and Petroleum Geology, v. 107, p. 163–184.

Visscher, P.T., Gallagher, K.L., Bouton, A., Farias, M.E., Kurth, D., Sancho-Tomás, M., Philippot, P.,

Somogyi, A., Medjoubi, K., and Vennin, E., 2020, Modern arsenotrophic microbial mats provide an analogue for life in the anoxic Archean: Communications Earth & Environment, v. 1, p. 1–10.

- Visscher, P.T., Prins, R.A., and van Gemerden, H., 1992, Rates of sulfate reduction and thiosulfate consumption in a marine microbial mat: FEMS Microbiology Letters, v. 86, p. 283–293.
- Visscher, P.T., Reid, R.P., and Bebout, B.M., 2000, Microscale observations of sulfate reduction: correlation of microbial activity with lithified micritic laminae in modern marine stromatolites: Geology, v. 28, p. 919–922.
- Visscher, P.T., and Stolz, J.F., 2005, Microbial mats as bioreactors: populations, processes, and products, in Geobiology: Objectives, Concepts, Perspectives, Elsevier, p. 87–100.
- Vorhies, J.S., and Gaines, R.R., 2009, Microbial dissolution of clay minerals as a source of iron and silica in marine sediments: Nature Geoscience, v. 2, p. 221–225, doi:10.1038/ngeo441.
- Westall, F., de Ronde, C.E.J., Southam, G., Grassineau, N., Colas, M., Cockell, C., and Lammer, H., 2006, Implications of a 3.472–3.333 Gyr-old subaerial microbial mat from the Barberton greenstone belt, South Africa for the UV environmental conditions on the early Earth: Philosophical Transactions of the Royal Society B: Biological Sciences, v. 361, p. 1857–1876, doi:10.1098/rstb.2006.1896.
- Widdows, J., and Brinsley, M., 2002, Impact of biotic and abiotic processes on sediment dynamics and the consequences to the structure and functioning of the intertidal zone: Journal of sea Research, v. 48, p. 143–156.
- de Winder, B., Staats, N., Stal, L.J., and Paterson, D.M., 1999, Carbohydrate secretion by phototrophic communities in tidal sediments: Journal of Sea Research, v. 42, p. 131–146, doi:10.1016/S1385-1101(99)00021-0.
- Winsborough, B.M., 2000, Diatoms and benthic microbial carbonates, in Microbial sediments, Springer, p. 76–83.
- Wooldridge, L.J., Worden, R.H., Griffiths, J., Utley, J.E., and Thompson, A., 2018, The origin of clay coated sand grains and sediment heterogeneity in tidal flats: Sedimentary Geology, v. 373, p. 191–209.
- Worden, R.H., Griffiths, J., Wooldridge, L.J., Utley, J.E.P., Lawan, A.Y., Muhammed, D.D., Simon, N., and Armitage, P.J., 2020, Chlorite in sandstones: Earth-Science Reviews, v. 204, p. 103105.
- Worden, R.H., and Morad, S. (Eds.), 2003, Clay mineral cements in sandstones: Malden, MA, Blackwell Pub, Special publication number 34 of the International Association of Sedimentologists.
- Yallop, M.L., Paterson, D.M., and Wellsbury, P., 2000, Interrelationships between Rates of Microbial Production, Exopolymer Production, Microbial Biomass, and Sediment Stability in Biofilms of Intertidal Sediments: Microbial Ecology, v. 39, p. 116–127, doi:10.1007/s002489900186.
- Zeyen, N., Benzerara, K., Li, J., Groleau, A., Balan, E., Robert, J.-L., Estève, I., Tavera, R., Moreira,

D., and López-García, P., 2015, Formation of low-T hydrated silicates in modern microbialites from Mexico and implications for microbial fossilization: Frontiers in Earth Science, v. 3, https://www.frontiersin.org/article/10.3389/feart.2015.00064 (accessed May 2022).



Conclusion

This thesis at the interface of geology and biology required a transdisciplinary approach. I had the chance to collaborate with specialists of different disciplines: geomicrobiology, geochemistry, mineralogy, etc. and to use a wide range of techniques. After the analyses and interpretations, I tried to synthesize my results into conceptual models that could be useful to other researchers. The general conclusions of this work are organized as answers to the research questions stated at the end of Chapter 1 in the following paragraphs.

<u>Topic 1.</u> Preservation of EPS, evolution of EPS properties and EPS-sediment interactions with depth (Chapter 3).

Q1: Are the EPS preserved at depth in the sediment? If so, how do EPS physicochemical properties evolve?

Q2: Do EPS still interact with sediments at depth? If so, are there modifications of EPSmineral complexes between the surface and deeper sediments?

Q3: Can we link the preservation of EPS and the sedimentary dynamics in an estuary? In other words, does the preservation of EPS depend on depositional conditions?

The preservation of EPS has rarely been studied below the first decimeters of the sedimentary column. In chapter 3, we present a characterization of EPS physico-chemistry several meters deep in estuarine sediments. Diatom biofilms secrete reactive colloidal EPS at the surface of the muddy intertidal zone of the Bordeaux Nord point bar (Gironde estuary, France). The studied EPS are mainly composed of polysaccharides and proteins, and include reactive functions such as carboxylic acids, amino groups or sulfates. Their concentrations were relatively low compared to similar studies in other estuaries. In chapter 3, EPS have been extracted from 16 samples along a 6 meters deep core extracted from the Bordeaux North tidal point bar. The quantity and reactivity of EPS are very high in the surface biofilm, and decrease sharply (10 to 20 times) at a depth of 50 cm, probably due to heterotrophic consumption. Nevertheless, some deeper samples (e.g., 4.3 m deep) have a quantity and reactivity equivalent to those of the surface biofilm. FTIR spectroscopy and acid-base titrations showed minor changes in the composition of EPS with depth: a local disappearance of carboxylic acid groups in some horizons, a shift of the amide II band with depth possibly linked to complexation of proteins with mineral surfaces, and an increase in amino groups relative to carboxyl or sulfate groups at depth. The concentration of EPS is not statistically correlated to sediment grain size or Total Organic Carbon (Q1).

EPS form aggregates with clay to sand-sized sedimentary particles, and these aggregates are observed at all depths in the core. In sandy horizons, EPS are found in detrital clay coats, i.e. thin envelopes of clay minerals and silts around sand grains. In muddy horizons, they are mixed with clay and silt particles. We observe no morphological or mineralogical modification of EPS-sediment complexes with depth. The interactions between EPS and sediment could be partly explained by the complexation of functional groups e.g., carboxyls, with mineral surfaces. Interactions with sediments is documented even in horizons with low EPS concentrations. This could be explained by a preferential complexation of refractory EPS to mineral surfaces, or more likely because minerals surfaces protect EPS from degradation (Q2). Finally, we observe a correspondence between the vertical gradients of EPS and the sedimentary facies and sequences. EPS concentrations are higher at the top of tide-dominated sedimentary sequences. This could be explained by an increased biofilm productivity when the estuarine point bar is stable and the muddy intertidal zone is most extensive. In contrast, river flood sedimentary periods could be associated with significant point bar migration and erosion of the intertidal zones. This instability could prevent significant development of biofilms and production of EPS. The preservation of highly reactive EPS could play a major role in early diagenetic organo-mineral reactions (Q3).

<u>Topic 2.</u> Geochemical gradient, microbial activity, EPS and related impacts in the sediment early diagenesis (Chapter 4).

Q4: How do microorganisms impact sediment geochemistry in the Gironde estuary? Can we link metal concentrations to a stratification of microbial metabolisms at depth? What is the impact of microbial metabolisms on the precipitation/dissolution of mineral phases in the sediments?

Q5: Is there a role for EPS in the precipitation/dissolution of metals in estuarine sediments?

In the sediment, microbial metabolisms are generally stratified according to a gradient of successive electron acceptors with decreasing potential free energy yield (i.e., oxygen, nitrate, Mn and Fe oxides, sulfate and methane). In **chapter 4**, we measured the concentration of some key ions in the porewater (Mn^{2+} , Mg^{2+} , Ca^{2+} , Fe^{2+} , Fe^{3+} , SO_4^{2-} , HS^- , NO_3^- , Cl^- , Na^+) of 3 cores with various length (0.3 to 6 meters). Some key elements show significant fluctuations that can be related to biogeochemical reactions organized as a function of depth: (i) oxygenic photosynthesis is performed in the diatom biofilm at the surface of the sediment; (ii) aerobic respiration is predominant down to approximately 5 cm; (iii) a first reduction of Mn oxides is

possibly coupled with nitrification (or sulfate reduction) between 5 and 10 cm, as indicated by the concomitant increase in dissolved Mn and nitrates; (iv) nitrates are subsequently reduced between 10 and 15 cm; (v) sulfate reduction is active at several depth intervals, but the main sulfate reduction zone occurs between 8 and 20 cm; (vi) the reduction and dissolution of iron and manganese oxides occurs in the zone of sulfate reduction, and is particularly active at 20 and 30 cm (Q4).

While metabolic activity decreases between the surface biofilm and the zone of aerobic respiration, it increases markedly in the zone of anaerobic respiration, between 10 and 30 cm. In contrast, the enzymatic activity peaks in the zone of aerobic respiration and is relatively constant and low in the anoxic part of the sediment column, revealing the typically observed sequence of different strategies for energy acquisition and organic carbon degradation among heterotrophs. EPS concentrations concomitantly decreased between the surface biofilm and 50 cm. This was confirmed by an inverse correlation between sugar and protein concentrations and metabolic activity (see also chapter 3 and **Topic 1; Q5**). Most metabolic reactions seem greatly reduced below 1 to 1.5 m deep (except for local sulfate or nitrate reduction). Consequently EPS, which serve as a source of organic carbon for heterotrophic microorganisms, have a greater potential for preservation at depths when buried below this depth range (Q4, Q5). Microbial metabolisms also impact sediment and pore water geochemistry, creating the conditions for the precipitation or dissolution of metal-rich mineral phases, such as sulfides or oxy(hydroxides). Using XRF, we document enrichments for some metals (e.g., Fe, Mn, Ca) and sulfur in the sediment, which are coincident with zones of or sulfate reduction (e.g., between ca. 15 and 50 cm, or at ca. 1.5 or 3 meters deep. Precipitation-dissolution modelling using phreeQC confirms that iron sulfides (e.g., mackinawite or pyrite) are supersaturated in the same depth intervals (Q4).

Dissolved Fe(III) increase with depth despite a supersaturation for iron oxides in porewater. We observe no statistical relationships between EPS and porewater chemistry. However, preliminary micro-XRF measurements show that Fe (as well as Ca, K or S) can be strongly bound to EPS. EPS are known to increase the solubility of iron oxy(hydroxides) and could potentially play an inhibitory role in Fe(III) precipitation (**Q5**). We finally propose a synthetic geomicrobiological model explaining potential interactions between microbial activity, porewater and sediment chemistry, and exopolymeric substances.

<u>Topic 3.</u> Role of EPS in the aggregation of clay and sand: an experimental approach (Chapter 5).

Q6: Do EPS produced in diatom biofilms interact with sedimentary particles (e.g., sand and clay) in the Gironde estuary? If so, what are the products of these interactions? *Q7:* What are the possible mechanisms of EPS-mineral interactions in estuarine sediments?

In estuarine environments, clay and sand are segregated by tidal processes. Yet, clay coats, thin clay and silt envelopes lining sand grains, are abundant in modern (including the Gironde estuary) and ancient estuarine deposits. Although assumed, the role of biofilms in the formation of clay coats was not demonstrated. In **chapter 5**, we have extracted and characterized the EPS produced by diatom biofilms at the surface of the sediment on the Bordeaux Nord point bar (see **Topic 1**). We subsequently propose a series of experiments at ambient pressure and temperature where we have mixed purified intertidal biofilm EPS with clay minerals and quartz reference materials. We have successfully produced clay-coated quartz sands similar to those observed in the Gironde, but also in some ancient estuarine sandstones. The imaging of experimental sediment-EPS mixes using cryo–scanning electron microscopy and atomic force microscopy demonstrates that EPS form organic bridges between clay and quartz (**Q6**).

We propose a scenario describing clay coat formation from molecular to sediment scale. The characterization of sediments from the point bar show that clay-EPS complexes are likely initiated at the biofilm-mud interface on low-energy mudflats. Erosion by tidal currents induces resuspension, transport and deposition of clay-EPS complexes on sand dunes during slack water periods, creating interfaces between clay-EPS complexes and sand in, e.g., interdune ponds. At the molecular scale, the potential interaction mechanisms between EPS and mineral surface are cation exchange, hydrophobic and electrostatic interactions. The reactive functional groups characterized in **chapter 3**, i.e. carboxyl, sulfates and amino-groups, could have a prominent role in aggregation (Q7).

During burial diagenesis, clay-EPS aggregates form potentially the source material for the precipitation of chlorite precursors such as berthierine. Detrital coats from ancient estuarine sandstones show textural similarities to the experimental clay-EPS complexes and could be considered as biosignatures of biofilm development and EPS production in ancient estuaries.