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# Cultivation and biorefinery of cyanobacteria isolates from Qatar for the production of pigments and bio-bitumen in desertic environment

Touria Bounnit

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# THESE DE DOCTORAT

NANTES UNIVERSITE

ECOLE DOCTORALE N° 602

*Sciences de l'Ingénierie et des Systèmes*

Spécialité : « *Génie des Procédés et Bioprocédés* »

Par

**Touria BOUNNIT**

**« Culture et bioraffinerie d'isolats de cyanobactéries du Qatar pour la production de pigments et de bio-bitume en environnement désertique. »**

Thèse présentée et soutenue en visio-conférence, le « 02 Décembre 2024 »

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# Résumé

## Résumé

### Introduction et état de l'art :

Face aux problèmes croissants liés au changement climatique, des solutions durables économiquement sont nécessaires. L'attention se tourne de plus en plus vers le potentiel remarquable des microalgues pour répondre à cette problématique, notamment en raison de leur capacité à capturer le dioxyde de carbone (CO<sub>2</sub>) tout en produisant des métabolites valorisables dans des domaines d'applications variés. Leur culture à grande échelle pourrait donc jouer un rôle clé dans la réduction des émissions de gaz à effet de serre. Parallèlement, l'intégration de concepts innovants de bioraffinerie, tirant parti de la riche biodiversité métabolique des microalgues, pourrait contribuer à la transition vers une économie circulaire et biosourcée.

Le Qatar met en place un programme de bioéconomie durable visant à répondre aux Objectifs de Développement Durable des Nations Unies. Cependant, son secteur énergétique demeure fortement dépendant des combustibles fossiles. Leur combustion et les différents processus industriels associés à leur extraction et leur transformation entraînent d'importantes émissions de CO<sub>2</sub>, principale cause du changement climatique. Le pays, qui a produit 102,6 millions de tonnes de CO<sub>2</sub> en 2022, s'est donc fixé comme objectif national de réduire ses émissions de gaz à effet de serre de 25 % d'ici 2030. Ainsi, l'exploration de solutions durables, dont la filière microalgues, pour relever ce défi environnemental et économique pressant s'impose. Grâce à son ensoleillement et à la disponibilité de terres non arables, et de larges opportunités de financement, le Qatar (et plus généralement la péninsule arabique) pourrait devenir un emplacement de choix pour la culture commerciale de microalgues. Celles-ci devront néanmoins être adaptées à l'eau de mer en raison de la rareté de l'eau douce dans la région, ainsi qu'aux températures extrêmes. Il est donc impératif de sélectionner des souches spécifiques pour éviter l'effondrement des cultures et permettre une production de biomasse à grande échelle.

Sans négliger leurs applications dans l'alimentation animale et humaine, les biocarburants et la bioremédiation, il semble intéressant de viser des produits à haute valeur ajoutée comme les phycobiliprotéines. Ces pigments, principalement présents dans les cyanobactéries, sont utilisés comme colorants naturels dans les secteurs de l'alimentation et de cosmétique, mais possèdent aussi des propriétés uniques valorisables dans les domaines des biotechnologies et du diagnostic médical.



## Résumé

Néanmoins, malgré leur importance croissante, leur production commerciale à grande échelle reste principalement limitée à quelques espèces telles que *Spirulina*, *Anabaena* et *Porphyridium*.

Une production efficace et rentable de phycobiliprotéines repose sur les points clés suivants : i) la sélection de la souche appropriée, ii) la définition des conditions de culture pour une expression maximale des pigments, et iii) l'application d'une stratégie de bioraffinerie pour une production rentable.

Pour permettre leur production à partir de biomasse cultivée dans des régions désertiques comme le Qatar, il est donc nécessaire d'isoler et d'identifier de nouvelles souches locales capables de les produire, et qui : i) soient naturellement adaptées aux conditions extrêmes, notamment en termes de température et de salinité, et ii) permettent de développer une chaîne de valeur complète, incluant la valorisation des coproduits après extraction des pigments. Cela garantirait non seulement une rentabilité économique, mais aussi une utilisation durable des ressources, contribuant ainsi à une bioéconomie circulaire et à la durabilité environnementale dans ces régions.

Pour cela, l'Université du Qatar a construit une Collection de Cultures de Cyanobactéries et de Microalgues (QUCCCM) qui regroupe des souches indigènes isolées dans différents environnements locaux, y compris dans les eaux marines.

Parallèlement, une approche récemment développée au GEPEA pour valoriser les résidus d'extraction de phycocyanine de *Spirulina* s'avère particulièrement intéressante. Elle consiste à réaliser une conversion hydrothermale à basse température ( $\approx 260^\circ\text{C}$ ), permettant un procédé intermédiaire entre la carbonisation hydrothermale et la liquéfaction hydrothermale, conduisant à une fraction huileuse dont la rhéologie est similaire à celle d'un bitume pétrolier, et donc baptisée bio-bitume.

----

**Partant de ces hypothèses formulées dans le chapitre 1 consacré à l'état de l'art, les travaux expérimentaux réalisés au cours de la thèse sont présentés dans les quatre chapitres suivants, le chapitre 2 décrivant la méthodologie et les chapitre 3, 4 et 5, les résultats obtenus.**

Outre toutes les techniques d'analyse et de caractérisation utilisées, le **chapitre 2** décrit notamment les conditions de culture et le procédé de conversion hydrothermale :

## Résumé

- Les conditions de culture, en laboratoire à l'aide de photobioréacteurs cylindrique de 1L où la lumière, la température et la salinité sont contrôlées dans le **chapitre 3**, et en extérieur en conditions solaires dans des raceways de 200L de culture, soumis aux variations d'intensité lumineuse, de température et potentiellement de salinité du fait de l'évaporation dans le **chapitre 4**.
- Les procédés d'extraction des phycobiliprotéines : un procédé d'extraction « doux », consistant à utiliser une machine de laboratoire « Tissue Lyser », excepté au **chapitre 5** où il est comparé à un procédé d'extraction « dur », simulé par un traitement ultérieur de la biomasse par broyage haute pression.
- Le procédé de conversion hydrothermale : compte tenu de la quantité limitée de biomasse disponible, un réacteur de 30 mL a été utilisé, permettant de travailler sur des lots de seulement 3 grammes de biomasse sèche. En revanche, le réacteur de 300 mL précédemment utilisé au GEPEA nécessitait des lots de 50 grammes.

## Principaux résultats :

Le **chapitre 3** a permis le criblage de quatre souches de cyanobactéries marines isolées au Qatar, en évaluant leur capacité à croître et à produire des phycobiliprotéines dans des conditions de température et salinité variées, représentatives d'une culture en extérieur. Les souches ont d'abord été identifiées grâce à l'analyse de leur séquence 16S rDNA et de leur composition biochimique. Les résultats ont révélé des similitudes génétiques importantes avec *Chroococciopsis* (QUCCCM34), *Pleurocapsa* (QUCCCM54), *Euhalothece* (QUCCCM77) et *Cyanobacterium aponium* (QUCCCM129).

L'étude de la croissance et de la production de phycobiliprotéines à différentes températures (jusqu'à 40 °C) et salinités (jusqu'à 60 ppt) a permis d'évaluer leur potentiel en tant que candidats viables pour une production en conditions de culture extérieure désertiques, tout en mettant en évidence leur thermo et halo tolérance. Les résultats ont également révélé que la synthèse des phycobiliprotéines était influencée par la salinité, la température et l'âge de la culture. Il est donc nécessaire d'ajuster de près les conditions de stress abiotiques environnementaux et le moment de la récolte pour optimiser la production des pigments. En outre, l'évaluation du potentiel antioxydant des extraits bruts de phycobiliprotéines en utilisant les tests DPPH et

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Trolox a révélé des propriétés antioxydantes remarquables, avec des valeurs observées dépassant celles rapportées pour diverses sources courantes d'antioxydants, largement utilisées dans la cuisine, les cosmétiques et plusieurs préparations thérapeutiques.

Les résultats obtenus ont conduit à sélectionner la souche *Pleurocapsa* sp., (QUCCCM 54). En plus de sa productivité élevée en biomasse, elle produit principalement de la phycocyanine (PC), un pigment particulièrement intéressant en raison de ses propriétés antioxydantes et de ses applications commerciales dans les aliments, les cosmétiques et les produits pharmaceutiques. La production de PC a été observée dès le stade précoce de croissance, ce qui est avantageux pour maximiser la production sur des périodes de culture plus courtes. Un autre avantage critique observé au cours de l'étude est la capacité naturelle de sédimentation de la souche QUCCCM54. Cette caractéristique facilite la récolte à grande échelle, en limitant le recours à des méthodes de séparation mécaniques ou chimiques additionnelles. Cette facilité de récolte améliore ainsi la viabilité économique globale de la souche pour les applications industrielles.

Cette étape de criblage a fait l'objet d'une publication parue en 2022 :

Bounnit, T., Saadaoui, I. Al Ghasal, G., Rasheed, R., Dalgamouni, T., Al Jabri, H., Leroy, E., Legrand, J., 2022, Assessment of novel halo- and thermotolerant desert cyanobacteria for phycobiliprotein production. *Process Biochemistry*, Vol. 118, pp. 425-437.

Le **chapitre 4** concerne la transition de la culture de *Pleurocapsa* sp. en laboratoire, utilisant des photobioréacteurs à lumière artificielle, vers sa culture en extérieur dans des conditions solaires désertiques. L'objectif est d'évaluer davantage son potentiel en tant que souche commercialement viable pour une production dans les conditions réelles du Qatar.

Tout d'abord, la capacité de la souche à croître en utilisant l'urée comme source d'azote à faible coût a été évaluée en laboratoire. Les résultats obtenus ont démontré son aptitude à croître en mixotrophie, utilisant l'urée non seulement pour l'azote mais aussi comme source secondaire de carbone, avec un impact positif sur le taux de croissance et l'accumulation de phycobiliprotéines. En conséquence, l'urée a été adoptée comme source d'azote pour les cultures solaires et son approvisionnement a

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été optimisé pour les conditions extérieures. Deux concentrations d'azote, 2X et 10X, ont été adoptées pour évaluer la faisabilité de réduire l'apport en azote dans la perspective d'une production à grande échelle.

Les résultats des cultures en conditions solaires dans les raceways ont souligné l'importance critique de la densité de l'inoculum pour assurer la survie de la souche et permettre la production de biomasse.

En outre, les variations de concentrations en azote (2X et 10X) ont eu un impact minimal sur la croissance et la biosynthèse des phycobiliprotéines, suggérant la possibilité de réduire l'apport en azote sans affecter la productivité, apportant ainsi des avantages économiques et environnementaux. Cependant, les rendements en biomasse étaient notablement inférieurs à ceux observés dans les études en laboratoire avec photobioréacteurs, pointant la nécessité d'une optimisation supplémentaire des pratiques de culture en extérieur, pour tenir compte des conditions environnementales réelles, notamment d'intensité lumineuse.

Le **chapitre 5** porte sur l'étude de la faisabilité de la valorisation des résidus de biomasse après extraction des phycobiliprotéines, par conversion hydrothermale pour la production de bio-bitume. Un résidu de biomasse industrielle de *Spiruline*, précédemment étudié au laboratoire a servi de référence. L'étude préliminaire a montré que le réacteur Swagelok de 30 mL produit un rendement en phase huileuse inférieur à ceux obtenus lors d'études antérieures menées avec des réacteurs agités de plus grand volume et mieux contrôlés. Néanmoins, ce petit réacteur permet de comparer les produits de conversion hydrothermale issus des résidus de biomasse des 4 souches cultivées en laboratoire, comme décrit au chapitre 3, après un procédé d'extraction pigmentaire « doux ».

Les rendements obtenus de la phase hydrophobe sont proches de 50 % similaires à ceux de la Spiruline. Toutefois, après la séparation de l'huile et des particules solides, les résultats ont montré des rendements en phase huileuse inférieurs, variant entre 9 et 28 %. Ce rendement réduit en huile semble être lié à une teneur en cendres plus élevée dans les biomasses avant conversion. De plus, il a été observé que seules les souches *Chroococidiopsis.sp* (QUCCCM34) et *Cyanobacterium aponium* (QUCCCM129) produisent des huiles au comportement de type bitume. En revanche, la souche *Pleurocapsa* (QUCCCM54) conduit non seulement au plus faible rendement

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en phase huileuse, mais elle ne se comporte pas comme un bitume en termes de rhéologie.

La deuxième partie de l'étude, axée sur la biomasse de *Pleurocapsa sp* cultivée en extérieur, s'est d'abord focalisée sur la teneur en cendres avant conversion hydrothermale. Il a été observé que seul le procédé simulé d'extraction de pigments « dur » réduit significativement cette teneur. Cette diminution a eu un impact majeur sur l'efficacité de la conversion hydrothermale, entraînant un rendement plus élevé en phase huileuse et une modification de la composition des huiles, en termes de distribution de masse moléculaire. En particulier, une fraction notable de molécules de masses supérieures à 1000g/mol apparaît, semblable à celles observées dans les huiles issues du résidu de Spiruline. De plus, le comportement rhéologique devient comparable à celui d'un bitume pétrolier conventionnel, suggérant que cette propriété est liée à la formation de molécules de masse moléculaire relativement élevée lors de la conversion hydrothermale. Il a également été observé que les conditions de culture, en particulier la concentration en urée, n'influençaient pas fortement le rendement des produits hydrothermaux. Cependant, les huiles bitumineuses obtenues diffèrent légèrement en termes de rigidité. Par conséquent, les conditions de culture peuvent affecter les applications potentielles du biobitume produit.

## Conclusions et perspectives

Ce travail a montré la pertinence de la démarche consistant à sélectionner une souche de cyanobactérie productrice de phycobiliprotéines au sein de la QUCCCM, en vue de sa culture en extérieur en conditions solaires désertiques, ainsi que la faisabilité de la co-production de bio-bitume par conversion hydrothermale des résidus après extraction des pigments. Néanmoins, plusieurs pistes de travail futures peuvent être envisagées :

- La méthodologie développée dans cette étude s'est concentrée sur l'étude de l'effet de la température et la salinité comme deux facteurs de culture, et que leur étude en laboratoire dans des photobioréacteurs contrôlés permettrait une transition en douceur vers la culture en extérieur. La productivité significativement réduite observée en extérieur suggère que l'intensité et la qualité de la lumière peuvent également être un facteur dominant. Il serait intéressant dans des études ultérieures d'inclure ce paramètre en laboratoire

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en utilisant une lumière artificielle capable de reproduire les conditions attendues en extérieur.

- La nécessité d'avoir des quantités de biomasse plus élevées afin d'explorer pleinement l'impact du procédé d'extraction des pigments sur la teneur en cendres et la conversion hydrothermale, en utilisant un réacteur de volume plus important et mieux contrôlé.
- La valorisation de la phase aqueuse et des particules solides, représentant plus de 50 % des produits de conversion hydrothermale, doit également être explorée. Cela permettrait à terme de modéliser l'ensemble du processus de culture et de traitement en aval, afin d'évaluer les avantages économiques et environnementaux d'une filière microalgue en milieu désertique.

# General Introduction

## General Introduction

In the face of escalating climate change and resource depletion concerns, sustainable solutions are urgently needed. The spotlight increasingly turns to microalgae's remarkable potential to address these challenges, notably due to their ability to capture carbon dioxide (CO<sub>2</sub>), while yielding to valuable products with diverse applications. Consequently, their cultivation presents a unique opportunity to simultaneously mitigate greenhouse gas emissions and promote sustainable resource utilization. According to the available statistics, global production of microalgae biomass has now exceeded 20 kt/year, with market prices typically ranging from 5 to 500 €/kg for applications such as food, feed, health-related and cosmetic [1]. In contrast, process for the low-value applications like biofuels, fertilizers, wastewater treatment and chemicals, are generally below 5 €/kg [1]. These price variations are driven by several factors, including the type of application, differences in strains, geographical conditions, cultivation systems, production scale, downstream processing, and technological advancements. To enhance the economic viability and competitiveness of microalgae products, it is crucial to optimize these factors and integrate biorefinery concepts taking advantage of the rich microalgal biodiversity. This approach promotes the development of a circular, bio-based economy and helps achieve sustainable outcomes.

Qatar is one of the first countries to establish a sustainable bio-economy program coping with the agenda of the United Nations Sustainable Development Goals. However, its energy sector remains heavily reliant on fossil fuels, and their combustion along with associated industrial processes, generates significant CO<sub>2</sub> emissions, a primary cause of climate change. In 2022 alone, the country produced 102.6 million metric tons of CO<sub>2</sub>, with more than 35 metric tons per person [2]. Conscient that it is by far the largest greenhouse gas emitter in the world per capita, the State of Qatar has set a national goal aiming to reduce its greenhouse gas emissions by 25 percent in 2030. To achieve this, exploring sustainable solutions that address these urgent environmental and economic challenges is essential. Local microalgae isolates are viewed as one of the most promising strategies to support the national vision, as the production of 1 kg of microalgae biomass can capture approximately 1.8 kg of CO<sub>2</sub> [3]. Moreover, the local environmental conditions, the availability of process inputs, and the funding opportunities make the region an appropriate place for algal biomass



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production for various applications such as feed and food, biofuels, bioremediation, and higher added value products.

Among the high value metabolites, phycobiliproteins (PBPs), are prominent pigments found mainly in cyanobacteria. Beyond their use as natural colorants in food and cosmetics, they possess unique properties that make them valuable in different fields ranging from biotechnology to medical diagnostics [4-6]. Despite their growing relevance in several markets, their production from biomass grown in desert regions such as Qatar has not been extensively explored, and the current large scale commercial production is mainly restricted to few species like *Spirulina*, *Anabaena*, and the rhodophyte *Porphyridium*, [7]. Consequently, there is a critical need to isolate and identify new strains to enhance the algae-value chain development process. In this context, Qatar University has built up a large local culture collection of purified cyanobacteria and green microalgae. Up to date, most research in Qatar has focused on applications such as aquaculture, biofertilizers, and bioenergy, often utilizing either whole algal biomass or targeted metabolites [8-15]. There has been limited emphasis on isolating and producing novel native strains for PBPs production.

Temperature and water salinity are among the cultivation parameters that can enhance the PBPs accumulation within the cells, as these pigments serve as defence mechanisms due to their strong antioxidant and protective properties [5, 16]. This is particularly promising for developing PBPs- rich biomass in Qatar's harsh desert climate, where using sea water for algal large-scale production is regarded as the most feasible solution, due to the freshwater scarcity, and the witnessed extreme temperatures —often exceeding 40 °C in summer— leading to evaporation and increased salinity. However, the extraction of PBPs generates substantial amounts of residual biomass, posing challenges for waste management and disposal. If not managed properly, this leftover biomass could have negative environmental impacts. Therefore, an algal biorefinery approach should be investigated to optimize feedstock utilization for various applications.

In this context, the present work aims to explore such a holistic approach by first producing PBPs a high-value product, and subsequently converting the residual biomass into a material that mimics the rheological behaviour of bitumen, referred to as bio bitumen, through a hydrothermal conversion process. Such a downstream

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processing strategy is expected to maximise profitability and minimise environmental impact, supporting a sustainable and circular resource utilisation approach, particularly if industrial by-products, such as urea, are used as nutrient during cultivation. By starting with the identification and screening of the local cyanobacterial isolates, to the large-scale production of the selected strain under Qatari environmental conditions, and finally the downstream processing (Fig.1), this study aims to provide a proof of concept for the feasible co-production of both valuable pigments and a biobased alternative to petroleum bitumen from marine desert cyanobacteria species in the area and in other desert regions with features similar to those of the Arabian Peninsula.

To achieve these goals, the present research work is subdivided in the following parts:

**Chapter 1** provides an overview on the potential of Qatar as a location for commercially viable algae production, highlighting the region's environmental conditions and resources. It reviews the current knowledge on cyanobacteria and their prospective use as a source of phycobiliproteins. The chapter also explores conditions affecting the cyanobacterial growth and PBPs production, with an emphasis on salinity and temperature, two main factors addressed in this study. Additionally, hydrothermal conversion and its application to bio-bitumen production from microalgal biomass is discussed.

Then, in **chapter 2**, we describe the materials along with the experimental methods and protocols used throughout the experimental work, and the obtained results are presented and analyzed in the subsequent three chapters.

**Chapter 3** begins with the molecular identification of the local strains, followed by a laboratory scale screening investigation of their growth and PBPs production under varying temperature and salinity. A well-controlled photobioreactor (PBR) was used during study. This approach enables the selection of the optimum conditions leading to the highest growth and PBPs concentration. By performing in parallel a bioprospection of the antioxidant potential of the produced PBPs by the four strains, this chapter will allow selecting one indigenous strain for outdoor solar cultivation.

**Chapter 4** explores the transition from indoor to outdoor cultures, which is one of the most crucial stages towards the development of commercial algae-based processes.

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Before assessing the ability of the candidate strain to grow and produce PBPs under prevalent desert climate conditions, its aptitude to assimilate urea as an alternative nitrogen source (as strategy to lower the production cost) was first investigated indoor. Following the indoor optimization, the transition to outdoor cultures and ability of the strain to grow and produce PBPs under prevalent desert climate conditions is investigated. The outdoor cultivation experiments also provide most of the biomass used in the last chapter.

**Chapter 5** focuses on the hydrothermal conversion of residual biomass left after PBPs extraction. Special emphasis was on the biomass obtained from the outdoor cultivation of the selected candidate, where two types of extraction process “mild” and “hard” are evaluated before hydrothermal conversion to assess their influence on the yields and characteristics of the final products. The goal is to evaluate how these extraction methods influence the composition and rheological properties of the oil phase, with the objective of achieving bitumen-like behavior.

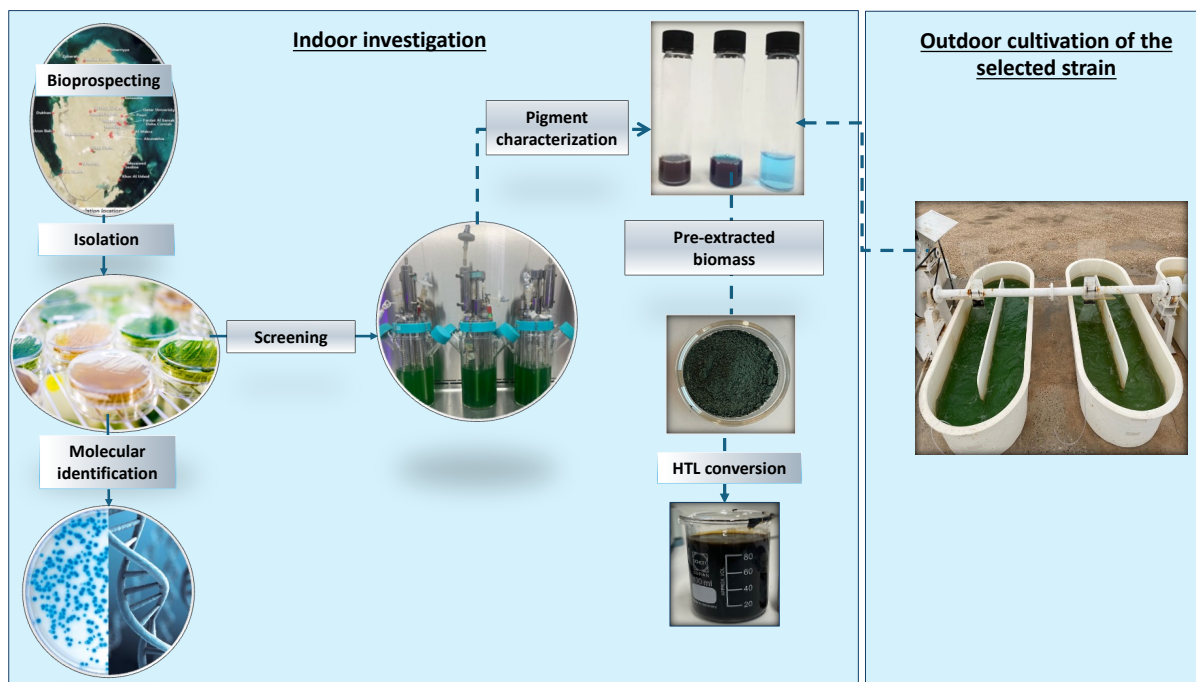


Figure 1: Schematic presentation of the methodology followed during this study.

# **Chapter 1: State of the art and objectives**

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### **Introduction**

This literature review provides the foundation for our study by highlighting the current state of research and development in both phycobiliprotein production and bio-bitumen.

We begin with a comprehensive overview of the significance of cyanobacteria as a feedstock, emphasizing on their role in sustainable practices and carbon capture while producing phycobiliproteins. The review also covers the large-scale production of the biomass, and the local constraints associated with, focusing on the influence of cultivation parameters on both growth and the expression of PBPs. Special attention is given to the influence of salinity and temperature regimes corresponding to outdoor large-scale cultivation conditions in Qatar's challenging desert environment, as well as the available local resources for sustainable biomass production.

Following this, we introduce details on the targeted pigment phycobiliproteins (PBPs), covering their industrial applications and benefits.

The last part of this chapter is dedicated to hydrothermal conversion as a process for valorizing residual biomass, and its application in producing of bio-bitumen, a valuable material with potential uses in construction and other industries.

## **Chapter 1: State of the art and objectives**

### **1. Microalgae as a feedstock for CO<sub>2</sub> capture and bioeconomy in Qatar**

The growing global demand for sustainable alternatives to petroleum-based fuels and chemicals, has spurred interest in exploring novel biomass feedstocks. This aligns with the concept of bioeconomy, which embodies a transformative shift towards a more sustainable and resource-efficient economic model, emphasizing the use of renewable biological resources to meet the need of societies. Unlike the traditional linear economy, where resources are extracted, used, and disposed of, a bioeconomy seeks to create a circular system, where biomass is harnessed to produce various bio-based products such as food, chemicals, materials, and waste is recycled into new resources. This approach seeks to create a closed-loop system where materials are kept in use for as long as possible, thereby reducing the need for new raw materials and minimizing environmental impact. It also supports long-term economic resilience and sustainability by decoupling economic growth from resource consumption.

Carbon capture is a pivotal component of the transition to a sustainable bioeconomy, particularly in regions like the Gulf Cooperation Council (GCC) countries, where fossil fuel dependency is high and environmental sustainability is increasingly prioritized. As these nations grapple with the challenges of climate change, rising temperatures, and water scarcity, implementing effective carbon capture strategies becomes essential for mitigating greenhouse gas emissions and fostering economic diversification.

Microalgae have emerged as a promising and versatile feedstock, playing a pivotal role in carbon capture through the process of photosynthesis while sustainably producing various bio-based products. They consist of both prokaryote (Cyanophyceae) and eukaryote (Chlorophyta) organisms with different sizes and shapes (Fig. 2), and can be cultivated in various aqueous media, including brackish water or seawater, thus minimizing strain on freshwater resources. Moreover, they can thrive in areas unsuitable for traditional agriculture, avoiding competition with food crops.

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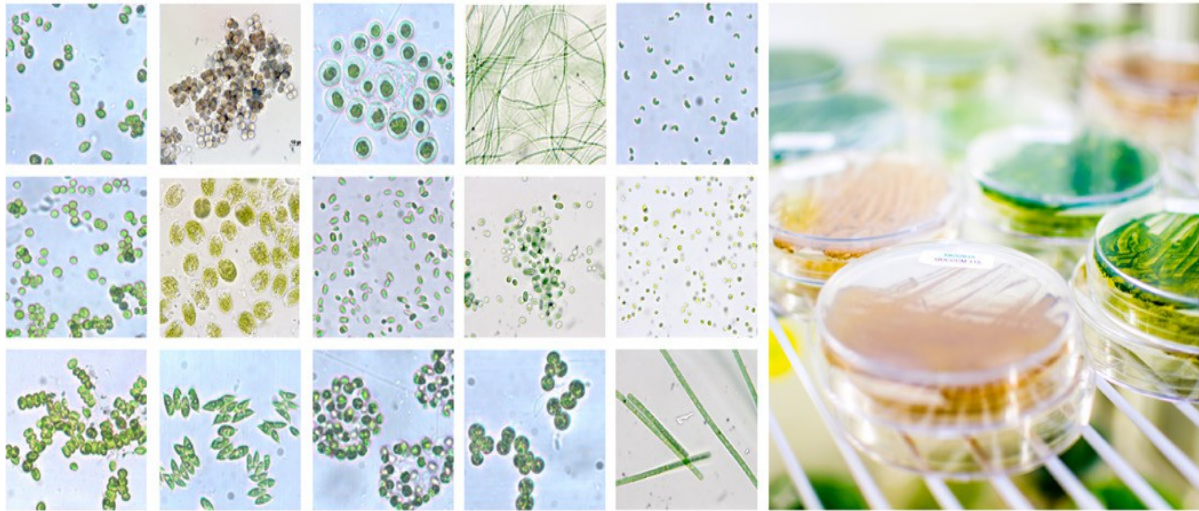


Figure 2: Microscopic observation of different forms of microalgae cells: unicellular picoplankton, multicellular cells and filamentous cells [8].

For their photosynthetic growth, microalgae require three major components: sunlight, water, and carbon source, generally CO<sub>2</sub> or carbonates, to rapidly produce biomass and contribute to the release of approximately 50% of the atmospheric oxygen worldwide, a process known as oxygenic photosynthesis [17]. The general equation for this process is:



Besides, they display high photosynthetic efficiency, areal productivity and proved able to outperform over other feedstocks by surviving in a wide range of temperatures, salinities, pH values, and light intensities [18]. This resilience presents significant opportunities for their production in desert climates like Qatar, where conventional agriculture faces numerous challenges.

In recent years, microalgae have gained great interest due to their versatile cultivation potential using organic waste, nutrients from wastewater, or byproducts from other industries which aligns with the principles of circular economy and sustainable resource management [19]. Furthermore, their cultivation can be tailored to yield specific bioactive compounds, including lipids, proteins, carbohydrates, and pigments, each with valuable applications in different industries, namely food, aquaculture, energy, nutraceutical, pharmaceutical, cosmetics, bioplastics, biofertilizer aside from their primary function in CO<sub>2</sub> mitigation (Fig. 3). These biobased chemicals are often

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biodegradable and environmentally friendly, reducing the environmental impact at various stages of their lifecycle, from production to disposal [20]. Last but not least, the current industrial applications of microalgae biomass span a wide range of sectors, providing opportunities for job creation and economic diversification, particularly in regions with suitable conditions for cultivation. However, challenges like strain selection, scalability, cost-effectiveness, and efficient harvesting methods still need to be addressed for widespread commercial adoption. Addressing these challenges will be crucial for unlocking the full potential of microalgae-based solutions and realizing their benefits on a large commercial scale.

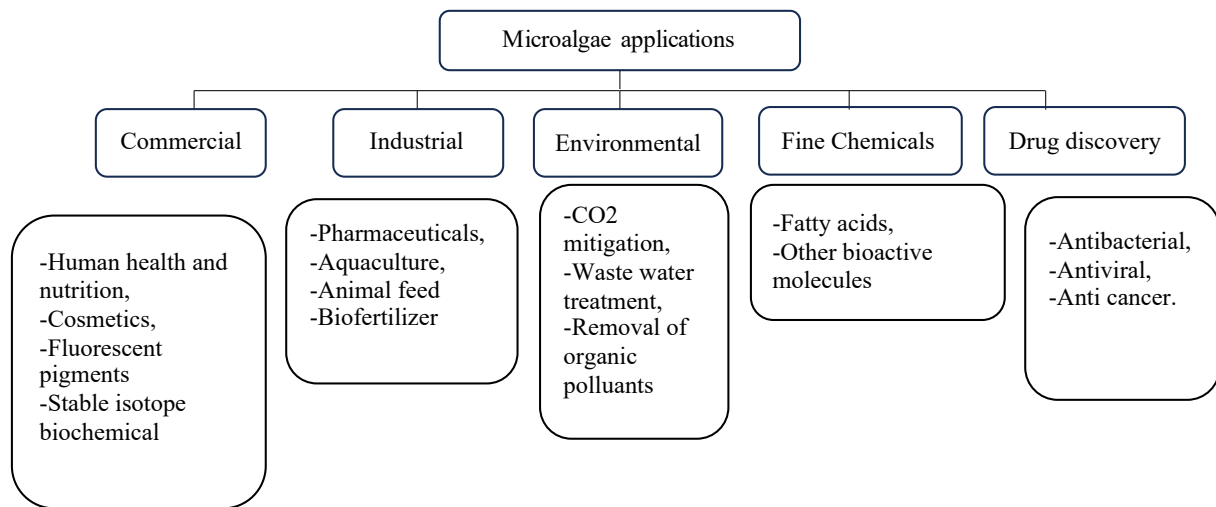


Figure 3: Flow chart showing applications of microalgae in different fields.

### 1.1. Sustainable inputs for successful algae production in Qatar

The economic feasibility of algae cultivation is largely dependent on the localization of production, being the driving factor behind choices in strain, cultivation systems, and products. Located in the west part of the Arabian Gulf, Qatar is a peninsula with a surface area of 11.437 km<sup>2</sup>. The successful production of algae in Qatar relies on sustainable inputs that support the growth and productivity of these microorganisms in the challenging desert environment. Notably, the place is characterized by the existence of large areas of non-arable land (Table 1), in addition to suitable climate with abundance of light year-round (Fig. 4A) and adequate temperature (Fig. 4B), making it ideal for algal production when compared to other top locations worldwide, such as USA and Australia. Due to the freshwater scarcity in the country, viable large-scale cultivation is based on seawater, which is easily accessible. Brackish groundwater and



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high-quality treated industrial wastewater can also be considered for low salt tolerance strains cultivation or for use to compensate the evaporation-loss.

Table 1: Overview of total and arable land area in the gulf area.

Country	Total area (km <sup>2</sup> )	Arable land (km <sup>2</sup> )
Bahrain	780	16 (2.1%)
Kuwait	17 820	80 (0.4%)
Oman	309 500	767 (0.2%)
Qatar	11 490	140 (1.2%)
Saudi Arabia	2 149 690	34 370 (1.6%)
UAE	98 648	423 (0.6%)

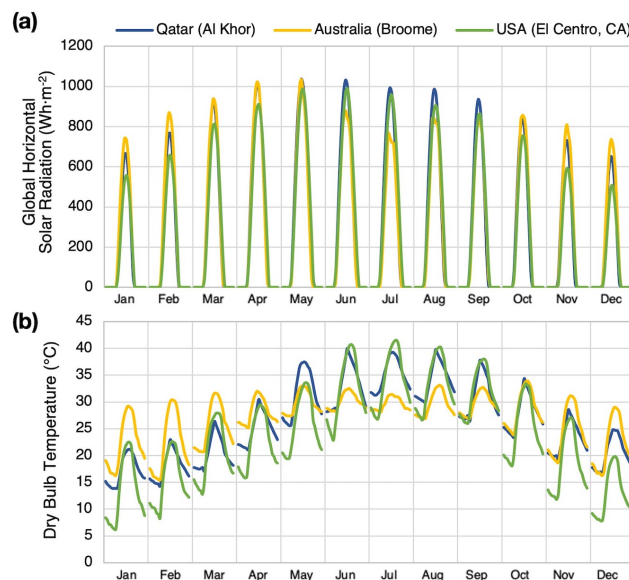


Figure 4: Comparing climate in global high-potential algae production regions. The figure shows the positioning of Qatar based on their monthly average 24h profile for (a) global horizontal radiations (Wh.m-2), (b) dry bulb temperature (°C).

The country also presents the highest CO<sub>2</sub> emission per capita with majority emissions originating from point-sources in the energy, construction, and manufacturing industries. Integration of algae culture with the existing industries could also provide sustainable process input. For example, bio-crude oil produced through hydrothermal liquefaction of microalgal biomass can be processed in existing oil refineries, either as sole process-feed, or blended with petroleum feedstocks [21]. This could open the door to a gradual transition to renewable fuels that are compatible with existing petroleum

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infrastructures. Furthermore, the governmental ambitions provide a significant opportunity for the establishment of algae value chains. For example, the local airline Qatar Airways has co-invested in a research project for the development of algae aviation fuels [22]. Following this, multiple projects were launched, with the government funding opportunities, to develop local aquaculture and livestock industry, where algae biomass a locally produced could become a sustainable partial alternative to imported feeds. Taking all these factors into consideration, the Arabian Peninsula could have a leading role in the establishment and development of algae value chains.

In recognition of this, Qatar university has built up a Culture Collection of Cyanobacteria and Microalgae (QUCCCM), which houses different indigenous strains [8]. This culture Collection is a unique resource, and the first of its kind in the Gulf Cooperation Council (GCC) region. Strains were isolated from various local environments, ranging from fresh to marine water, as well as from soil, sabkha and rocks with the aim to identify promising candidates for commercial application. The harsh environmental conditions favored the evolution of various types of microalgae and cyanobacteria, highly adapted to extremophilic conditions, offering a potential reservoir of thermo-, photo-, and halotolerant strains to be selected for industrial cultivation [23, 24]. The collection contains new species within existing genera, as well as several novel isolates with low relation to existing genera [8].

### 1.2. Biomass production and Harvesting techniques

Enabling large-scale cultivation will decisively contribute to the development of a sustainable feasible and economically viable microalgae-based industry. Cultivation systems can be grouped into two different categories: open and closed systems. Each has its own advantages and drawbacks, and the choice between them depends on factors such as the type of algae being cultivated, the desired end product, the location, and the available resources.

On the “low tech” side, open pond systems involve cultivating algae in large, shallow ponds accounting for 90% of global microalgae production due to their economic feasibility [1]. They are made of concrete, and white liners are used and are relatively simple, easily expandable, and operating costs are relatively small [25]. Open pond culture systems can be classified broadly as: shallow lagoons and ponds, inclined (Cascade) systems, circular central-pivot ponds, and raceway ponds being by far the most widely used system (Fig.5).

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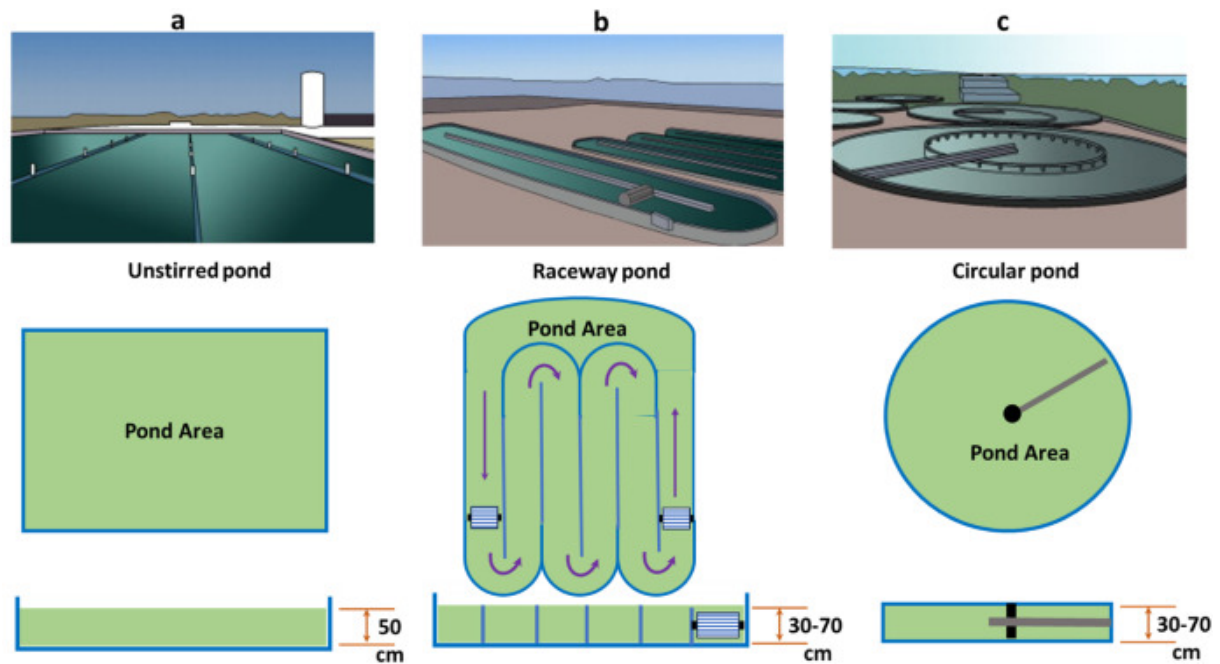


Figure 5: Example of open Cultivation Systems: a) open undisturbed pond b) paddle wheel racing pond, and c) Circular Ponds [26].

Open ponds cultivation relies on the natural sunlight, reducing the need for artificial lighting. However, the system presents some drawbacks, in particular culture contamination; significant loss of water due to evaporation leading to salinity increment; limited diffusion of  $\text{CO}_2$  from the atmosphere, in addition to the difficulty to control environmental factors like light and temperature [27]. To overcome some of the issues selection and cultivation of tolerant microalgae can be applied to maintain reasonable productivity [28]. Indeed, commercially successful microalgal species that thrive in open ponds are those that can withstand harsh environments and competition such as *Dunaliella*, *Spirulina*, and *Chlorella*.

Some constraints of open pond cultivation, in particular the lack of sufficient control mechanisms to optimize the growth of microalgal cultures can be avoided by using controlled closed systems called photobioreactors (PBRs). These PBRs can be tubular, flat-panel, or even plastic bags for less high tech PBRs (Fig.6)

## Chapter 1: State of the art and objectives

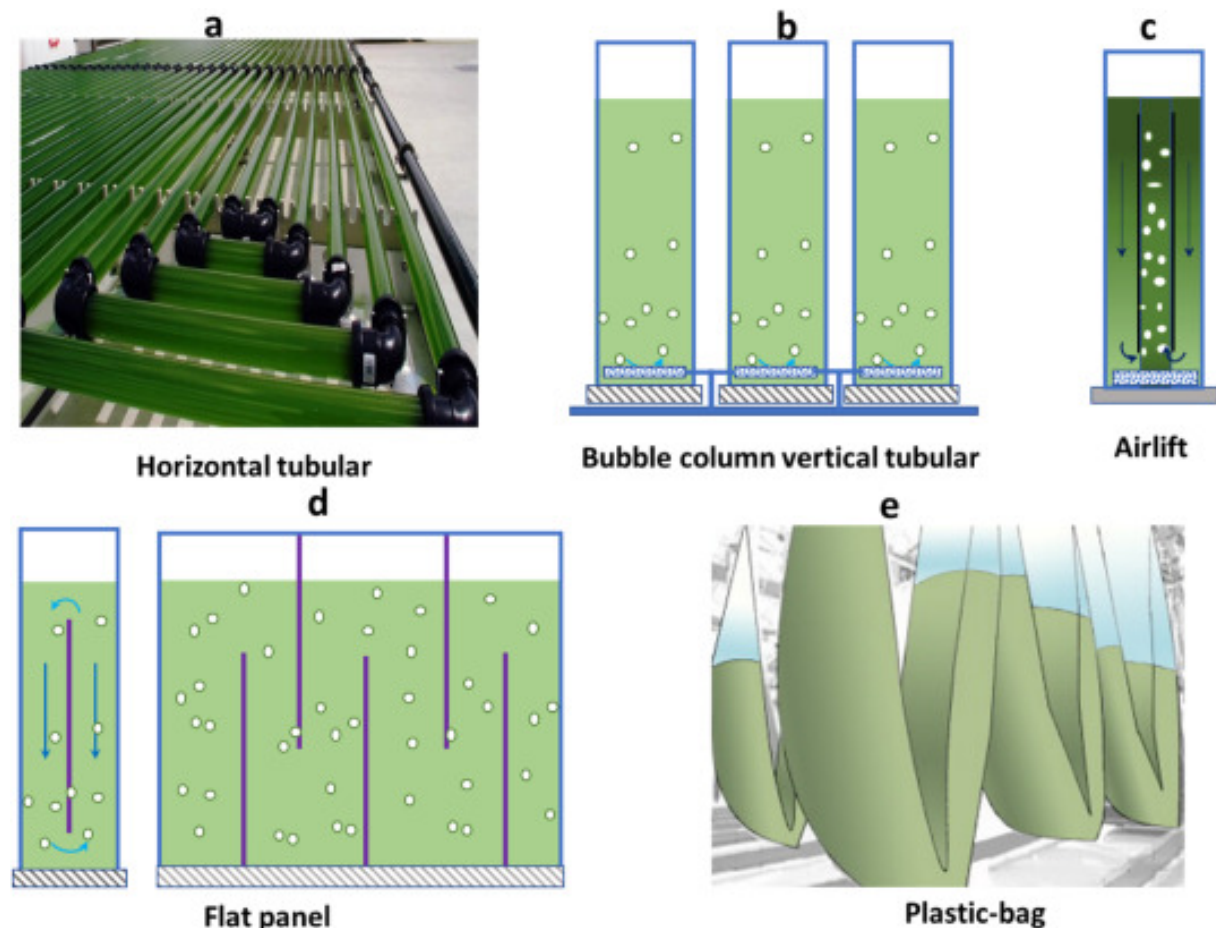


Figure 6: Closed Cultivation System: a) Horizontal Tubular b) Bubble column c) Airlift tubular; d) Flat panel, and e) Large-scale plastic-bag photobioreactors [26].

PBRs allow a greater control over environmental factors, including light intensity, temperature, and CO<sub>2</sub> concentration, reduce the risk of contamination from external microorganisms and allow higher biomass concentration [29]. They are generally used to produce high-value and biochemical metabolites. However, their construction and operation cost is very high and in general prohibitory for low-cost feedstock production (e.g., biofuel) [29]. Scalability of PBRs is another obstacle for biomass large scale production. Some strains tend to stick to the surface wall, form biofilms covering the PBR surface and blocking the incoming light, leading to reduced biomass productivity and necessity to regular cleaning of the PBR interior. Another major issues is the heat build-up, during the summer in temperate regions such as Qatar which require excessive cooling energy [29, 30], in addition to the oxygen accumulation in these closed-system. Table 2 compares the production of microalgae in open and closed photobioreactors.

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Table 2: Comparative study between open and closed photobioreactors system regarding the microalgae cultivation [26].

Factors	Open system	Closed photobioreactors
Space requirement	10,000 m <sup>2</sup>	100 m <sup>2</sup>
Evaporation rate	High	No evaporation
Water losses	Very high	Low
CO <sub>2</sub> -losses	High	Low
Biomass concentration	0.5–1 kg/m <sup>3</sup>	5 kg/m <sup>3</sup>
Photoconversion efficiency	± 1.5 %	± 5 %
Energy (power and heat) requirement	± 57 MJ m <sup>-2</sup> y <sup>-1</sup>	±207 MJ m <sup>-2</sup> y <sup>-1</sup>
Volumetric algal productivities	0.12–0.48 g. L <sup>-1</sup> ·d <sup>-1</sup>	0.2–3.8 g. L <sup>-1</sup> ·d <sup>-1</sup>
Temperature	Variable	Cooling is required
Weather dependency	High	Low
Contamination	Higher	None
Quality of biomass	Variable	Reproducible
Light-efficiency	Poor	Good

The Algal Technologies Program at Qatar University has already demonstrated successful growth trial of several potential microalgae strain up to 500 m<sup>2</sup> (100,000 L culture) raceway in the Qatari desert [15, 23, 31]. A number of strains have been investigated for their potential for large-scale outdoor production for biofuels and fish feed [15, 23, 31-33]. Nevertheless, there is still a great deal for potential native strains and applications that remain largely unexplored.

For the successful commercialization of microalgal products, efficient biomass harvesting is crucial. This efficiency must be achieved in a cost-effective manner, balancing high biomass production with low operational costs, minimal maintenance requirements, and reduced energy consumption. Different techniques exist with varying costs and energy requirements. Most commonly used methods are physical including centrifugation, filtration, and sedimentation [34]. Biological methods are also employed, such as bio/ auto flocculation using the natural tendency of cells to

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aggregate. It can also be through introduction of bacteria/fungi [35]. Chemical harvesting methods using flocculant agents such as aluminum sulfate or ferrous sulfate are also existing [35]. Electrochemical and magnetic technologies are a new approach for biomass harvesting. Electrochemical technologies are considered as a cheap, innovative, and efficient method in comparison with other methods which need complex operational parameters and costly chemicals [34]. They are mainly based on the generation of metal ions because of the oxidizing metal electrodes, which create the destabilization of colloid suspension and subsequent coagulation/ flocculation or floatation of microalgal biomass [34]. On the other hand, the magnetic separation is based on tagging a non-magnetic substance like cells with magnetic particles that show paramagnetic movement with the help of a magnetic field. The cells are therefore isolated from the medium in an external magnetic field [36]

Choosing the appropriate harvesting method is paramount and mainly depend on the end products. The method should effectively separate biomass from the culture medium while minimizing associated costs and maintaining the quality. Key factors to consider include cell size and density, potential cell damage, salt concentration, and moisture content. For cost-effective biomass harvesting, a combination of two or more methods can be employed. Additionally, it is crucial to avoid contamination during harvesting and consider the possible recycling of the culture medium to further reduce costs and enhance sustainability.

### 1.3. Post-harvest and downstream processing

The post-harvest and downstream processing of microalgae are critical steps in converting the harvested biomass into products such as biofuels, bioactive compounds, bioplastics, etc. These processes involve several stages, including drying, extraction, and purification, each tailored to maximize yield and quality.

#### 1.3.1. Drying:

Drying is the first step after harvesting, aiming at reducing the residual moisture content of the biomass to enhance its shelf life, and ease the extraction of substances. Various methods are used for drying [37], including:

- **Solar drying:** A cost-effective method that relies on natural sunlight. It is suitable for regions with abundant sunlight and where less stringent time constraints are acceptable. However, it is weather-dependent and may not be

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suitable for all applications, as it might cause degradation of valuable compounds.

- **Spray drying:** Involves atomizing the biomass into a hot air chamber, which rapidly removes moisture. This method is efficient in preserving the integrity of sensitive bioactive compounds but is energy intensive.
- **Freeze drying:** This method sublimates water from the biomass at low temperatures and pressures, preserving the structure and composition of the product. It is extremely energy-intensive but produces high-quality products with minimal degradation of bioactive compounds.
- **Cross flow drying:** Uses a stream of hot air passing across the biomass. The method requires precise control over drying conditions to minimize thermal degradation of valuable compounds, balancing efficiency and energy consumption.
- **Vacuum-shelf drying:** Involves placing the biomass on shelves in a vacuum chamber. This method also requires precise control of drying conditions to protect bioactive compounds from thermal degradation, balancing efficiency and energy use.

Each method has its advantages and limitations, with choices often balancing efficiency, energy consumption, and the preservation of valuable compounds.

### 1.3.2. Cell Disruption Methods

Cell disruption is an important step towards the extraction of cell components such as lipids, proteins, and carbohydrates from the biomass. Common methods include [38]:

- **Mechanical extraction:** Techniques like bead milling and high-pressure homogenization break down cell walls to release intracellular contents.
- **Chemical extraction:** Uses solvents such as acids, alkali, hypochlorites, antibiotics, detergents, etc, which interact with components of the microalgal cell wall causing deformations and promoting cell disruption.
- **Enzymatic extraction:** Utilizes enzymes to degrade cell walls gently, releasing valuable compounds without harsh chemicals or high energy inputs.
- **Other processes** such as: Freeze thaw, microwave-based extraction, ultrasonication, electroporation, etc.

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The adoption of specific method depends on the targeted products and on the rigidity of the cell wall. Very often, the integration of more than one method is required.

### 1.3.3. Extraction and purification:

Several extraction methods can be employed, often in combination with cellular disruption techniques. These methods include [39] :

- **Solvent extraction**, which uses organic solvents to selectively dissolve desired compounds.
- **Ionic liquids and deep eutectic solvents**, which offer eco-friendly alternatives with enhanced selectivity
- **Supercritical fluids extraction**, known for their efficiency in extracting high-value compounds under controlled pressure and temperature conditions.

Each method offers unique advantages depending on the target compounds and desired purity. Purification processes might be required to refine the crude extracts and obtain high-purity compounds. By optimizing these processes, microalgae can be effectively utilized for a range of sustainable and valuable applications.

## 2. Pigments expression as a high valuable product

The current industrial applications of microalgae biomass span a wide range of sectors providing opportunities for job creation and economic diversification, particularly in regions with suitable conditions for cultivation. Fig. 7 highlights the trade-offs between volume, cost, and application across different levels of products: Biomass used in energy segment and chemical production have higher potential volume and lower cost, catering to broader applications, while higher-value products often serving specialized markets, such as pharmaceuticals, food and feed, or bioplastics usually have lower volumes and higher costs due to added processing such as purification.



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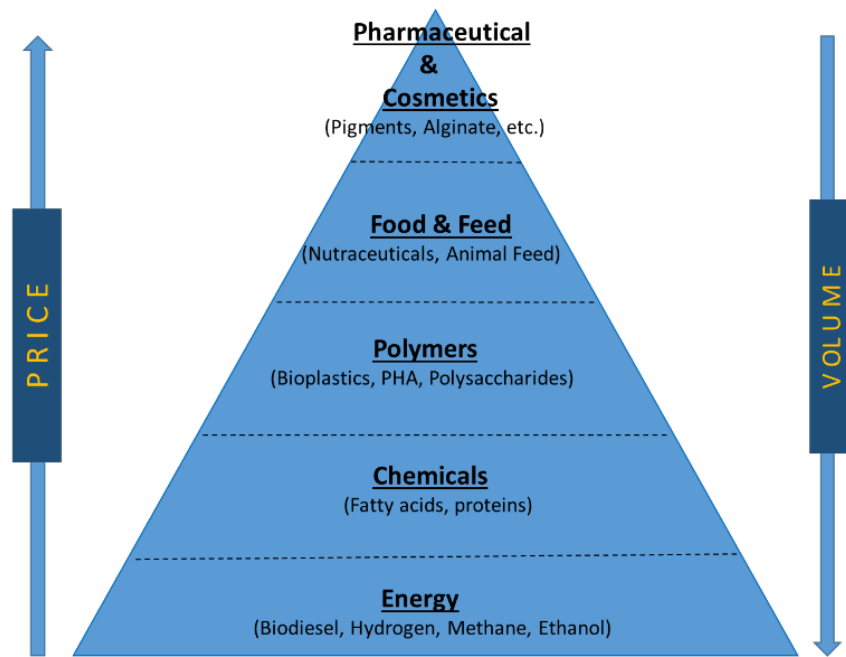


Figure 7: Product hierarchy with their cost and volume. More volume products are lower priced, while higher value products are produced at lower volumes.

Several algae strains are currently used industrially for these purposes. Some of the main ones are listed in table 3.

Table 3: Examples of the algae strains industrial applications [40].

Strain	Applications
<i>Chlorella</i> sp	Health supplements, food additives, and biofuels due to its high protein, lipid, and Polyunsaturated fatty acids (PUFAs) content.
<i>Arthrospira</i> sp (spirulina)	Protein source used in dietary supplements, Phycocyanin production.
<i>Haematococcus pluvialis</i>	Astaxanthin production, used in the aquaculture, nutraceutical and cosmetic industries due to its antioxidant properties.
<i>Dunaliella salina</i>	Beta-carotene producer, a precursor of vitamin A, often employed in food coloring and supplements.
<i>Nannochloropsis</i> sp	Biofuel and PUFAs production.
<i>Porphyridium</i> sp	Production of bioactive compounds, including polysaccharides with potential health benefits.

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<i>Isochrysis</i> sp	Aquaculture as a feed source for fish and shellfish larvae.
<i>Scenedesmus</i> sp	Wastewater treatment and biodiesel production
<i>Chlamydomonas reinhardtii</i>	Biofuel production

The market for algal pigments, located at the top of the pyramid (Fig.7), has experienced steady growth in recent years, propelled by rising consumer interest in health and wellness, the expanding dietary supplements industry and the rising demand for natural food colors, and sustainable products. In the food and beverage industry, algal pigments are sought after as natural colorants to replace synthetic dyes and microalgae such as *Spirulina* and *Chlorella*, not only provide visually appealing colors but also offer potential health benefits [41, 42]. Additionally, the use of algal pigments in cosmetics and personal care products aligns with the demand for natural ingredients in beauty formulations [43]. The pharmaceutical industry has also shown interest in algal pigments, exploring their health-promoting effects, further contributing to the expanding market [44].

The capacity of novel strains to produce pigments can be a key selection criterion when evaluating them for possible large-scale cultivation and valorization. Chlorophyll is the most well-known microalgal pigment. It captures sunlight and converts it into chemical energy, driving the primary energy source for the algae [45]. Beyond chlorophyll, microalgae boast an array of pigments such as carotenoids and phycobilins, each with distinct colors and roles [45]. Carotenoids, including beta-carotene and astaxanthin, not only contribute to the algae's coloration but also exhibit antioxidant properties with potential health benefits for organisms higher up the food chain [46]. Phycobilins, like phycocyanin and phycoerythrin, are responsible for the brilliant blues and reds seen in certain microalgae, they play a critical role in light absorption for photosynthesis in addition to their use as natural food coloring and in health supplements [47].

If glance into the market of microalgal pigments,  $\beta$  -carotene, Astaxanthin and C-phycocyanin (C-PC), are the three major high-value pigments with commercial uses in different industries [48, 49]. The main algae strains used as these pigment producers are as follows:

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- *Dunaliella salina* is a green microalga known for the production of beta-carotene, which provides an orange-red color when accumulated within the cell. The strain is the main source of the natural  $\beta$ -carotene, accounting for 14% of its biomass with a price between 300 and 3000 \$/kg [40]. The pigment is used as natural food coloring, dietary supplement and as an additive in cosmetic products [50, 51].
- *Haematococcus pluvialis* is considered the best natural source of astaxanthin, a bright red secondary carotenoid from the xanthophylls group, which accounts up to 4% of its biomass [52] and costs around 7000 \$/kg [53]. The pigment is known for its antioxidant properties and is commercially used in nutraceutical and cosmetic fields, in addition to its use as feed supplement in the aquaculture and poultry industry to improve their quality and boost their reproductive performance [54].
- *Spirulina*, scientifically known as *Arthrospira platensis*, is a blue-green microalga rich in the blue pigment C-phycoyanin, which constitutes up to 20% of its dry weight. Phycocyanin is mainly used as a natural food coloring with a price of \$500 /kg [55, 56].

We will now go into more depth about the phycobiliprotein family. In fact, the native strain that was selected and cultivated in this thesis is a phycocyanin producer as we will see later in chapters 3 and 4.

### 2.1. Phycobiliproteins: properties, extraction and applications

Phycobiliproteins are colored accessory pigments synthesized by cyanobacteria and red algae to help them carrying out photosynthesis in environments where solar energy is not optimal [57]. These intensely colored pigments are homogenous family of light harvesting proteins which absorb visible light in the range of 450 to 670 nm. On the basis of amino acid sequences as well as the identity and similarities in protein folds, it was suggested that the PBPs evolved from an ancient globin family of proteins [58]. Their basic structure includes protein subunits, consisting of two polypeptide chains known as  $\alpha$  and  $\beta$  (Fig. 8). They are organized into complex structures called phycobilisomes, which are large antenna complexes situated on the outer surface of the thylakoid membrane and enhance light absorption and funnel energy to the photosynthetic reaction centers [59]. Based on their structure and light absorption wavelengths, they are divided in 3 main types, [60-63]:

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- Phycoerythrin (PE) is a red or pink pigment with an optimum absorbance in the 540-570 nm range and a fluorescence emission optimum around 575– 590 nm. It consists of two major protein subunits known as  $\alpha$  (alpha) and  $\beta$  (beta), which are assembled in a hexameric structure ( $\alpha_3\beta_3$ ). Phycoerythrin contains phycobilins known as phycoerythrobilin and phycoerythrobilin, which are responsible for its red color.
- C-Phycocyanin (C-PC) is a blue pigment with an optimum absorbance in the 610– 625 nm range and a fluorescence emission optimum around 575– 590 nm. Similar to phycoerythrin, it also consists of  $\alpha$  and  $\beta$  subunits that form a hexameric structure ( $\alpha_3\beta_3$ ). Phycocyanin contains phycocyanobilin, which imparts its blue color.
- Allophycocyanin (APC): is a bluish-green color pigment with an optimum absorbance in the 650– 660 nm range and a fluorescence emission optimum around 645– 653 nm. It works in conjunction with C- phycocyanin to capture light energy. It also consists of  $\alpha$  and  $\beta$  subunits forming a hexameric structure ( $\alpha_3\beta_3$ ). Allophycocyanin also contains phycocyanobilin and a modified form of this pigment called phycocyanorubin.

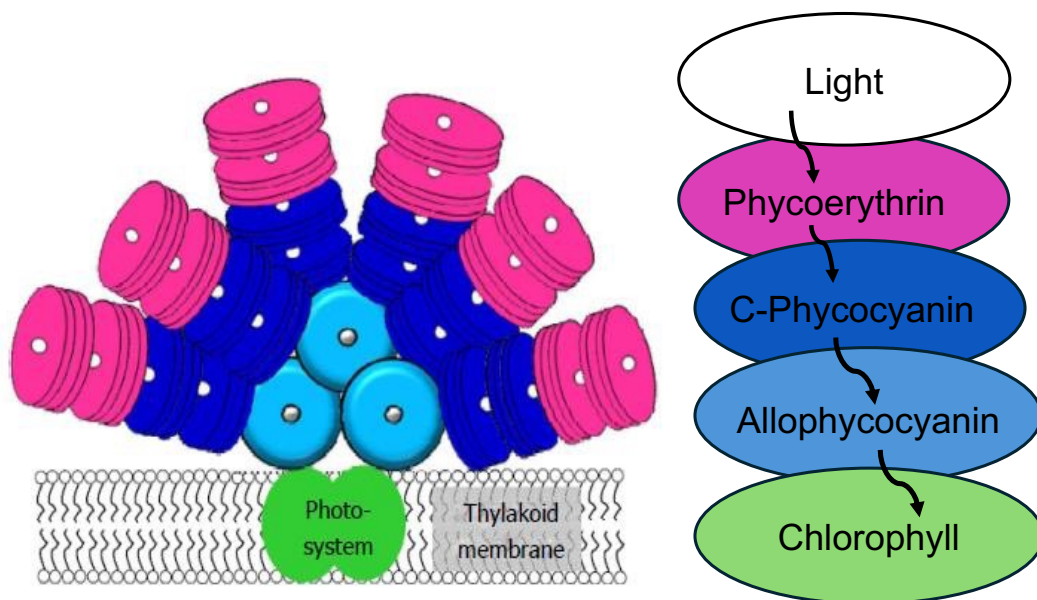


Figure 8: Schematic diagram of phycobilisome situated on the thylakoid membrane [64].

PBPs play a crucial role in the photosynthetic metabolism by capturing light energy and enabling it to be transferred to chlorophyll molecules according to the following pathway: PE → C-PC → APC → Chlorophyll “a” with C-PC the major constituents of phycobilisomes, whereas APC constitutes the core of phycobilisomes and function of

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the bridging pigment between phycobilisomes and the photosynthetic lamella [57, 65]. PE is found in the periphery of phycobilisomes and serve as major light-harvesting proteins in numerous cyanobacteria. It extends the light-harvesting capability of the phycobilisome from red region to the green region of the visible light spectrum [66] (Fig. 8). Many cyanobacteria have shown a tendency to respond to a change in light quality by changing the composition of their antenna in such a manner that it increases the proportion of the pigment with absorption spectrum more nearly complementary to the available light [58]. Quality of incident radiation and efficient energy transfer involved in light harvesting evolve PBPs to gain the ability to survive in broad range of environments including different depths of sea and fresh water, thus, populate ecological niches that could not be colonized by organisms with only chlorophyll as their main photosynthetic pigment.

The extraction of phycobiliproteins can be obtained by chemical, enzymatic and/or mechanical treatments. The choice of methodology depends on the organism as well as its cell wall stability and resistance [65]. Generally, the classical extraction is based on the high solubility of phycobiliproteins in aqueous solvents, among them phosphate buffer is the most common used one [67]. The enzymatic extraction can be done by subjecting the biomass to an enzymatic digestion with lysozymes, however, the recovery demands an extraction step of separation of the enzymes [68]. Combination of these methods with other mechanical ones such as sonication, homogenization, high-pressure, French press, glass beads-assisted extraction were used in order to improve the phycobiliproteins extraction [65].

The commercial exploitation of cyanobacteria to produce phycobiliproteins has increased in recent decades leading to a growing interest in strains large-scale production [69, 70]. Several microalgal strains are known for their high phycobiliprotein production, particularly phycocyanin and phycoerythrin. *Arthrospira platensis* (*Spirulina*) is one of the most well-known sources of phycocyanin, followed by *Leptolyngbya* sp, *Anabena* and some other species of *Nostoc* [65]. *Porphyridium* sp, on the other hand, is the commonly studied and utilized strain for phycoerythrin production. However, only few species of cyanobacteria are exploited for phycobiliprotein production at the commercial level, with PC occupying the largest market area [71]. For instance, phycocyanin extracted from *Spirulina* is advertised as

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'Lina blue' by Dainippon ink [72], and is also marketed by the french company Algosource as a phycocyanin rich extract called Spirulysat® [73]. It is noteworthy to mention that seawater strains remain largely uninvestigated. Hence, identification of more PBPs producing cyanobacteria could expand the opportunity of selecting suitable species from a broad range for commercial exploitation.

As technology and cultivation methods advance, the production of phycobiliproteins from microalgae is likely to become more efficient, contributing to the broader adoption of these pigments in various industries. Indeed, they have already been commercialized for the development of value-added products across a wide range of sectors, including food, pigments, nutraceuticals, pharmaceuticals, biomedical, and biotechnology industries [7]. The study of phycobiliproteins as bioactive compounds has been expanding in the past years, with a growing range of health-related applications such as [65, 70] :

- **Antioxidants** through the inhibition of free radicals and lipid peroxidation-induced damage [74].
- **Anti-inflammatory** agent, with different mechanisms of action in enzymes expression and activation, and in modulation of macrophages function, inhibiting the pro-inflammatory signals [7].
- **Anticancer** potential as apoptosis activators and leukemia inhibitors [75]. The fluorescence nature of PC has been widely accepted for the inhibitory role on malignant cell of leukemia (K562).
- **Anti-diabetes** properties [65], eye protection by inhibition of sodium selenite-induced cataract [76] and cardio protection by inhibition of ischemia-reperfusion-induced damage by free radicals have also been reported. PC exhibits remarkable inhibition for heart malfunctions by alteration of blood enzymes aspartate amino transferase, alanine amino transferase, and malondialdehyde [77].
- The fluorescence properties of PBPs have been utilized as **marker** in antibody receptor and **donor** in immunological research laboratories [78].

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### 2.2. Effect of cultivation parameters on growth and Phycobiliproteins expression

The growth and metabolism of PBPs is regulated by environmental factors such as light, nutrient availability, and stress conditions such as pH, temperature and salinity. [5, 16]. Despite these microorganisms being adaptable to a variety of environmental conditions, it's important to carefully consider and optimize a number of growth conditions in order to produce the PBPs effectively [65].

#### 2.2.1. Light

Light is a crucial factor influencing algal growth due to its central role in the process of photosynthesis, where algae convert light energy into chemical energy in the form of ATP (adenosine triphosphate) and NADPH (nicotinamide adenine dinucleotide phosphate). These molecules serve as essential energy carriers that drive various metabolic reactions within the algal cells, facilitating growth and biomass production. [79].

Different wavelengths of light can have varying effects on algal growth. Blue and red light are most effective for photosynthesis, while green light is less efficiently used [80]. Increased light intensity can boost microalgae growth, up to a certain point that varies depending on the species [81]. However, there is a threshold beyond which excessive light becomes detrimental. This phenomenon is known as photoinhibition, where the photosynthetic machinery becomes damaged due to the accumulation of reactive oxygen species, ultimately slowing down or halting growth [82]. Proper management of light exposure is hence essential to ensure optimal algal growth and prevent photoinhibition or other light-related stress. In general, optimum level of light intensities for most of the microalgae species are about 200 – 400  $\mu\text{mol photons/m}^2/\text{s}$  [83]. The number of photons reaching the culture's surface ( $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ ) enables the determination of incoming light intensity as a function of photon flux density (PFD). How much PFD can be absorbed by cells is dependent on cell density, cell optical properties and culture mixing. Appropriate penetration and uniform distribution of light is also needed to avoid the self-shading effect, in which algae at lower layers of culture are shaded from the light by upper layers. The remaining photons are either reflected or transformed to heat. Ultraviolet light (UV; 215–400 nm), on the other hand, adversely affects the algal growth, by damaging to the photosynthetic machinery within the cells, with UV-B (215–380 nm) causing more damage to the cells compared to UV-A radiation (380–400 nm) [84]. In response to UV radiation, algae employ several

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defense mechanisms, such as migration, the development of protective cell walls, and enhanced synthesis of carotenoids and other pigments to mitigate damage [84].

Algae also require a balance between light and dark periods for growth. While continuous light cycles at optimum light intensities lead to improved biomass productivity, this approach is not universally beneficial. It is essential to consider the specific requirements and tolerances of the algae species in question and optimize light intensity and duration accordingly. Experimental trials are often necessary to determine the best lighting regime for a given cultivation setup. Most studies have shown that 16 h light/8 h dark cycle is most suitable for algal growth [85].

Light affects not only biomass production but also its composition [86]. Various studies have shown that the lipid content is related to the environmental light intensity. In *Nannochloropsis* sp., grown under low light conditions, 40% of the total lipids were found to be galactolipids and 26% were found to be triacylglycerols. In the same system, high light ( $550 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) conditions resulted in an increased synthesis of triacylglycerol with a reduction in galactolipid synthesis [87]. *Nannochloropsis* cells under low light conditions were characterized by high lipid content and high proportions of eicosapentaenoic acid [87]. Confirming this observed trend, another study on the same species reported an increase in unsaturated fatty acids mainly due to an increase in EPA (from 44.3% to 60.7% of the organic content) and a decrease in protein content, with decreasing irradiance.

Besides lipids, light influences also the PBP's expression. Different light intensities can influence the expression of specific types of phycobiliproteins. It has been reported that low and medium light intensities was found to boost the biosynthesis of these pigments as they are synthesized to expand the range of light absorption In low-intensity conditions [88]. A light intensity of  $150 \mu\text{mol photons/m}^2/\text{s}$  was found to be optimum for phycobiliprotein production in the cyanobacterium *Arthrocnemum africanum* with 16:8 light: dark regime as the optimal photo-period [89]. Another research work reported concentration of phycoerythrin and phycocyanin, which were inversely related to growth irradiance for *Synechococcus* WH7803 under intensities from 200 to  $2000 \mu\text{mol m}^{-2} \text{s}^{-1}$  [90]. Some species, however, have adapted to intense light, a process known as light acclimation. This adaptive response ensures optimal light capture and photosynthetic efficiency under varying environmental light regimes. This is the case of *Arthrocnemum africanum* and *Rhodomonas* sp., which both accumulated



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more PBPs under higher light intensities [91, 92].

Light quality is another factor influencing the expression of specific types of phycobiliproteins. Studies has demonstrated that exposure to blue light increases the productivity of PBPs in various species of *Anabaena*, *Synechococcus*, and *Nostoc* [93-95]. Conversely, some *Nostoc* species like *Nostoc sphaeroides* produced more PCs when exposed to red light and more PEs when exposed to green light [95].

The duration of illumination also displayed an effect on photosynthetic activity and, consequently, on the PBPs increase. Excessively high continuous light intensities lead to photoinhibition, usually occurring when the rate of light-driven energy capture exceeds the rate of energy utilization, mainly in nutrients limited media. Photoinhibition can cause the damage of photosynthetic apparatus and as response, some species might downregulate PBPs expression to reduce the risk of photodamage [96].

In summary, light is essential for both algal growth and PBPs expression. Effective management of light in algal cultivation systems is crucial to optimize growth rates and PBPs productivity while avoiding issues like photoinhibition.

### 2.2.2. Nutrients

Nutrients are essential for algal growth as they provide the building blocks for cell development, energy production, and various metabolic processes. The availability and balance of nutrients significantly influence the growth rate and overall productivity of algae. Different microalgae species may vary in their nutritional needs; however, the basic requirements are same for all species. Nitrogen (N), phosphorus (P), and carbon (C) are classified as macronutrients required for algal growth which form the backbone of microalgae ( $\text{CH}_{1.7}\text{O}_{0.4}\text{N}_{0.15}\text{P}_{0.0094}$ ) [18], and considerable variation in the algal biochemical composition can be observed under conditions of their limitation [97]. 50% of microalgae biomass is comprised of carbon; however, it may vary between strains and based on the culture conditions. As an example, microalgal biomass with high lipid content will have more carbon compared to others.

Carbon dioxide ( $\text{CO}_2$ ) supply is crucial for microalgae culture due to its essential role in photosynthesis, the fundamental process that drives algal growth. It is a critical carbon source that algae use to convert light energy into chemical one. Proper  $\text{CO}_2$  supply contributes to maintaining optimal growth conditions, high biomass productivities and helps in maintaining balanced pH levels in cultures, preventing pH

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fluctuations caused by photosynthetic activity. In mass microalgae cultivation, relying on carbon dioxide absorption from the atmosphere through the surface of a raceway is entirely insufficient to support photosynthesis during sunlight and enable intensive microalgae farming [98]. For instance, reaching a biomass productivity of  $25 \text{ g} \cdot \text{m}^{-2} \cdot \text{d}^{-1}$  requires an average of  $45.8 \text{ g CO}_2 \cdot \text{m}^{-2} \cdot \text{d}^{-1}$  to be supplied to the growth ponds (assuming a biomass carbon content of 50% w/w). This is substantially higher due to the low concentration of  $\text{CO}_2$  in the atmosphere (0.03–0.06%) and limited pond surface area. Therefore, it is imperative to investigate other alternatives to avert carbon limitation. Inorganic carbon sources like industrial streams (flue gas) and soluble carbonate solutions can be used rather than using commercial  $\text{CO}_2$  due to the expensive cost of which is estimated to be between 8 and 27% of the total algae production [99]. Provision of more efficient delivery mechanism is also vital for effective algae production as approximately 50% to 90% of the  $\text{CO}_2$  delivered to the pond exits to the atmosphere [24, 99]. Moreover, it is important to achieve a balance between the  $\text{CO}_2$  demand of microalgae growth and its supply in the culture as high concentrations can reduce the medium pH and eventually limit microalgae growth [100].

While the most of microalgae are phototropic, some of them still could use various types of organic compounds as source of carbon and energy, in the presence of light, the phenomenon is known as mixotrophy. Some studies reported that shortage in carbon can increase the PBPs synthesis as they serve as defense machinery against damage caused by light when carbon assimilation is restricted [101]. In contrast, Zeng et al. [102] reported an enhancement in PBPs production for *Spirulina platensis* after introduction of  $\text{CO}_2$  into culture medium which stimulates the carboxylase activity in rubisco to fix carbon and produce biomass rich in PBPs.

In addition to carbon, nitrogen and phosphorus, are the other macronutrients required for growth and metabolism. Nitrogen is considered a key factor due to its essential role in various cellular processes and biochemical reactions and accounts for 7%–20% of cell dry weight. Once taken up, nitrogen undergoes metabolic processes such as transamination, and deamination to incorporate it into cellular constituents or convert it into other nitrogenous compounds. These nitrogen-containing compounds serve as precursors for the synthesis of proteins, nucleic acids, and other essential biomolecules. Nitrogen is also an essential element in photosynthesis as it represents a major component of chlorophyll, and is also necessary for PBPs production [103].

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Therefore, it is crucial to control and optimize the nitrogen source and/or concentration in the production process to ensure sustainable biomass and phycobiliprotein production. Under nitrogen-replete conditions, the cells growth and reproduction increase, leading to an increase in phycobiliprotein synthesis. This is the case for most species including *Phormidium ceylanium*, *Limnothrix* sp., *Oscillatoria* sp., and *Eubhalotheca* sp., where a direct correlation between nitrate concentration and PBPs accumulation was observed [104-106]. Insufficient nitrogen can result in chlorosis, where algae exhibit yellowing or loss of green coloration due to reduced chlorophyll production, resulting in growth inhibition and reduced algal productivity [107]. However, for certain applications, limitation is applied to enhance specific metabolites productions. For example, nitrogen starvation shifts the lipid metabolism from membrane lipid synthesis to accumulation to neutral lipid storage which can be used in the biodiesel production [108]. Conversely, excessive input of nitrogen causes eutrophication leading to algal blooms and ecological imbalances.

Algae assimilate nitrogen from the environment in various forms, including ammonium ( $\text{NH}_4^+$ ), nitrate ( $\text{NO}_3^-$ ), and urea. This assimilation is strain-dependent, with certain strains being more susceptible than others. Khazi et al. [109] examined growth and PBPs production under 3 nitrogen sources: potassium or sodium nitrates and ammonium chloride for *Arthrospira platensis*, *Pseudoscillatoria* sp. and *Phormidium* sp. The findings revealed that, both growth and PBPs content was maximal for *Arthrospira platensis* using sodium nitrate, whereas both strains *Pseudoscillatoria* sp. and *Phormidium* sp. accumulated better PBPs content with ammonium chloride.

Due to the high nitrogen requirement for algal cells in mass cultivation, it is advised to replace this nutrient from basic culture media to other low-cost sources to improve the economic viability of microalgae biomass production. Urea ( $\text{CH}_4\text{N}_2\text{O}$ ), a nitrogen-containing compound, is a polar chemical which is ubiquitous in nature. Because of its widespread availability and low cost, urea can be incorporated into algal culture systems, making the large-scale algal production cost-effective [110-112]. It can also help in reducing the environmental impact of nitrogen-containing waste products, as it can be converted into ammonium and nitrate by algae strains, which can be used later to support plant growth. Davis et al. [113] found that urea can be an excellent organic compound that would serve as suitable nitrogen sources for *C. pyrenoidosa* without

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causing pH fluctuations in the medium. Similarly, urea has been reported to positively influence the growth and phycocyanin production of *Spirulina* [114]. Furthermore, the use of urea as a nitrogen source has resulted in high productivity and pigment accumulation in species such as *Neochloris oleoabundans*, *Microcystis aeruginosa*, and *Chlorella* sp [111, 115-117]. As the significance of urea as a nitrogen source for microalgae becomes more recognized, it has been demonstrated that urea can also serve as an effective combined nitrogen and carbon source. The intracellular breakdown of urea by urease releases CO<sub>2</sub>, which can be utilized as an additional carbon source to support algal growth. [98]. Indeed, the production of species such as *S. Platensis*, *Neochloris oleoabundans*, *M.aeruginosa* and *Chlorella* sp. under urea as nitrogen source resulted in high productivity and pigment accumulation [111, 115-117].

Phosphorus is also an essential nutrient for algal growth and the production of phycobiliproteins. It constitutes 1% of dry weight of algae and is assimilated from the environment in the form of phosphate ions (PO<sub>4</sub><sup>3-</sup>). It is a key component of ATP which is generated during photosynthesis and is essential for algal growth and metabolism proteins including PBPs, lipids, and carbohydrates [118]. Phosphorus availability regulates various both cell division and PBPs accumulation. Limitation can inhibit algal productivity, as cells prioritize essential functions under nutrient stress conditions. Therefore, optimal phosphorus availability is necessary.

There are few other essential micronutrients (i.e. Fe, Zn, Cu, Mn, Co, Mo) that are only required in trace amounts. Iron is the most important metal among them as it acts as redox catalyst in photosynthesis and nitrogen assimilation and mediates electron transport reactions in photosynthetic organisms [119].

### 2.2.3. pH

The pH of the culture media is another important factor affecting microalgae growth since it determines the solubility and availability of CO<sub>2</sub> and other essential nutrients, and thus can have a significant impact on algal metabolism [100, 120]. Microalgae species have different pH requirements. Although, most grow well in the neutral pH, some cyanobacteria species have been found to have preference for a basal pH, which ranges between 7.5-8.5 [120]. Monitoring and adjusting pH levels within an optimal range are crucial for ensuring healthy and productive algal cultures.

Acidic conditions can alter nutrient uptake or induce metal toxicity and consequently

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affect the algal growth [121]. Coleman and Colman studied the effect of external pH on photosynthesis of *Coccochloris peniocyctis* and found a significant decrease in total accumulated carbon and oxygen evolution at pH 5.0 and 6.0, which suggested the reduction in photosynthesis in this cyanobacteria at these pH ranges [122]. Normal growth rate was restored after the pH was lowered.

The expression of phycobiliproteins is affected by pH through its influence on nutrient solubility and bioavailability, as well as on substance transport across the cell membrane [123]. The pH range for PBP's biosynthesis is reported to be between 6 and 10 depending on the species, with pH ranges in the alkaline region being more appropriate. Hence, maintaining a suitable pH is important for maximizing pigment production. For instance, the ideal pH values for *Rhodomonas* sp., *Lyngbya* sp., *Spirulina* sp., and *Synechocystis* sp., are 7.0, 8.0, 9.5, and 10, respectively [89, 92, 124]. Extreme pH can denaturize the phycobiliproteins influencing their stability [123]. Therefore, maintaining an appropriate pH is crucial for optimizing both algal growth and pigment production.

### 2.2.4. Temperature

Temperature is considered as one of the major ecological factors limiting growth and metabolic productivity in most microalgae by influencing their respiration rate, membrane fluidity and nutrient uptake [125]. It is an important factor for large scale cultivation specially in open pond culture and need careful monitoring as the algae experience significant temperature change over time. The optimal growth temperature depends on the adaptation and tolerance of each strain [126]. Previous researches demonstrated that most of the microalgae species are able to carry cellular division within a limited temperature range, typically between 15 and 30°C, with optimal conditions from 20 and 25°C [127]. Growth at temperature optima results in minimal cell size and the efficiency of carbon and nitrogen utilization decreases at non-optimal temperatures [128]. The effect of temperature increase above optimal growth range is often reported to be more harmful than low temperature. One of the most commonly observed changes with temperature shift is the alteration in the level of unsaturation of fatty acids in the lipid membrane [129, 130]. *Dunaliella salina* has shown a considerable increase in fatty acid unsaturation in response to decrease in temperature from 30 to 12 °C [130]. Specific cyanobacteria species from desert environment has a temperature tolerance up to 40 °C e.g. *Spirulina platensis*; up to 42 °C for other

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strains isolated from hot spring e.g. *Chaetoceros calcitrans*, and *Leptolyngbya* HS-36 [131-133]. Different strategies are deployed to tackle the effect of increased temperature such as energy re-balancing and cell shrinking [134]. Nonetheless, algal viability and photosynthetic activity are reported to be negatively impacted above threshold of 43 °C. This is due to degradation of key enzymes and membrane denaturation. High temperature disrupts bonds within cell walls and also denature proteins [135, 136]. Temperature can also be used to induce the production of valuable metabolites. This rise with increasing temperature is generally attributed to cellular response, enhanced active free oxygen radical formation or increased biosynthetic enzyme activity [135, 137, 138].

Phycobiliproteins synthesis in cyanobacteria is regulated by temperature and optimum temperature for maximum production of phycobiliproteins production is strain specific. Temperatures outside this range can lead to reduced enzyme activity, altered gene expression, and changes in cellular metabolism. Previous research work highlighted those temperatures ranging between of 30–36 °C were more suitable to enhance phycobiliproteins yield in most cyanobacteria [65, 139]. For example, 35 °C was found to be most suitable for the phycobiliproteins synthesis for *Nostoc* sp., [59]. However, it was dramatically slowed down at lower temperatures since these conditions reduced the metabolism of the organism by affecting enzyme activity, membrane fluidity, and gene expression [140]. According to Sakamoto and Bryant [141], only *Synechococcus* sp., PCC 7002 proved to be more responsive and presented a preference to low temperatures (22 °C). Higher temperatures (>40 °C), on the other, induce heat stress, causing PSII inactivation, PBS dissociation, and ultimately their denaturation leading to low/no phycobiliprotein expression [59]. Heat stress can also cause damage to cellular membranes, affecting the stability of phycobilisomes, where phycobiliproteins are located.

Nevertheless, there are some thermophilic cyanobacteria that can acclimate to changing temperatures over time. This involves adjusting gene expression and cellular processes to optimize performance under different temperature conditions [140]. For instance, *Synechococcus lividus* PCC 6715, *Thermosynechococcus vulcanus* NIES 2134, *Thermosynechococcus elongatus* NIES 2133 etc., have all demonstrated their ability to grow optimally at 45–60 °C, with a phycobiliprotein synthesis in these upper temperature ranges [142]. Both in vitro and in vivo studies have examined the

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thermostability of PBPs with PC being the most studied PBP. As a result, thermophilic PBPs may exhibit specific structural features, and the cyanobacteria showed a strong coupling of PBPs to photosystem compared to the mesophilic ones. These features include increased numbers of stabilizing intermolecular interactions (e.g., hydrogen bonds, salt bridges) within the protein complex, as well as enhanced hydrophobic interactions that help maintain protein structure even at higher temperatures [143]. Thermophilic organisms often produce heat shock proteins (HSPs) that help prevent protein denaturation and assist in refolding damaged proteins. These HSPs play a role in maintaining the integrity of PBPs under high-temperature conditions [143, 144]. To date, the most thermostable PC examined *in vitro* was isolated from *S. lividus* sp. which reportedly remained stable at » 70 °C [145, 146]. In spite of this, prolonged exposure to extreme temperatures can still result in the reversible dissociation of PBPs from photosystems or, under severe stress, their irreversible denaturation can occur [147].

Qatar is located in the northern hemisphere and is climatologically defined as subtropics region characterized by high temperatures. The country's temperature variations are notable, with distinct seasonal differences. A short cooler winter from December to February with a mean daytime temperature of 22°C, and a hot season from April to October, within which we can distinguish a very hot period from May to mid-October characterized by intense rising temperatures which goes above 40 °C and can reach up to 50 °C [148]. Nights are warm but may still be relatively hot compared to many other regions, with temperatures often above 30 °C. March and November are considered transitional months, warm but without excesses with daytime temperatures typically range between approximately 25 to 35 °C.

Moreover, Qatar experiences distinct wind patterns that significantly influence its temperature dynamics throughout the year. During the summer months, a moist northwest wind prevails, contributing to increased humidity levels and occasionally providing relief from the intense heat. In contrast, a hot and dry south wind dominates primarily from spring to summer. This wind can raise temperatures substantially, reaching up to 30°C in winter and surpassing 40°C from April to October. Importantly, it drastically lowers relative humidity levels, exacerbating the sensation of heat and contributing to the dryness of the environment. Additionally, the south wind is known

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for stirring up dust and sandstorms, further impacting visibility and air quality during its seasonal prevalence [149].

Sea water temperatures in Qatar typically remain lower than ambient air temperatures and are influenced by several factors throughout the year, including seasonal changes, solar radiation, wind patterns, and ocean currents. Variations between surface and deeper layers are also evident. During winter, sea surface temperatures tend to be cooler, ranging from 20°C to 25°C, due to lower air temperatures and reduced solar radiation, while deeper waters maintain relative stability. As the months progress, increasing sunlight and warmer air temperatures gradually raise sea surface temperatures, which often exceeding 30°C with water surface temperature reaching up to 2°C lower than ambient temperature (Own observation).

As previously discussed, large-scale algal production in Qatar primarily occurs in open raceway systems where controlling factors such as temperature is challenging. Therefore, it is essential to select microalgae strains capable of tolerating significant diurnal temperature fluctuations across seasons to ensure sustainable production. However, the physiological functions of photosynthetic organisms are also influenced by salinity, which itself is greatly affected by high temperatures. This dual challenge underscores the importance of selecting robust strains that can thrive under fluctuating temperature conditions while managing salinity levels effectively.

### 2.2.5. Salinity

Water salinity is another abiotic factor altering the growth and metabolite production of marine cyanobacteria. Salinity levels can influence the growth through several mechanisms and each microalgal species has an optimal range of salinity for growth. The adaptation to this factor was also shown to be strain dependent and related to geographical regions of origin [150]. Some microalgal species such as *Dunaliella salina* and *Tetraselmis* sp., could grow at elevated salinity. Cyanobacterial plasma membrane plays a role in the sensing of high salinity and acclimates against adverse environmental conditions by regulating the extracellular and intracellular ion homeostasis. For instance, it is commonly known that *Synechocystis* adapts to unfavorable environmental conditions by changing the permeability of its plasma membrane in reaction to high salinities (Singh et al. 2022). The adaptation can also be through generation of an antioxidative defense system which could mitigate the



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negative effect of saline environment. Furthermore, it was reported that the salt tolerant microorganisms accumulate several solutes to compensate for the difference in water potential that helps cells to take up water, establishing or maintaining turgor pressure. Moderately halotolerant marine strains are characterized by glucosylglycerol (GG) as their main compatible solute and sometimes glucosylglycerate (GGA) as a secondary compatible solute, whereas halophilic strains that are able to grow in saturated salt concentrations usually synthesize glycine betaine (GB) or glutamate betaine [151]. Nonetheless, not all the strains present the ability to acclimatize to salinity, and the effect can be depressive as high salinities causes both osmotic and ionic stress on microalgal cells. Water tends to move out of the cells in response to higher external salinity, affecting cell turgor pressure. This hinder the water and nutrient uptake, leading to reduced growth and eventually death [152]. Changes in salinity concentrations also affect chloroplast structure and the alter metabolic activities, mainly protein synthesis [153].

The expression of phycobiliproteins in different salinity environments has not been examined extensively. Different species of photosynthetic organisms might respond differently to salinity changes. In general, high salinity levels usually disrupt the balance of ions within cells. This can impact enzyme activity, protein stability, and cellular processes, inhibiting the phycobiliprotein synthesis [154]. Previous research showed that salinity increase provoked a decrease in the production of these pigments with 80% less phycobiliproteins synthesis [139]; the same negative effect has been also described for *Limnothrix* sp. [155]. On the other hand, some photosynthetic cyanobacteria have evolved mechanisms to adapt to varying salinity levels and enhancement of PBPs expression was observed under high salinities. For example, an increase of phycobiliprotein production by *Phormidium* sp. was reported when the salinity raised to 0.5 M (» 30 ppt) [75]. Similar trend was found for *Oscillatoria* sp. [156].

Qatar has one of the lowest annual rainfall levels in the world, which makes water resources a critical factor for the country development and sustainability. Waste water could be considered as resources as several wastewater treatment facilities produce almost 253.2 (1000 m<sup>3</sup> /year) of treated wastewater (TWW) [157], mostly used for green spaces irrigation. However, biomass grown in TWW may have trace amount of toxic and heavy metals or even pathogens both as cell components and also adsorbed

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to the biomass [158], hence such biomass cannot be suitable for human consumption and use as feed or for the production of high valuable products.

Saline water, on the other hand, can be considered for the outdoor algal cultivation as marine environments cover majority of the country with a length of its coastal line of about 1000 km. Moreover, Qatar falls within a low elevation coastal zone (0–28 m above sea level). Therefore, pumping seawater will be easier and requires less energy. While the average salinity in the Middle East is 40 ppt (part per thousand), the average rate is 55 ppm in Qatar. Fig. 9 shows the variation in water salinity across Qatar coast. Depending on the specific location, the salinity goes from a minimum of 39 g/L, usually observed in the northern area to a maximum of 58 g/L in south-eastern regions of the country. However, if we are considering open ponds system for saline microalgae cultivation which relies solely on sea water and recycled media, salinity will increase overtime due to evaporation. The rise in air temperature and subsequent increase in evaporation rate, result in salinity increment ranging from 0.5 to 1.5 ppt [159]. To maintain a consistent salinity and culture volume, the addition of freshwater to the culture is necessary. However, this poses challenges in Qatar due to freshwater scarcity, making it impractical for large-scale operations. Therefore, selection of strains with a wide salinity tolerance is required for the sustainable and economical production of PBPs. In this case, even if the daily evaporation loss is balanced by adding seawater, growing these strains remains feasible. Moreover, elevated salinity levels reduce the risk of contamination from competing microalgae and predators.

Previous studies have demonstrated successful large scale of saline microalgae across different salinity ranges. For instance, a local halotolerant *Tetraselmis* sp. was cultivated in semi-continuous outdoor in the Qatari desert using recycled media in salinities up to 80 ppt, with the aim to produce biocrude oil [23] (Das et al., 2019). However, local data on the impact of salinity as a growth parameter on the production of cyanobacterial strains and their PBPs synthesis are currently lacking. This gap underscores the need for further research in this area to optimize cultivation conditions and maximize the potential of saline microalgae cultivation in Qatar's unique environmental context.

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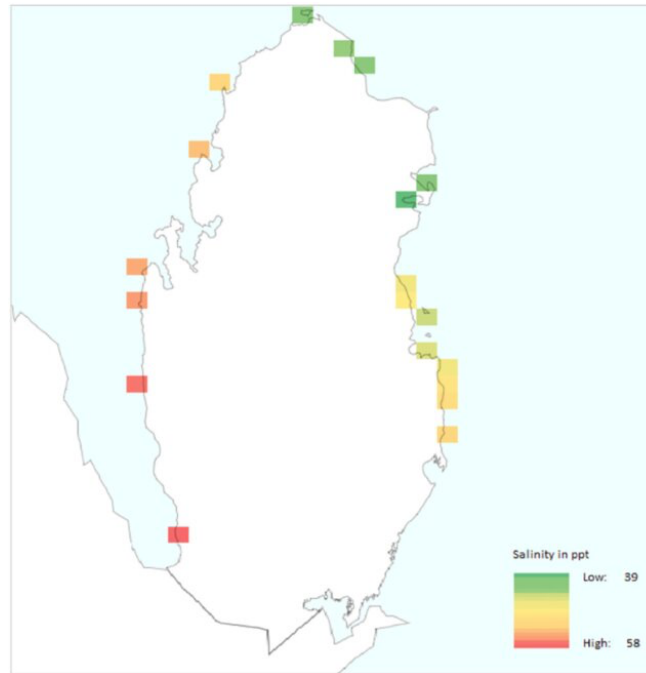


Figure 9: Salinity variation across Qatar, [159]

### 2.2.6. Mixing

Mixing is a critical factor in algal system production, and proper mixing plays a significant role in achieving optimal growth conditions [160]. In open pond systems or photobioreactors, mixing prevents the settling of algae at the bottom and maintains a well-distributed algal population. As the culture density increases, the top layer absorbs all the light energy, leaving the bulk of the culture in darkness and resulting in low overall biomass density. Thus, appropriate mixing of culture is essential for enhancing light access to suspended cells, ensuring that all algae receive sufficient light for photosynthesis [161]. Efficient mixing allows also uniform distribution of nutrients and CO<sub>2</sub> in microalgae culture preventing nutrient gradients and depletion in certain areas and assuring a uniform CO<sub>2</sub> is dispersion throughout the culture medium. Additionally, mixing enhances the dissolution of oxygen into the culture medium. Adequate oxygen levels are essential for aerobic metabolism and respiration in algae, especially when the algal population is dense [162].

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### 3. The Importance of a biorefinery approach

Despite the remarkable efforts demonstrated in laboratory and pilot scale addressing the prospective applications of microalgae, the economic barriers unfortunately prevented several products from their commercial viability. It is particularly relevant for pigments, which represent only a very small fraction of the microalgae biomass. To counteract this issue, one or more extra market-driven products needs be produced to offset the high production costs. This can unlock new possibilities for sustainable production and avoid the inflation of microalgae bioproducts mainly in markets where the synthetic pigments involve lower production costs [52].

Over the past decade the algal biorefineries have been investigated as an integrated approach to optimize the use of multiple components of the feedstock for various applications. This holistic approach mimics the concept of traditional petroleum refineries but uses renewable biological resources, thereby supporting environmental sustainability, economic viability, and technological innovation. It does not only enhance the economic viability of biomass utilization but also supports global efforts towards achieving sustainable development and energy security. As such, the continued development and implementation of biorefinery technologies are essential for building a sustainable future.

Various methods exist for converting biomass into valuable products, each utilizing different approaches depending on the desired outcome. **Biochemical conversion** processes, such as fermentation and anaerobic digestion, use biological organisms or enzymes to produce biofuels, biochemicals, or fertilizers. **Thermochemical conversion** involves heat and/or chemicals, with methods like hydrothermal liquefaction, hydrothermal carbonization, pyrolysis, gasification, and combustion transforming biomass into bio-oil, syngas, and biochar. **Chemical conversion** techniques, such as transesterification and catalytic cracking, chemically alter biomass-derived compounds into biodiesel, biofuels, or chemicals. Each method serves different applications, ranging from bioenergy production to bioplastics and biochemicals, contributing to sustainable resource use.

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In this work, only hydrothermal liquefaction under subcritical conditions using microalgae biomass residues will be discussed.

### 3.1. Biomass valorization through hydrothermal liquefaction for sustainable biorefinery application

Hydrothermal conversion is a promising strategy applied in biorefinery processes for the conversion of biomass into valuable products such as biofuels, biochemicals, biopolymers and soil amendments, with high conversion yields and selectivity. It involves conversion of biomass at certain high temperatures and pressures. Hydrothermal Liquefaction (HTL) can directly convert wet biomass and thus avoids extra energy consumption during the drying process [163]. In such conditions, water becomes subcritical and acts as both a solvent and a reactant to facilitate the decomposition of organic or carbonaceous feedstock. The subcritical state obtained between 150°C and 350°C, and under sufficient pressure to remain in the liquid state, affects the physicochemical properties of water. Under normal conditions, water is polar and can't dissolve organic substances. By increasing the temperature, in the subcritical conditions (Fig.10), it becomes an effective solvent for nonpolar substances [164].

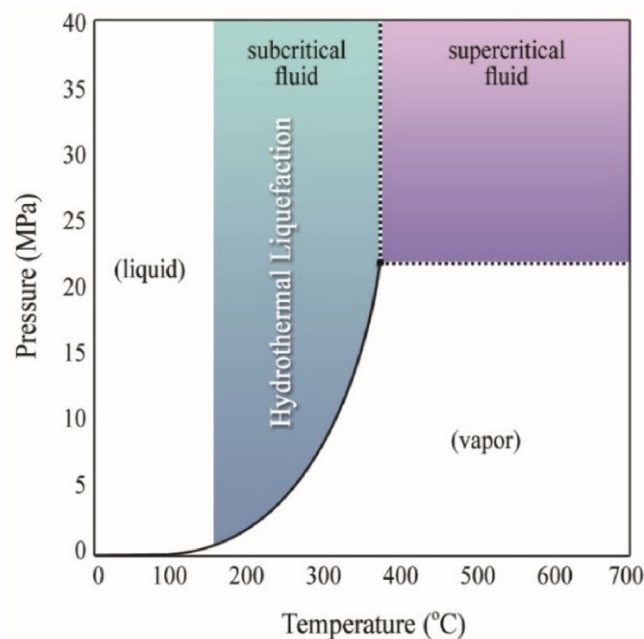


Figure 10: Hydrothermal liquefaction processing conditions within the temperature-pressure phase diagram of water [165].

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As can be seen in Fig. 11, the dielectric constant of water decreases as temperature approaches its critical point, until it reaches polarity values comparable to those of nonpolar solvents [166]. For example, the dielectric constant ( $\epsilon$ ) decreases from 78.5 at 25°C to reach 21 at 300°C. Close to the critical temperature, the ionic product of water ( $K_w$ ) is approximately 3 times more than at room temperature. As a result, the concentrations of  $H^+$  and  $OH^-$  ions are higher, thus promoting reactions catalyzed by acids and bases. Subcritical conditions also influence water viscosity which usually decreases when the temperature increases (from 1 g/cm<sup>3</sup> at 25 °C to 0.75 g/cm<sup>3</sup> at 300) leading to an increase in the speed of chemical reactions and in the diffusion coefficient of water which acts as a catalyst [164].

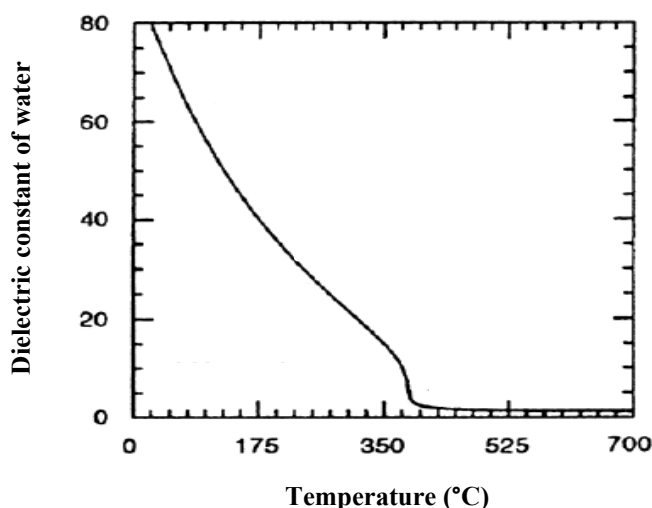


Figure 11: Schematic representation of the changes in the dielectric constant of water as per the temperature increase [166].

### 3.1.1. Application of HTL conversion to algal biomass

HTL was applied first to microalgae in 1994 [167] and is generally used to convert biomass into biocrude oil for energy use [168], with a biocrude yield typically higher than those of other methods (e.g., pyrolysis) [169]. Various number of microalgal strains have been investigated as feedstocks including *Nannochloropsis* sp., *Tetraselmis* sp., *C. vulgaris*, *Scenedesmus* sp., *Spirulina* sp., *Botryococcus branunii*, with most of the work carried out on a laboratory scale in batches using small volume reactors [170-173]. The obtained data revealed that the resulting HTL products are influenced by the type of microalgal and its chemical composition. On a large scale, the hydrothermal liquefaction of microalgae is not yet developed. In 2012, a continuous

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hydrothermal treatment unit for two microalgae *Chlorella* and *Spirulina* was tested at the University of Sydney in Australia, under temperatures between 250- 350 °C, and pressures of 150 – 200 bars [174].

The conversion of microalgae to biocrude during HTL comprises of conversion of three major components: lipids, proteins and carbohydrates [175]. The major reaction pathways involved in the HTL reaction are simplified in Fig. 12. They involve hydrolysis, dehydration and decarboxylation, cracking and fragmentation, and finally condensation reactions. The condensed compounds contribute to the formation of biocrude, a mixture of hydrocarbons, oxygenated compounds, and nitrogen-containing compounds [168]. The high temperature and pressure that the biomass undergo cause the hydrolysis of complex organic molecules. Proteins represent the largest fraction of microalgal biomass [176]. They are composed of one or more peptide chains which are polymers of amino acids. Proteins are the main source of nitrogen in liquefaction biocrude, which affects odor, combustion (if applied as biofuels). The peptide bonds are very stable. Consequently, their hydrolysis is slow below 230 °C [177]. Protein hydrolysis begins with a gelation step forming water-insoluble aggregates. Under hydrothermal conditions, these aggregates are degraded and release polypeptides which will subsequently be hydrolyzed to form amino acids [178]. After a first step of hydrolysis of amino acids, decarboxylation and deamination were observed [179]. These two reactions are potentially advantageous since they contribute to the elimination of oxygen and nitrogen, hence improve the quality of the products obtained. Deamination aims to produce ammonia and organic acids while decarboxylation produces carbonic acids and amines. The main decomposition products were acetaldehyde, diketopiperazine, ethylamine, methylamine, formaldehyde, acetic acid, propanoic acid, n-butyric acid and iso-butyric acid [180]. Proteins can enhance the yield of biocrude. Studies have shown that nitrogenous compounds mainly pyridine, indole, pyrrole were also present. These compounds were obtained by recombination with other biomass degradation products [180]. However, the existence of these nitrogen containing compounds is undesirable since they increase in the nitrogen content in the biocrude reducing its quality.

Triglycerides are the major form of lipids found in microalgae, and they are broken down through the process of hydrolysis into glycerol and free fatty acids, except when broken down under high heat, the glycerol exists in water phase [181]. Under ambient

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conditions, lipids are insoluble in water. However, as mentioned previously the decrease in the dielectric constant of water in hydrothermal conditions ensures better miscibility [164]. Below 300 °C, fatty acids are considered to be highly stable in water but at above this temperature, they decompose to form long hydrocarbon chains [181]. The biocrude issued from the lipid rich strains contained a large quantity of fatty acids and different long-chain hydrocarbon alkanes [182]. The fatty acids are mainly palmitic acid and palmitoleic acid, while the identified alkanes are mainly pentadecane, nonadecane and docosane. The decarboxylation of long-chain hydrocarbon fatty acids generates the corresponding alkanes [183]. But beyond the critical point of water, certain unstable fatty acids (oleic acid, linoleic acid, arachidonic acid, etc.) break down into a variety of compounds such as glycerin, acetaldehyde, propaldehyde, etc [184]. The hydrolysis of triglycerides also produces glycerol which generates products such as alkanes, aldehydes and light alcohols contributing mainly to the aqueous phase [185]. The reaction between the carboxylic acid and glycerol promotes the production of esters. Indeed, Xiu et al. showed that glycerol improves ester yields since it promotes esterification reactions of carboxylic acids in bio-oil [186].

Carbohydrates in the form of polysaccharides and starch which are broken down into simpler sugars, which undergo further reactions, including dehydration, isomerization, and fragmentation. The degradation products of sugars to alcohols, carboxylic acids, furans, phenols and aldehydes depending on the specific reactions occurring [187]. Although the contribution of polysaccharides to the yield of the oil phase is regarded to be low, their importance should not be neglected given that their degradation products (cyclic ketones, phenols, etc.) have a significant effect on the viscosity of the bio-oil produced. This directly influences its flow properties and then its commercial applications. Carbohydrates contribute also to the formation of char through dehydration and carbonization processes and the gas fraction may contain volatile compounds derived from their breakdown.



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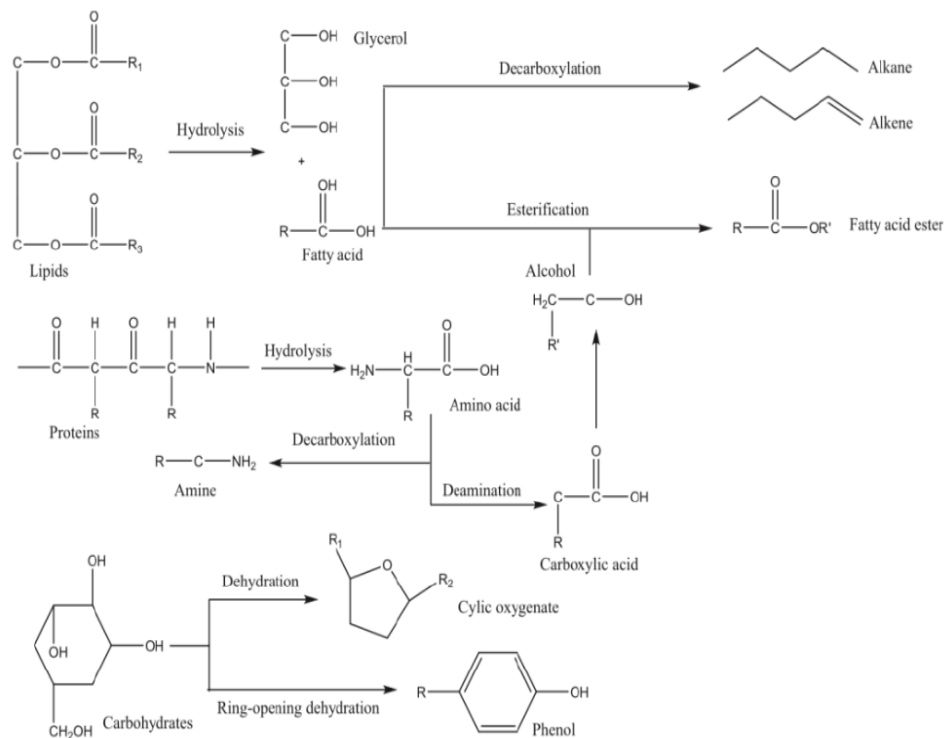


Figure 12: An overall reaction mechanism for HTL process from microalgae biomass [188].

### 3.1.2. HTL products

HTL converts the biomass into four different products: (1) biocrude, (2) aqueous phase, (3) solid residues, and (4) gases. The performance and the outputs of the HTL process are greatly influenced by factors such as temperature, pressure, residence time, feedstock type, nature of solvents and catalysts used.

#### 3.1.2.1. Gaz Phase

The gaseous fraction can yield up to 20 % of the original organics present in the microalgae feedstock [189]. It is composed of volatile gases including carbon dioxide  $\text{CO}_2$ ,  $\text{H}_2$ ,  $\text{CH}_4$ ,  $\text{N}_2$ ,  $\text{C}_2\text{H}_4$  and  $\text{C}_2\text{H}_6$ . [190]. Those compounds are generated from decarboxylation and cracking reactions of biomass during liquefaction treatment [168].  $\text{CO}_2$  is the main gas product of HTL [172] and could be recirculated to the microalgae production system to enhance the sustainability of microalgal production. Following  $\text{CO}_2$ , hydrogen is the second mostly detected gas and might be used for further hydrotreating of the produced biocrude [189].

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### 3.1.2.2. Aqueous phase

The aqueous phase is a significant fraction of HTL outputs. Its composition varies according to feedstock and reaction conditions, but it mainly consists of water and a range of light water-soluble organic compounds [168]. The main chemical functional groups found in the aqueous phase are: carboxylic acid, aromatics and alcohols [191]. The high content of organics in the aqueous phase might be recycled to the culture medium, however its direct integration requires further treatments [192]. Another interesting handling alternative is its gasification at supercritical conditions to obtain a hydrogen-rich fuel gas [193]. After this process, most of the nutrients would ideally still remain dissolved in the outcoming aqueous phase, allowing their recycling as well to the cultivation medium [194].

### 3.1.2.3. Solid residues

Besides the aqueous phase, a solid by-product is also generated during the HTL process, commonly denoted as biochar. The formation of biochar involves the carbonization of organic components present in biomass, leading to the formation of a carbon-rich solid material with a porous structure. This porous structure gives biochar its high surface area and adsorption capacity, making it useful for various applications in agriculture, environmental remediation, and energy production. Biochar can be used as soil amendment to enhance soil fertility, water retention, and carbon sequestration. It has applications in agriculture and environmental remediation. Moreover, they may be used as feedstock for subsequent thermochemical processes, such as pyrolysis or gasification, that would produce other energy, while the remaining ashes could be recycled as nutrients for microalgal growth.

### 3.1.2.4. Biocrude

Biocrude or bio-oil is the most sought-after products of HTL. It is a dark and viscous liquid characterized by low oxygen content, high calorific value with an energy content of 70–95 % of that of fossil crude oil [195]. It is similar to fossil crude oil and can be further refined into transportation fuels such as gasoline, diesel, and aviation fuel [188]. This process was first introduced by the oil company Shell in the 1980s [196]. A study proposed that HTL could convert up to 24 to 64 wt % of biomass into biocrude, which could be composed of 50–80 % carbon, 5 to 18 wt % oxygen, 4 to 8 wt % nitrogen [188]. Chemical analysis of the biocrude oil revealed the existence of different types of

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compounds in particular straight and branched aliphatic compounds, aromatics and phenolic derivatives, esters, nitrogenous ring structure, carboxylic acids and heavy insoluble materials (Algaenan derivatives) [188].

The composition and yield of the biocrude oil are affected by the type of microalgal species and amounts of protein, lipids, and carbohydrates within the microalgal biomass [188]. Likewise, they are impacted by the reaction parameters of hydrothermal liquefaction such as reaction interval time, temperature, and biomass to water ratio [188]. It is noteworthy that since in HTL the entire microalgal biomass is converted, it is not understood which component of is leading to better conversion and products quality. The analysis of results using a multiphase component activity model revealed that lipids and protein fractions significantly contributed their conversion to the biocrude or bio bitumen with high conversion coefficients of 0.85 and 0.45 respectively, while carbohydrates showed the lowest conversion coefficient of 0.22 [197]. Moreover, it was concluded that the biocrude yield could increase up to 15 % in lipid rich microalgae biomass [198]. In this sense, as limitation in nitrogen is responsible for lipid accumulation, it may be interesting to play on this cultivation parameter to increase the yield.

### 3.1.3. Biocrude oil as alternative binders to petroleum bitumen

#### 3.1.3.1. Examples of bitumen from other resources

Bitumen, sometimes referred to as asphalt, is a semi-solid or liquid form of oil that is black, viscous and extremely sticky in nature. This organic material which is used for many building applications (road paving, waterproofing, roofing, corrosion protection..), consists of a high number of organic compounds structured in a very complex form [199]. Its elemental composition depends on its geographical source, but mainly includes carbon (80–88 wt%) and hydrogen atoms (8–12 wt%) with a hydrogen-to-carbon molar ratio (H/C) of 1.5, in addition to a small amount of heterocyclic species and functional groups containing sulphur, nitrogen and oxygen atoms [200]. Currently, most of the commercially used bitumen is obtained from petroleum industries. However, it is strongly influenced by socio-economic and geographical issues. Moreover, the recent past has shown supply issues for this heavy fraction of petroleum distillation, since its conversion to gasoline is attractive in case of a high level of the crude oil market [201].

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Despite being one of the important producers and exporters of crude oil and other refined petroleum products, the state of Qatar is till date importing asphalt exclusively from abroad through the “State fuel company” and distributed to contractors [202]. The daily real demand varies according to government projects involving the road network and seasons [203]. As example, in 2020, total imports of bitumen to Qatar amounted to \$ 25 billion given to the increase consumption in road projects and the heavy construction that happened prior to the world cup event. Fig. 13 summarizes the top trading partners the country is dealing with to import its bitumen.

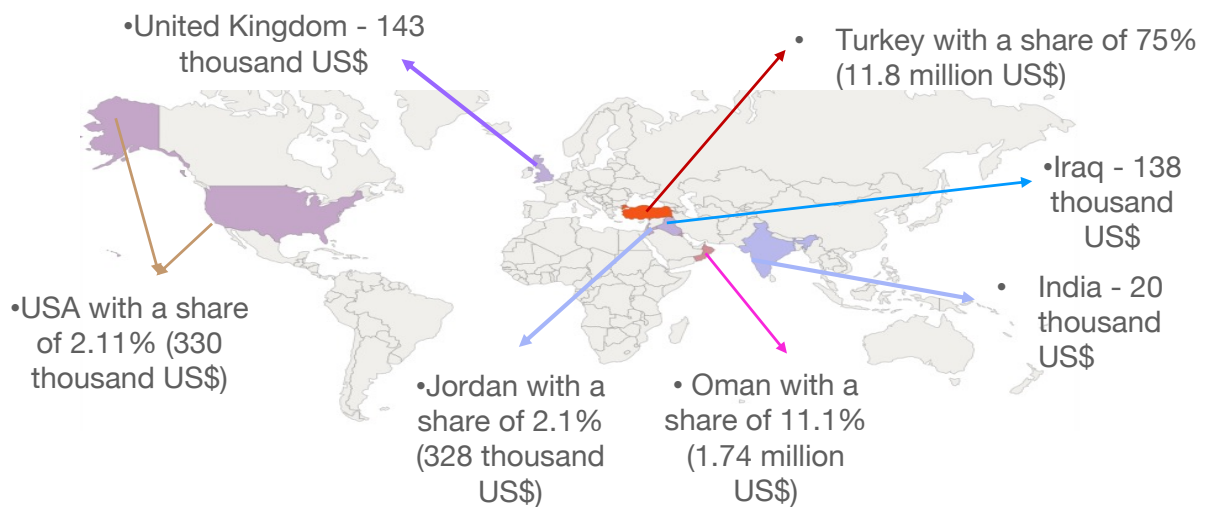


Figure 13: Top trading partners from where the state of Qatar imports its bitumen.

The bitumen derived from oil refining processes, presents several challenges and limitations [204]:

- **Environmental Impact:** Heating bitumen to make it usable can release greenhouse gases such as carbon dioxide and methane, contributing to global warming and climate change. In 2018, the production of one ton of asphalt generated the emission of 13 kg of CO<sub>2</sub> as presented in the environmental report of bitumen published by Route de France [205]. Additionally, bitumen extraction methods, such as oil sands mining, can have significant environmental impacts, including habitat destruction and water pollution.
- **Resource Depletion:** Crude oil, the primary source of bitumen, is a finite resource. As global demand for energy continues to rise, there is concern about the depletion of crude oil reserves. This depletion can lead to increased prices and geopolitical tensions as nations compete for dwindling resources.

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- **Energy Intensive Processing:** The extraction, processing, and transportation of bitumen require significant energy inputs, further contributing to environmental impacts and greenhouse gas emissions. Additionally, the refining of bitumen into usable products such as asphalt requires energy-intensive processes.
- **Health and Safety Concerns:** The production and use of bitumen-based products can pose health and safety risks to workers and nearby communities. Exposure to bitumen fumes and dust can lead to respiratory issues, and accidents during transportation or processing can result in spills and environmental contamination.

Taking all this into account, prospection of efficient routes to produce bitumen substitutes from renewable biomass sources has become a crucial necessity to reduce this dependence on this fossil energy, tackle the climate change issue and minimize the greenhouse gas emissions. Various governments have supported and incentivized research into novel alternatives to non-renewable energy sources, to achieve the low carbon energy transition [206]. Indeed, to have a long-term impact on carbon capture efforts, processes are required to: a) fixate CO<sub>2</sub> for the long-term; b) have significant capacity and c) are economically attractive.

The choice of an optimal composition of a bio sourced bitumen (partially or totally modified) must take into account a certain number of issues (aging, rheological behavior at high and low temperatures, mechanical fatigue, etc.). From an economic point of view, a viable bio sourced binder must be competitive with the petroleum bitumen. The feedstock should be available in important quantities and does not compete with any other applications (mainly nutrition). As of now, the total substitution of petroleum bitumen to produce purely bio-based binders was not investigated and most of the research work targeted the partial substitution of bituminous binders using different biomass feedstocks (Table 4), with the aim of modifying its mechanical properties.

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Table 4: Examples of biomass residues used as additives to conventional bitumen

Biomass types	Conditions applied	References
Wastes from both piggery farms and <i>Spirulina</i>	Hydrothermal liquefaction (300°C, with a shear mixing proportion 1:8)	[207]
Used cooking oils	Esterification, mixture: 33.3% biomass, 30.3% bitumen, 36.4% other	[208]
Used cooking oils, rubber powder from tires	Mixture (15%), additive (palm oil-based biofuel ash), Mechanical mixer (900 rpm/ 120 min)	[209]
Oak wood	Pyrolysis, High speed mixer (600 rpm/ 30 min), 25 to 75% biobinder	[210]
Cedar wood	Pyrolysis + different proportion of mixing with the conventional bitumen up to 50 %.	[211]

Fig. 14 shows a demonstration example of bio-asphalt on large scale in Zeeland. The work was the first demonstration of lignin- bio asphalt road, where 50% substitution of bitumen was replaced by soda lignin, a by-product of wood pulp. The first lignin bio-asphalt road was established in July 2015 and still behaves very good up to 2019.



Figure 14: Example of road demonstration of bioasphalt mixed with conventional one in Zeeland, southwestern Netherlands. The pictures showed (a) the start of putting the mixture on road in 2015, (b) how the road looked in 2015 and (c) in 2019.

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Another example of demonstration is in Fig. 15, and it is showing a 1 Km cycling path that was built in 2017 and is still up to date at Wageningen campus. The road was used by mixing 3 Lignin types with the conventional bitumen and required 10 tons of lignin and 220 tons of bio asphalt.



Figure 15: Example of cycling pavement built by mixing conventional bitumens with biobitumen issued from 3 different lignin biomass.

### 3.1.3.2. Microalgae biomass as feedstock for bio bitumen production

While biocrude production is generally involving temperatures above 280°C, recent research has shown that bitumen-like oil can also be produced by performing HTL. The concept was introduced recently in 2015, where a process was established to valorize algae-biomass and produce bio bitumen through hydrothermal liquefaction using *Scenedesmus* sp [173]. The work was continued by other researcher from laboratories in GEPEA and University Gustave Eiffel, where other biomass residues of *Spirulina* were tested [172, 205].

Fig. 16 shows a Black diagram for different samples of hydrophobic oil obtained after HTL conversion of biomass residues at 260 °C, 280 °C and 300 °C, in comparison to the conventional bitumen. As presented in the data, the behavior from the samples obtained at both 280°C and 300°C differs significantly from the one at 260 °C, which is the one close to the rheological state observed for standard bitumen with a continuity of the isotherms in Black as a function of temperature. Such a diagram is obtained by measuring the viscoelastic modulus  $E^*$  of the material with a real part  $E'$ , related to its solid behavior and an imaginary part  $E''$  related to its viscous behavior.

$$E^* = E' + iE''$$

The tangent of the phase angle  $d$  is given by:

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$$\tan \delta = \frac{E''}{E'}$$

At low temperature, the material is solid, so that the phase angle  $\delta$  is close to zero and  $|E^*|$  is very high. At high temperature, the material is liquid with a phase angle close to  $90^\circ$  and a small  $|E^*|$ . In between, there is a continuous  $\delta$  vs  $|E^*|$ . However, this characteristic rheological behavior is not observed for the products of HTL experiments at  $280^\circ\text{C}$  and  $300^\circ\text{C}$ , for which “simple” biocrude oils are obtained.

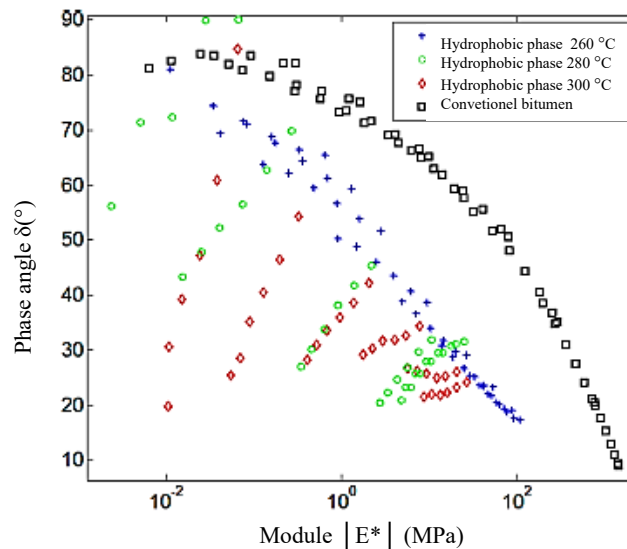


Figure 16: Rheological behaviors of hydrophobic phases of algal biomass obtained at different HTL temperatures compared to standard bitumen [205].

Therefore, the optimal temperature which made it possible to obtain viscoelastic behavior similar to petroleum bitumen is  $260^\circ\text{C}$  and lies on the crossover region between HTL and hydrothermal carbonization as mentioned in Fig. 17,18. In this type of process, the water is in a sub-critical state and plays a dual role as solvent and reagent. This research work has opened to the possible partial or complete substitution of conventional bitumen by microalgae as sustainable feedstock. However, up to date the number of studies exploring this area remains limited, with a few studies on feedstocks isolated from freshwater environments namely *Scenedesmus* sp and *Spirulina* sp [172, 173, 205]. Therefore, expanding the scope of research to include marine microalgal species could broaden our understanding and unlock additional opportunities for sustainable innovation in this field. With a product lifetime of over 20 years, the sequestered carbon within the product is stored for a long-term. Moreover, similar to the recycling of conventional bitumen, it might be possibility of recycling the bio bitumen into new roads, leading to an even longer retention of the sequestered



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CO<sub>2</sub>. However, this approach requires further studies to assess the effects of recycled asphalt on its properties, performance, and durability.

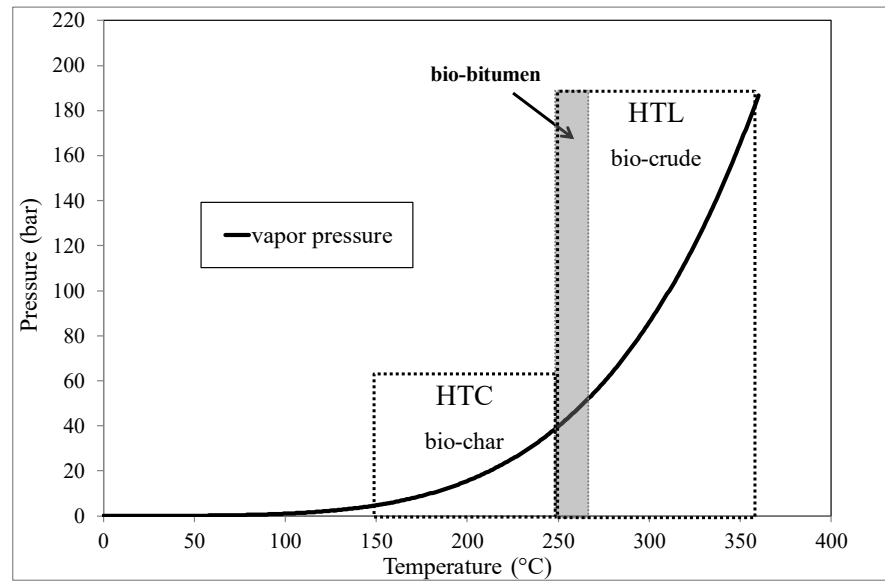


Figure 17: Schematic presentation for the HTL working conditions (temperature and autogenous pressure). The conditions leading to the formation of biobitumen are also mentioned in the graph [172].

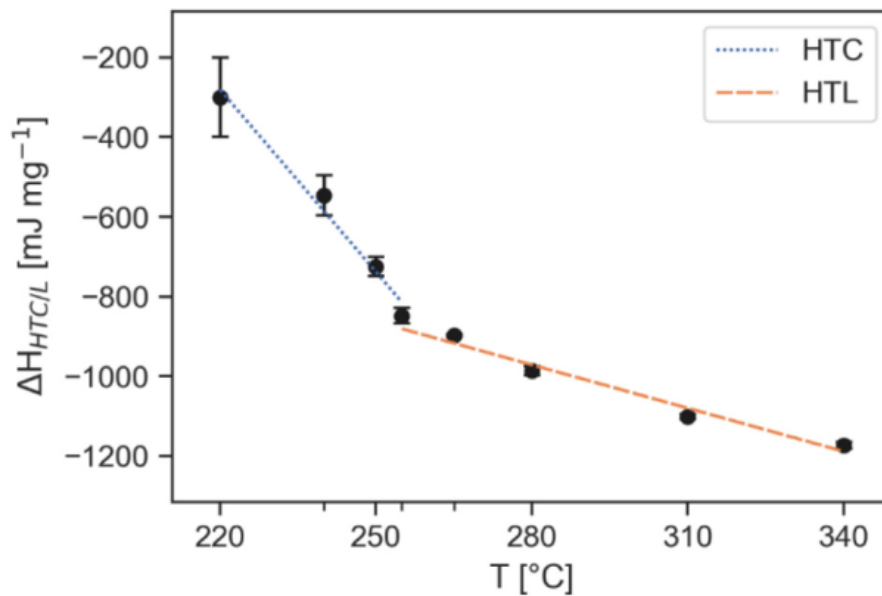


Figure 18: Graph showing the crossover region between HTC and HTL processes, and highlighting the thermodynamic transition, in overall process enthalpy occurs at the transition from HTC to HTL, with enthalpy changes of  $\sim 0.3\text{--}0.9$  and  $\sim 0.9\text{--}1.2$  kJ g<sup>-1</sup> for HTC and HTL.

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### Conclusion

This literature review highlights the significant potential of cyanobacteria as a viable source for phycobiliprotein production, with applications ranging from food colorants to potential disease-prevention agents in humans. While the current advances in production technology are key for the large-scale cultivation of cyanobacteria for PBPs extraction, the potential of Qatar as a commercially viable location for algae production appears promising. However, there is a notable gap in research concerning the isolation of novel cyanobacteria strains capable of producing PBPs.

To be truly sustainable, the commercial production of cyanobacterial biomass in Qatar should be performed using open ponds. Previous attempts using PBRs were unsuccessful due to the excessive heat generated under outdoor conditions, resulting in culture crashes (internal findings). Open ponds, by contrast, have proven to be more suitable for biomass production in the harsh local climate. They are easier to build, operate, and maintain, though they lack control over physicochemical outdoor variations, potentially impacting culture stability.

Qatar has a dry climate with two distinct seasons, long extreme summer characterized by intense dry heat and short winter with mild temperatures. Additionally, freshwater scarcity in the region, and the challenges associated with using treated water, make marine strains a more viable option for biomass production. However, the inability to control the ambient temperatures in open ponds under outdoor conditions, and the resulting evaporation, requires the selection of strains that can withstand diurnal temperature fluctuations throughout the seasons, and tolerate varying salinity levels that occur naturally in outdoor environments. Optimizing these parameters is essential for enhancing algae production processes in Qatar and can begin at a laboratory scale using controlled photobioreactors and artificial light to establish optimal culture conditions. Once the optimal conditions are identified, the process can be scaled up to real outdoor conditions to produce sufficient biomass. This would enable further exploration of an algo-refinery approach, where residual biomass from PBPs extraction is utilized to produce bio-bitumen through hydrothermal conversion.

# **Chapter 2: Materials and Methods**

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### **Introduction**

This second chapter presents the materials, experimental techniques and devices used to obtain the results discussed in Chapters 3, 4 and 5.

First, the materials used in the study are introduced, starting with the microalgal sampling, molecular identification and cultivation both on a laboratory and a larger scale. This section details the growth medium and indoor cultivation conditions, as well as the protocols followed for outdoor cultivation.

The second part focuses on the various analyses conducted throughout the study. It outlines the methodologies used for estimating biomass productivity, pigment analysis, elemental analysis, and metabolites evaluation.

Finally, the last section is dedicated to the hydrothermal conversion experiments. It describes the methodology followed, products recovery protocols used to estimate yields, and the techniques applied to characterize the hydrophobic phases in terms of composition, structure, and rheological properties.

## Chapter 2: Materials and Methods

### 1. Strain sampling, isolation and cultivation

#### 1.1. Indoor investigation

##### 1.1.1. Sampling, isolation & morphological analysis

The cyanobacterial strains were obtained from seawater, sand, and clay samples collected from al Zubara, al Dhakhira and al Wakra districts in Qatar (Fig. 19). Table 5 shows the details of the different strains screened during this study. Samples were enriched using Guillard F/2. The composition of the medium (per liter) was:  $\text{NaNO}_3$  0.075 g,  $\text{NaH}_2\text{PO}_4$  0.005 g,  $\text{Na}_2\text{CO}_3$  0.03 g, and 1 mL trace metal mix ( $\text{FeCl}_3$  3.15 g,  $\text{Na}_2$  (EDTA) 4.36 g,  $\text{CuSO}_4$  0.0098 g,  $\text{Na}_2\text{MoO}_4$  0.0063 g,  $\text{ZnSO}_4$  0.022 g,  $\text{CoCl}_2$  0.01 g,  $\text{MnCl}_2$  0.18 g) [212]. Later, samples were purified using a previously described protocol [8], and the axenic cyanobacteria isolates were first identified morphologically using light-microscopy (Primo Star HAL Microscope, full Köhler, stage drive R, FOV 20, Carl Zeiss, Germany).



*Figure 19: Qatar Map highlighting the different places of sampling. The blue line indicates the different places of sampling, while the red dots correspond to places from where the 4 shortlisted strains were isolated*

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Table 5: List of selected strains from Qatar culture collection for screening

QUCCCM Numbers	Environnent	Habitat	GPS coordinates (N, E)
<b>QUCCCM 34</b>	Marine	Sea-water	N 25.59.183, E 51.0101.418
<b>QUCCCM 54</b>	Marine	Soil (Sabkha)	N 25 59 125, E 051 01 617
<b>QUCCCM 77</b>	Marine	Clay (Sabkha)	N 25 43 894, E 051 33 336
<b>QUCCCM 129</b>	Marine	Sea-Water	N 25 59 146, E51.011.328

### 1.1.2. Inoculum preparation

The inoculum of purified strains was prepared in flask level using F/2 media [212] supplemented with ten times concentrated in both nitrogen and phosphate (10P10N) to avoid macronutrient limitation. Normal sea water was used for the 40 ppt salinity, while %NaCl (w/v) was added to achieve media with 60‰ salinity. The media was then autoclaved and cooled before inoculation. All species were cultivated in 250 ml Erlenmeyer flasks with an initial optical density ( $OD_{750nm}$ ) of 0.2. The cultures were incubated in illuminated Innova 44 Shaker (New Brunswick Scientific) with 200 rpm agitation and photon flux of  $200 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  light irradiance on a 12 h: 12 h light: dark cycle and at 30 °C temperature.

### 1.1.3. Photobioreactor setup and experimental conditions

The shortlisted strains namely QUCCCM 34, QUCCCM 54, QUCCCM 77 and QUCCCM 129 were cultivated in aseptic 1 L photobioreactor (DASGIP parallel bioreactor system, Eppendorf, Inc., USA) (Fig 20). Two different salinities 40, 60 ppt, and three temperatures (20, 30 and 40°C) were investigated in biological duplicate. The culture inoculum was re-suspended in a volume of media culture to an initial  $OD_{750nm}$  between 0.2- 0.3 and was continuously sparged with  $3 \text{ L} \cdot \text{h}^{-1}$  air enriched with  $\text{CO}_2$  concentration controlled to maintain a pH of 8. Illumination was provided by 3 internal DASGIP LED Sticks, with a 3-channel emission-spectrum (Channel A, 660, 780 nm; Channel B, 572, 625, 640 nm; Channel C, 453 nm) under 12:12 h light: dark cycles. Set-points were 2.00, 1.244 and  $2.00 \mu\text{mol} \cdot \text{photons} \cdot \text{s}^{-1}$  for channels A, B and C respectively, which is equivalent to a light intensity of  $240\text{-}300 \mu\text{mol m}^{-2} \text{ s}^{-1}$ , described

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previously as the optimum light intensity for biomass and PBPs productivity for cyanobacteria [32]. Mixing was set to 200 rpm (pitch-blade impeller). The  $OD_{750nm}$  was measured every other day and samples were taken in parallel for dry weight analysis, PBPs estimation. Cultivation was conducted under the above-mentioned conditions over a period of 12 days or until reaching stationary phase (whichever comes first), after which cells were harvested by centrifugation at 3500 rpm for 10 min, washed with 0.5 M ammonium formate to eliminate the residual salt and freeze dried (Labconco, Freezone, Kansas City, MO, USA) prior to analysis.

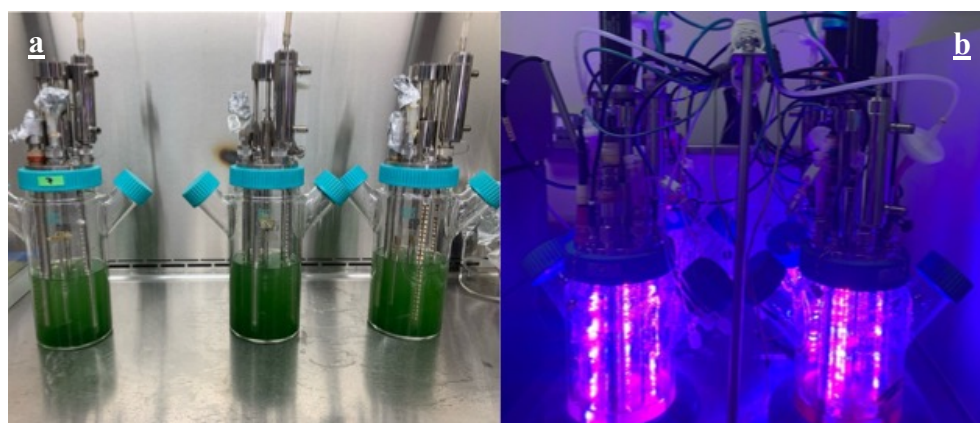


Figure 20 : Picture of PBRs system used for the strain cultivation without (a) and with illumination (b).

### 1.1.4. Effect of nitrogen source on the growth and PBPs expression

The marine strain *Pleurocapsa* sp. was screened for its ability to utilize urea as nitrogen source. Urea was provided by Qatar Fertilizer Company (QAFCO). Strains were cultured in F/2 media [212], using locally sterilized seawater with a salinity of 40 ppt. The F/2 media was ten times concentrated in both nitrogen and phosphate (10P10N). Sodium nitrate was used as nitrogen source in control culture, while the other culture had urea added with similar molar nitrogen concentration to that used for control cultures. Both conditions were inoculated in duplicate with similar initial inoculum density  $OD_{750nm} = 0.2$ . Cultures were kept in an illuminated Innova 44 Shaker Incubator (New Brunswick Scientific) under agitation of 200 rpm, a photon flux of  $240\text{-}300 \mu\text{mol m}^{-2} \text{s}^{-1}$  in a 12:12 h light: dark cycle and 30 °C temperature. After 12 days of growth, cultures were harvested and freeze dried for analysis.

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### 1.2. Outdoor cultivation

The selected strain was cultivated outdoor in 200L open ponds during the month of April- May 2021. For all the outdoor trials, the urea was adopted as the sole nitrogen source. F/2 media was used during the experiment with two different nitrogen (N) and phosphorus (P) concentrations: Two times concentrated F/2 media (2XF/2), and ten times concentrated F/2 (10XF/2). Initially, inoculum of *Pleurocapsa* sp. for each cultivation condition was prepared in duplicate indoor. The cultures received a constant light intensity of 600  $\mu\text{mol E/m}^2/\text{s}$  from white, fluorescent under a light period of 16 h per day and were maintained under a temperature of 30 °C. The mixing for the flask was done using the shaker, while for the 1L, 5L and 10L bottles it was using 0.5 v/v/m air supplied by a compressor (EDON; model ED550-100L, 1.65 kW) and enriched with 2 % CO<sub>2</sub>. The subculture for each culture volume was done each 7 days. Inoculation volumes of 10 % and 20 % (v/v) were tested for outdoor large production in the university facility located in Al-Khor, Qatar (N 25°69, E 51°51). The cultures were inoculated in 1 m<sup>2</sup> (2.5 m × 0.4 m), 200 L) raceway ponds system with Agitation was achieved through a 4-blade paddle system rotating at 32 rpm, resulting in an average linear liquid velocity of 26.2 cm.s<sup>-1</sup> [23]. Inoculums were mixed with sterilized seawater supplemented with either 2X or 10X fresh F/2 growth media as shown in (Fig. 21). The studied conditions were performed in duplicate and were cultivated in batch with similar starting densities, in the presence of carbon dioxide, automatically injected based on pH set at 8 considering that a pH-value of 8 minimizes nitrogen from volatilizing and phosphate from precipitating [213]. Temperature and light were recorded throughout the experiment using a HOBO Pendant MX Temperature/Light Data Logger (MX2202). Due to evaporation, the water loss was measured daily and compensated using fresh water and the cultures were periodically checked under microscope for any possible contamination. Samples were taken each alternative day for cell density and PBPs content. Once the pigment reached highest concentration, the biomass was harvested by sedimentation. At first, the paddle of the raceway tank was stopped, and the biomass was allowed to settle for an hour. The culture was drained from the bottom of the tank by a drainage valve (2 cm from the tank bottom) and was transferred to a 100 L bucket where the biomass was allowed to settle again. The supernatant in the bucket was decanted, and the remaining slurry was concentrated using a US filtermax centrifuge with an operating volume of 2 L followed by washing steps using ammonium



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formate to eliminate any debris of salts. The biomass was finally freeze dried using a Labconco freeze drier (Labconco, Freezone, Kansas City, MO, USA) and stored in  $-20\text{ }^{\circ}\text{C}$  fridge for further analysis. This biomass was called here after **raw biomass**.

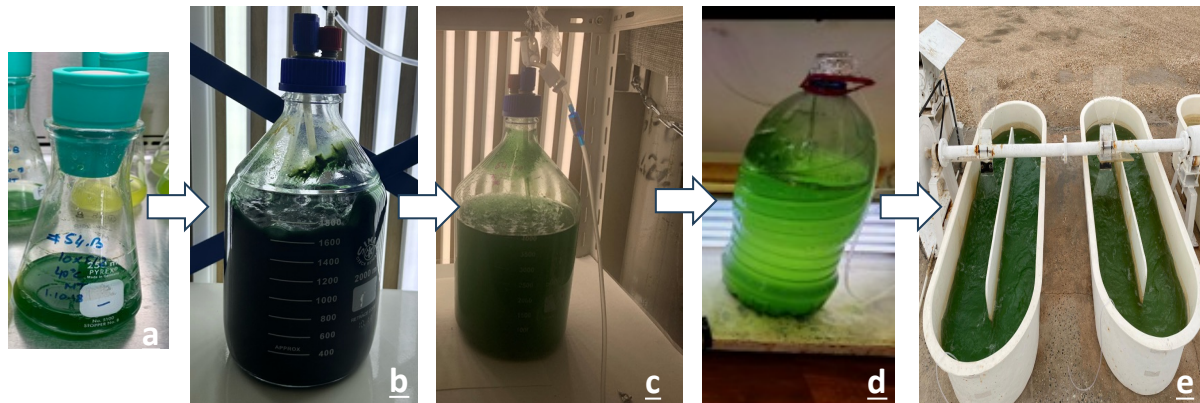


Figure 21: Different steps of inoculum preparation of *Pleurocapsa* for the large scale cultivation: from flask level (a) to 1L-5L-10L (b,c,d) than 200L ponds (e).

### 1.3. Simulation of pigment extraction by high pressure cell disruption

Cell disruption was performed for the biomass issued from outdoor culture, using a high-pressure disrupter Cell-D 2.2 kW (series B, Constant systems Ltd., Warwick, (UK)). Samples of suspension with 10 % dry matter in water were treated at a pressure of 120 MPa. The hydraulic system drives the piston up and down (Fig. 22). As the piston moves down, the compression chamber is filled with suspension. As the piston rises, the suspension is compressed to the set pressure and forced through the valve and forms a jet directed towards a target, causing cell lysis. The suspension is then recovered and centrifugated. Previous studies on *Porphyridium cruentum* showed that these conditions allow an efficient lysis of the cells for the recovery of water-soluble pigments [214].

Consequently, we decide to use this method in chapter 5 to simulate the effect of a “hard” pigment extraction process on biomass residues composition (in particular on ash content). In practice it will be applied after the “mild” pigment extraction with the tissue lyser apparatus (see section 2.6)

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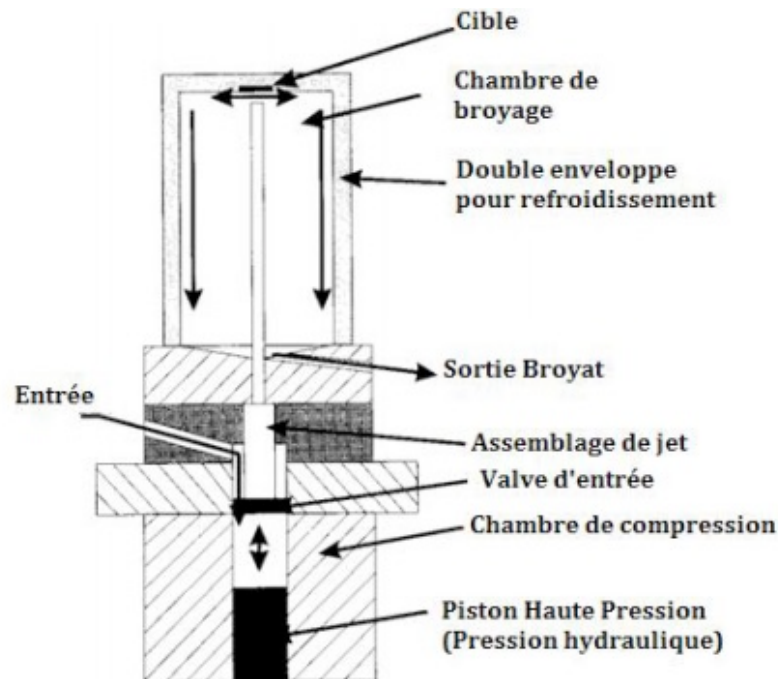


Figure 22: Schematic overview of high-pressure cell disruption device reproduced from the work of Zinkone [215].

The collected biomass was centrifuged at speed of 6000 rpm for 10 min before subjecting it to HTL conversion. This biomass served as second feedstock for the HTL conversion.

## 2. Biomass analysis

### 2.1. DNA extraction and gene sequencing of the selected strains

After purification, the strains QUCCCM 34, QUCCCM 54, QUCCCM 77 and QUCCCM 129 were identified using PCR-sequencing. The genomic DNA of the axenic strains was isolated using GenElute™ Plant Genomic DNA Miniprep kit (Sigma, USA). 10 mg of genomic DNA was used to perform Polymerase Chain Reactions (PCR) of 16SrDNA gene using the forward primer BS1F (5' GATCCTKGCTCAGGATKAACGCTGGC3') and the reverse primer 920R (5' TTT-GCGGCCGCTCTGTGTGCC 3'). PCR amplification was achieved using the SuperFi™ PCR Master Mix, (Thermo Fisher Scientific, Waltham MA). The PCR run with an initial denaturation at 95 °C (5 min), 35 thermal cycles of denaturation at 95 °C;

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(1 min), annealing at 56 °C (1 min), extension at 72 °C. Purification of PCR products was performed using ExoSAP-IT PCR Product Cleanup Reagent (Affymetrix, Santa Clara, California, USA), and the DNA concentration was determined by NanoDrop 2000c/2000 UV–Vis Spectrophotometer (Thermo Scientific, Wilmington, USA). The purified PCR fragments were sequenced by Genetic Analyzer 3500 (Applied Biosystems, California, USA), using the same primers used for the PCR amplification in addition to two other internal primers: forward primer BSL4F (5'GYAACGAGCGCAACCC 3'), and reverse one BSL8R (5'AAGGAGGTGATCCAGCCGCA 3').

### 2.2. Phylogenetic analysis

The obtained 16S rDNA sequences were aligned using Basic Local Alignment Search Tool (BlastN) and the aligned sequences were downloaded from the GenBank data base. Alignment was performed through MUSCLE [216] as implemented in MEGA X software. Phylogenetic tree and molecular evolutionary analysis were conducted by MEGA X using a neighbor-joining method [217, 218].

### 2.3. Biomass productivity and carbon capture rate

Biomass concentration  $C_X$  (g. L<sup>-1</sup>) was evaluated at the beginning and end of culture from dry weight measurements. Before filtration, the GF/C filters (Whatman GF/F™ Ø 55 mm) were washed with 0.5 M ammonium formate than dried at 90 °C for 24 h and the dry weights of filters were determined. Later 5mL of samples were filtered under a constant vacuum through pre-dried (24 h, 95 °C), pre-weighed, and washed with 0.5 M ammonium formate, glass microfiber filters. The filters were later washed with a double volume of 0.5 M ammonium formate to remove any salt debris, dried (24 h, 95 °C), and then cooled in a desiccator (>2 h) prior to weighing them. The biomass dry weight (DW) was determined as the difference between the weight of the dried filters prior to and after biomass filtration and drying. All measurements were performed in duplicate.

Biomass productivity was calculated as per equation (a) according to Griffiths and Harrison [219]:

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$$(a) P_X = \frac{C_{end} - C_0}{t}$$

In which:  $P_X$  is the biomass productivity in  $g L^{-1} d^{-1}$ ,  $C_0$  is the biomass concentration ( $g. L^{-1}$ ) at the start point of cultivation,  $C_{end}$  the biomass concentration ( $g. L^{-1}$ ) at the end of the culture and  $t$  the duration of cultivation in days.

$CO_2$  fixation rate ( $R_{CO_2}$ ,  $mg. L^{-1}. d^{-1}$ ) was calculated using Equation (b) by correlating biomass productivity, where  $P$  is biomass productivity,  $M_{CO_2}$  is the molecular weight of  $CO_2$ ,  $M_c$  is the molecular weight of C, and  $C_c$  is the carbon content [220].

$$(b) R_{CO_2} = C_c * P * \left(\frac{M_{CO_2}}{M_c}\right)$$

### 2.4. Total organic carbon

Total organic carbon (TOC) was analyzed using a TOC analyzer (Shimadzu TOC-LCSH). Briefly, the samples were collected and centrifuged first to separate the media and biomass. biomass was further washed with ammonium formate and freeze dried prior to analysis. Two to three injections were performed for statistical confidence.

### 2.5. Ash content, elemental analysis and higher heating value

The ash content was determined accordingly: 1g of freeze-dried biomass was placed into crucible and weighed ( $W_1$ ) then kept in a furnace (1500 series, USA), at  $600\text{ }^\circ\text{C}$  for 4h, after which the crucible was removed, allowed to cool down in room temperature and re weighed ( $W_2$ ). The ash content was calculated using the following formula:

$$(c) \text{ Ash Content (\%)} = (W_1 - W_2) \times 100$$

The elemental analysis of total carbon (C) hydrogen (H) and nitrogen (N) in the biomass was performed using a Thermo Scientific Flash 2000 Organic elemental analyzer coupled to a CHN analyzer (Germany). Aspartic Acid (Thermo Scientific, Germany) was used as a standard (C= 36.09%, N= 10.52%). Note that other expected elements are mostly Oxygen, and possibly Sulfur.

The amount of oxygen(O) present in the sample was estimated using the equation below:

$$(d) \% O = 100 - \% C - \% N - \% H - \text{ashes}$$

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This allowed calculating the higher heating values (HHV) using the Dulong's formula [221, 222].

$$(e)\text{Higher heating value } \left(\frac{\text{MJ}}{\text{kg}}\right) = 0,3491 \%C + 1,1783 \%H - 0,1034 \%O - 0,0151 \%N - 0,0211 \%Ash$$

### 2.6. SEM-EDX analysis

Scanning electron microscopic and energy dispersive X-ray (EDX) observations were performed with Nova NanoSEM 450 (FEI) (Czech) using an energy dispersive X ray detector (Bruker 127, Germany) for elemental semi quantitative analysis. The EDX method of analysis is one of the QU-CLU accredited methods ISO/IEC 17025:2017 for Chemical Testing, Certificate Number 2924.01. For sample preparation, the biomass was washed with water and centrifuged to form a pellet. The pellet was subjected to a primary fixation using 2.5% glutaraldehyde and an incubation at room temperature for 1 h. This was followed by a secondary fixation using 1% Osmic acid and an incubation for 4 h at 4 °C. A gradual dehydration procedure was done by washing the sample with gradients of ethanol starting from 30% to absolute concentrations. The sample was allowed to air dry and coated with gold particles using Sputter coater (Germany) before proceeding for SEM analysis.

### 2.7. Phycobiliproteins quantity and quality

10 mg freeze dried biomass from each strain and culture conditions were taken for pigments analysis. PBPs were extracted using phosphate buffer (pH: 7.4). Briefly, the biomass was placed in 2 mL eppendorf tubes containing 0.25-0.5 mm glass beads, with 25% filling ratio. 1mL phosphate buffer (pH: 7.4) was added and the tubes were placed in tissue lyser (Qiagen, Hilden Germany) for cell lysis. The cell disruption is achieved through shear forces caused by high-speed solid beads. This process is referred as “**mild**” pigment extraction process in chapter 5. Each cycle of lysing is of 1 min at 30 Hz frequency, to avoid overheating of the extract. After finishing, the tubes are centrifuged at 4000 rpm for 10 min at 4°C, and the supernatants were carefully transferred into clean tube for PBPs determination. The process of extraction was

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repeated until no significant amount of PBPs was present in the supernatant. The crude extract was analyzed by reading the absorbance of the pigment: phycoerythrin allophycocyanin and c- phycocyanin, and PBPs concentrations (w/v) ( $C_{C-PC}$ ,  $C_{APC}$  and  $C_{PE}$ , mg.L<sup>-1</sup>) were calculated as per Bennett and Bogorad [223] by the equations (f), (g) and (h):

$$(f) C_{C-PC} = \frac{A_{615} - 0.474 (A_{652})}{5.34}$$

$$(g) C_{APC} = \frac{A_{652} - 0.208 (A_{615})}{5.09}$$

$$(h) C_{PE} = \frac{A_{562} - 2.41(C_{PC}) - 0.849 (C_{APC})}{9.62}$$

Here  $A_{615}$ ,  $A_{652}$ , and  $A_{562}$  are the absorbance of extract at 615 nm, 652 nm, and 562 nm, respectively.

Throughout this study, phycocyanin as a general term refers to the sum of C-PC and APC [224]. The data of C-PC and APC separate are presented in appendix.

The PBPs yield ( $Y_X$ , mg.g<sup>-1</sup>) was calculated using the equation (i) [225]:

$$(i) Y_X = \left( \frac{C_X * SV}{\text{Weight of the biomass}} \right)$$

Where:  $X$  corresponds to the pigment type,  $C_X$  is the pigment concentration (mg. ml<sup>-1</sup>) and  $SV$  the solvent volume.

In addition to the yield, each PBPs percentage ( $\%_X$ ) was evaluated using the equation

$$(j) \%_X = Y_X * 100$$

Where:  $X$  corresponds to the pigment type,  $Y_X$  the yield (mg. g<sup>-1</sup>).

The purity index of each PBPs indicates the extract purity with respect to forms of contaminating proteins. It was conducted for the day showing the highest PBPs content using a spectrophotometer, and was calculated by the following equations, [226]:

$$(k) C - PC_{PI} = A_{615}/A_{280}$$

$$(l) APC_{PI} = A_{652}/A_{280}$$

$$(m) PE_{PI} = A_{562}/A_{280}$$

Where: PI is the purity index of each type of phycobiliprotein,  $A_{615}$ ,  $A_{652}$  and  $A_{562}$  indicated the absorbance wavelengths of C-PC, APC, and PE respectively, whereas  $A_{280}$  referred to absorbance of the total proteins in solution.

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### 2.8. Metabolites analysis

The obtained biomass from cultivation at 30 °C- 40 ppt, which corresponds to the average weather conditions in Qatar, was studied for metabolites and elemental analysis. The cultures were collected at the end of cultivation, freeze dried and subjected to metabolites study as detailed in Rasheed et al. [9], briefly:

- i) The quantification of carbohydrates was carried out following the method reported by Dubois et al. [227]. The freeze-dried biomass was dispersed in glacial acetic acid and incubated for 20 min at 85 °C, to remove all color interference with the colorimetric assay. The colorless pellet obtained after treatment was hydrolyzed, using hydrochloric acid (HCl 4 M), at 90 °C for 2 h. Then, the supernatant was subjected to calorimetric assay, using phenol sulphuric. The concentration is estimated by referring to a standard curve obtained using known concentrate of D-glucose.
- ii) For total protein, the dried biomass was hydrolyzed overnight at 60°C, using 5 mL sodium hydroxide (NaOH 0.1 M) from Sigma-Aldrich (St. Louis, MO, USA) [228]. The total protein content was determined for using Folin ciocalteau reagent [229]. The concentration is estimated by referring to a standard curve obtained using known concentrate of bovine serum.
- iii) Total lipids were extracted using the method of Folch, with some modifications, where freeze-dried biomass was treated with sodium chloride solution (0.88%) and adequate volume of methanol then incubated overnight at 4°C. Post overnight incubation, double the volume of chloroform (Analytical grade; Sigma-Aldrich, St. Louis, MO, USA) was added to the mixture, and cells were lysed using a tissue lyzer (Qiagen, Hilden Germany). The mixture was centrifuged at 5000 rpm for 5 min, and supernatant was transferred into tube. The extraction was repeated till complete removal of lipids from the biomass and the organic phase was separated from the aqueous phase by adding an adequate volume as per the ratio 8/4/3 of chloroform/ methanol/ water. The organic phase was then collected, transferred into preweighed tube, dried and the tube was re- weighed. The total lipid (TL) content was measured gravimetrically following the equation (n):

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$$(n) TL (\%) = \frac{\text{Lipid content (g)}}{\text{Biomass amount (g)}} * 100$$

### 2.9. Trolox equivalent antioxidant capacity (TEAC) determination

The antioxidant assay consists of the formation of a ferryl myoglobin radical from metmyoglobin and hydrogen peroxide, which oxidizes the ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) to produce a radical cation, ABTS<sup>+</sup>, a soluble chromogen that is green in color and can be determined spectrophotometrically at 405 nm. For this study, the TEAC assay was performed using the sigma Aldrich kit (CS0790, USA). Antioxidants suppress the production of the radical cation in a concentration dependent manner and the color intensity decreases proportionally reflecting the ABTS<sup>+</sup> radical-scavenging capacity of the extracts. Trolox, being a well-characterized antioxidant, is used as a standard reference compound to quantify the antioxidant activity of the sample. It is added to the reaction mixture in different known concentrations and the optic densities of 405 nm were determined using a synergy hybrid multi-mode microplate reader (Bio-Tek, USA). For every culture condition, only the day showing the highest pigment content was investigated and similar concentration of 1 mg/mL was kept for all the crude extracts tested. The TEAC value is determined by measuring the absorbance caused by the antioxidants in the sample and comparing it to a standard curve generated using Trolox. The TEAC of the crude extracts was determined using the following formula:

$$(o) X(mM) = (A_{405nm} - \text{Intercept}) / \text{Slope} \times \text{dilution factor}$$

Where: X (mM): Antioxidant concentration [(mM) relative to the concentration of the Trolox standard], A 405 nm: the average absorbance of the test Samples at 405 nm measured using synergy hybrid multi-mode microplate reader (Bio-Tek, USA), and the Intercept and slope are those of the standard curve. The results of each crude extract were later expressed in terms of Trolox equivalent antioxidant capacity TEAC, mM Trolox equivalents by g<sup>-1</sup> d.w.

### 2.10. DPPH radical scavenging activity



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The free radical scavenging activity of the extract was determined based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical [230]. Similar to the TEAC, only the day showing highest pigment content for each culture condition was investigated and similar concentration of 1 mg/mL was kept for all the crude extracts tested. 3 mL of pigment extract was added to 1 mL of a 0.1 mM Methanolic DPPH. Absorbance was read at 517 nm after 30 min, and the inhibition percentage of the samples on the DPPH radical is calculated by converting the absorbance as a percentage of the scavenging activity (SA%), according to the equation (p):

$$(p) SA(\%) = \left( \frac{A_0 - A_1}{A_0} \right) * 100$$

Where:  $A_0$  - absorbance of the control,  $A_1$  - absorbance of the test sample. DPPH without the test sample was used as a control.

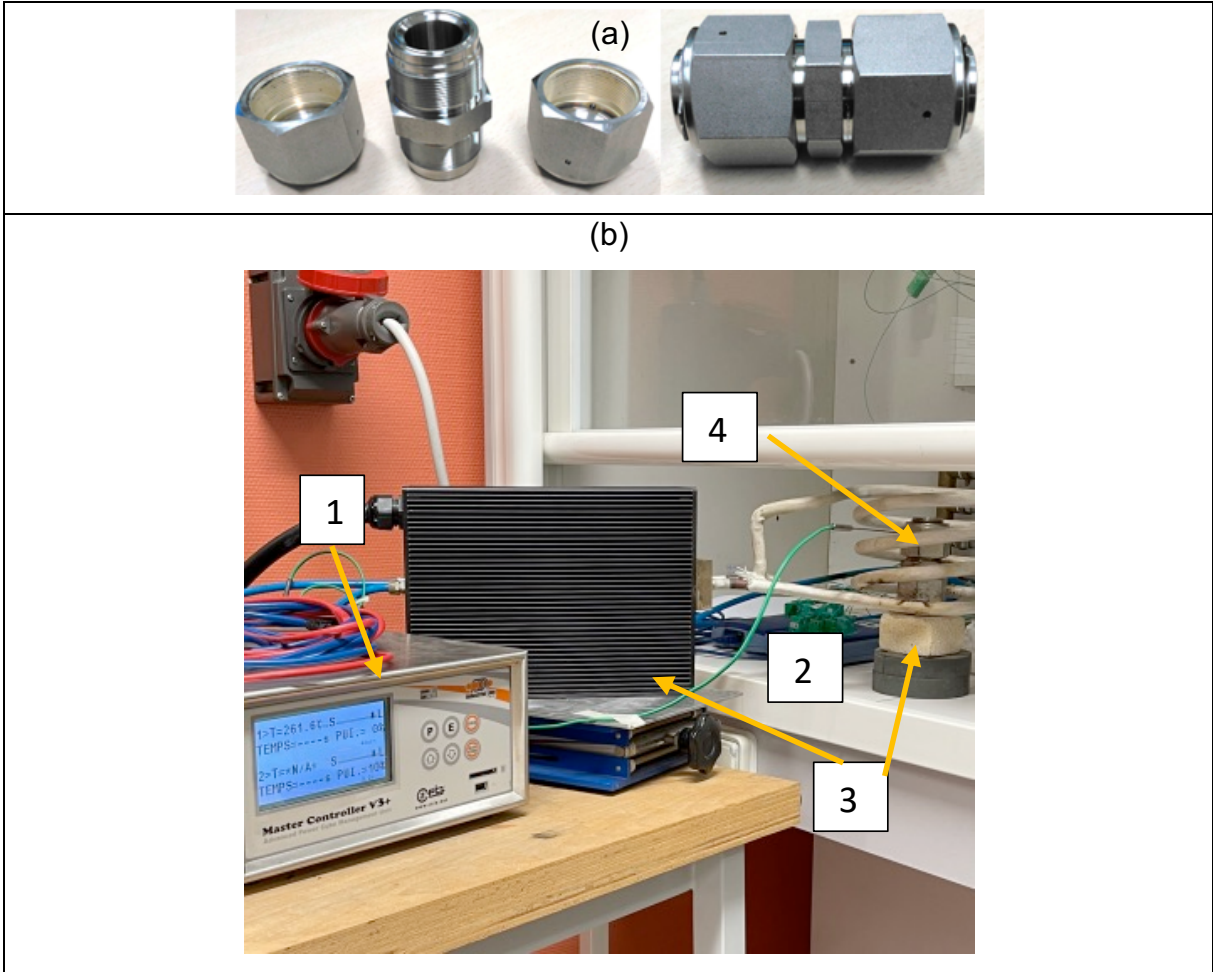
### 3. Hydrothermal liquefaction (HTL) experiments

#### 3.1. HTL conversion and products recovery

All the HTL experiments were conducted on the pre-extracted biomass (PBPs free biomass). 3 g dry biomass samples were converted using a 30 mL swagelok sealed tube as reactor (Swagelok, France), heated with an induction system allowing a fast heating from 25 °C to 260 °C at approximately 30 °C/ min (Fig.23). This system was designed by Antoine Rolland in his thesis [231], for small biomass samples. However, compared to previous works with larger instrumented HTL reactors [232], only the temperature of the steel is measured with a thermocouple and regulated.

Biomass was suspended in 12 mL of deionized water and placed in the Swagelok with a loading volume of 50 %. The biomass/ water ratio was kept constant during all experiments. The temperature program was that first describing bio-bitumen production in literature [205] : heating to 260 °C, isotherm at 260 °C during 60 min and slow cooling overnight in order to reach a temperature of approximately 25 °C before opening the reactor for recovering the products.

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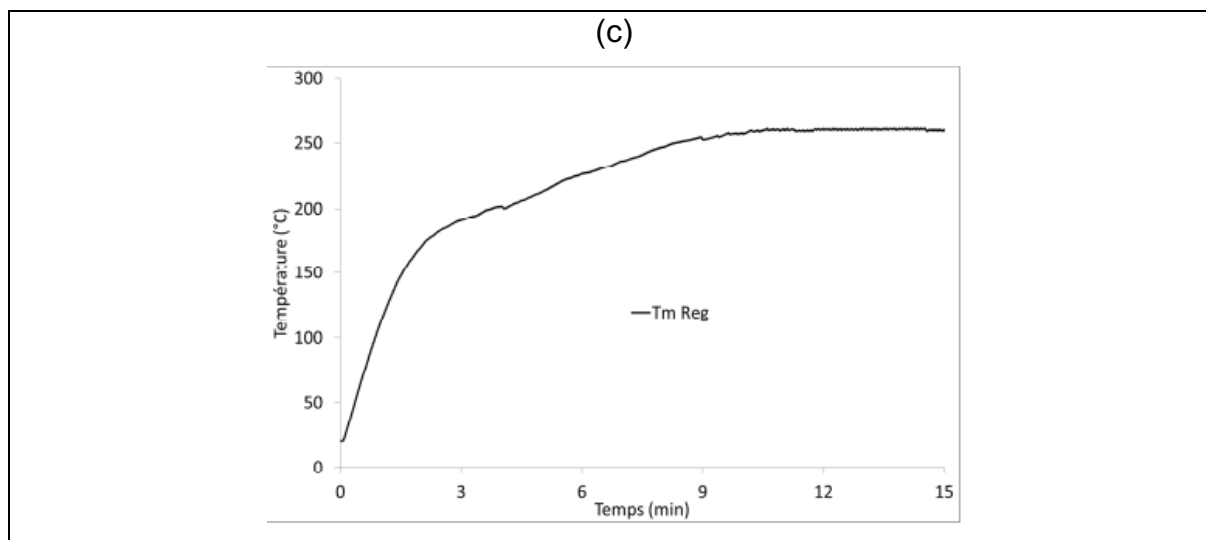


Figure 23 : Swagelok elements used to make the 30 ml sealed reactor **(a)**, picture of the full system used for HTL conversion **(b)**: 1 Control unit, 2 Thermocouple, 3 Induction system, 4 Swagelok reactor placed inside the induction system; and **(c)** heating profile to 260°C.

When opening the reactor, the gas phase was not estimated and was vented into the atmosphere. This phase consists mostly of CO<sub>2</sub> according to previous studies [172]. After releasing the gas, the reactor is opened, and two phases were collected: an aqueous orange colored phase containing water soluble products which was poured in a beaker as shown in “Fig. 25” and a hydrophobic phase which was further dissolved in dichloromethane and separated into biocrude oil and solid residues. Here’s a little scheme to better understand the fractionation we made (Fig.24):

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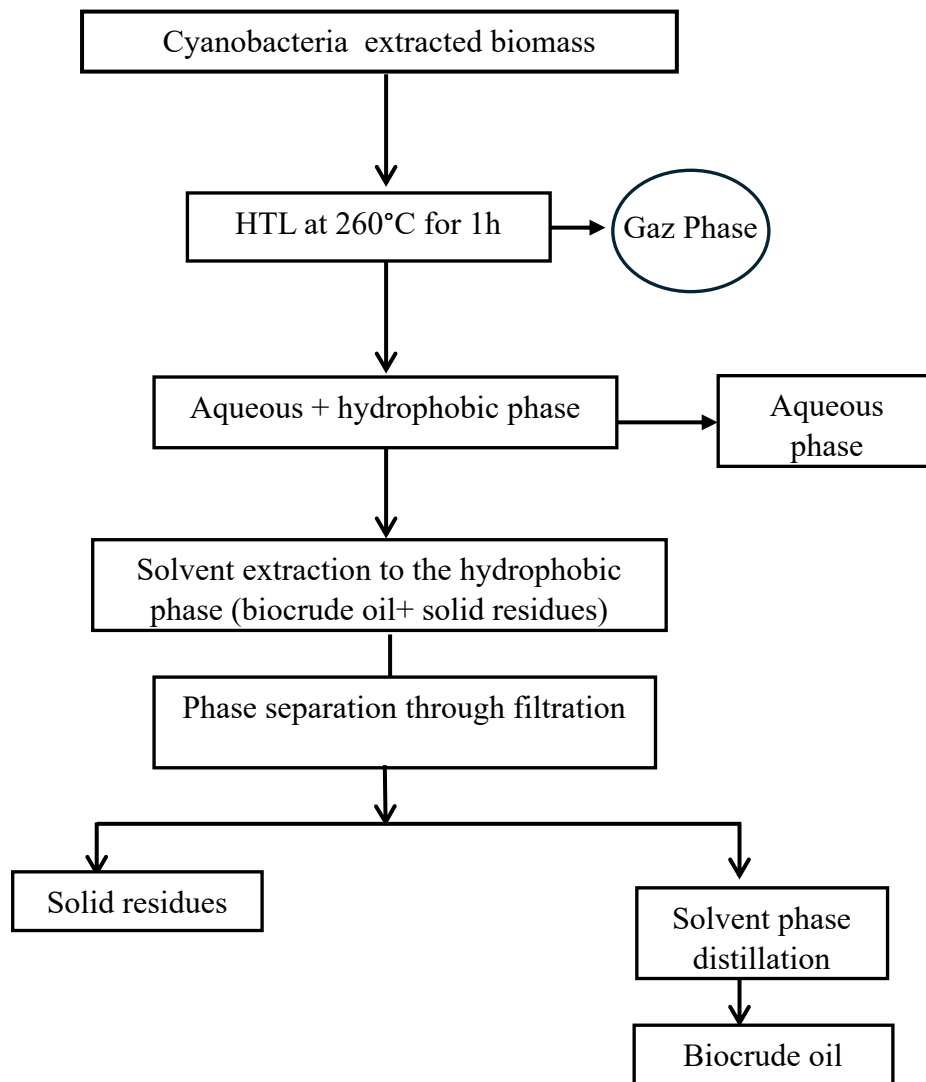


Figure 24 : Schematic presentation of the process followed to collect the different fraction of HTL conversion.

Assuming that the density of the aqueous phase remains equal to 1 (density of the initial di-ionized water), the aqueous phase yield  $Y_{aqueous}$  was estimated as below:

$$({}^o)Y_{aqueous} = \frac{m_{aqueous\ phase} - m_{water}}{m_{biomass}}$$

Where:  $m_{aqueous}$  is the mass of aqueous phase recovered, and  $m_{water}$  is the initial mass of water equal to 12 g.

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Figure 25: Aqueous phase collected after HTL reaction at 260 °C, 1h duration.

Later, the dichloromethane was added to the reactor to dissolve and collect the water insoluble phase. The reactor was further rinsed twice with dichloromethane to allow complete recovery of the remaining hydrophobic solid residual attached to the reactor's wall. These washings were combined and constitute the hydrophobic phase "Fig. 26".



Figure 26: Hydrophobic phase dissolved in dichloromethane.

The hydrophobic phase is composed of biochar "solid residues" and biocrude oil. The mixture was then filtered through a Whatman filter paper (3- 4  $\mu\text{m}$  porosity) to separate the insoluble solid residues "Biochar" from the organic phase as mentioned in "Fig 27". Several washes with dichloromethane were performed in order to ensure a complete removal of all dichloromethane soluble products. This step is challenging as the filters can be blocked with the tinny size of the solid residues and would require the several filters to avoid that. Finally, the solvent was eliminated from the filters by drying them in a hot air oven at 90 °C, and % of the solid residues of size higher than 3-4  $\mu\text{m}$  was calculated according to the equation (q):

$$(q)\% \text{ solid residues: } \frac{m_{\text{filter+residue}} - m_{\text{empty filter}}}{m_{\text{biomass}}} \times 100$$

Where:  $m_{\text{hydrophobic}}$  is the mass of hydrophobic phase resolubilized in dichloromethane and recovered after evaporation.  $m_{\text{filter}}$  is the initial mass of the filter, and  $m_{\text{filter+solid}}$  is the mass of the filter after filtration after drying it in the oven a at 90 °C.

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On the other hand, the organic oil phase (biocrude) was recovered after evaporation of dichloromethane under reduced pressure, using a rota evaporator (Hei-VAP Core model), coupled with a LABOXACT anti-corrosion modular vacuum system SEM 810, a condenser controlled by a Huber minichiller to 4°C and a regulated water bath. 250 mL flask containing 100mL of the organic phase dissolved in dichloromethane is immersed in the water bath set the boiling point temperature of dichloromethane to allow its evaporation. The flask is rotated at a moderate rate and the vacuum is turned on in order to reduce the pressure and facilitate the solvent evaporation from the sample. After complete evaporation of the dichloromethane, the pressure is gradually released and the flask is removed. The obtained biocrude yield was determined gravimetrically as mentioned below:

$$(r) \% \text{ Biocrude oil phase} : \frac{m_{oil \text{ phase}}}{m_{biomass}} \times 100$$

Where:  $m_{hydrophobic}$  is the mass of organic oil phase resolubilized in dichloromethane and recovered after evaporation.

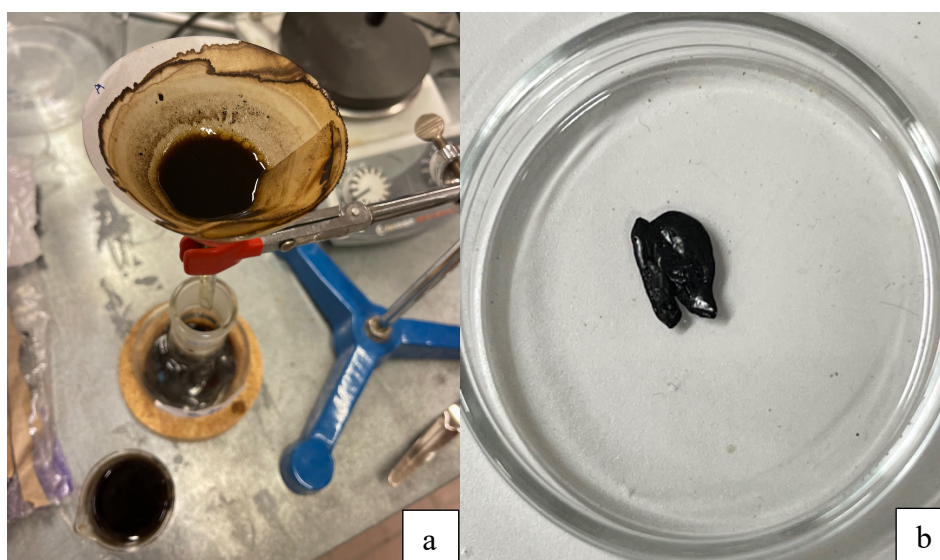


Figure 27: Picture of **(a)**: filtration process of hydrophobic phase: the solid residues remain in the filter and the organic phase is collected in the rotaevaporator bulb flask, and **(b)**: biocrude oil obtained after evaporation of dichloromethane.

During the recovery of the different phases, losses in each fraction are prone to happen and together with the gaseous phase were estimated as in equation (s):

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$$(s) \%_{Loss} = 100 - \%_{hydrophobic\ phase} - \%_{Aqueous\ phase}$$

### 3.2. Characterization of the oil and solid fractions fraction

#### 3.2.1. Elemental analysis, ash content, energy recovery

Similarly to the biomass, the ash content (% Ash) and organic elemental analysis (%C, %H, %N) of the oil and solid fractions were measured as mentioned in paragraph 2.5. The energy recovery (ER) for each HTL run was determined by equation below:

$$(t) ER (\%) = Biocrudeyield (\%) \times \frac{HHV (biocrude)}{HHV (feedstock)}$$

#### 3.2.2. GC/MS analysis

The chemical composition of the biocrude samples was characterized through gas chromatography mass spectrometry (GC/MS), using a 7890A Agilent technology gas chromatography coupled with a 5973 Mass Spectrometry detector. The injector temperature was kept constant at 300 °C. An autosampler was used to inject a 1 µL sample (in splitless mode) onto the GC column (30 m × 250 µm × 0.25 µm Rxi-5Sil MS column). Helium carrier gas was used at 15 psi pressure and a flow velocity of 1.67 mL/min. The oven temperature was initially held at 60 °C for 2 min, then was increased at 6 °C/min until it reached 300 °C, where it was held for 20 min. The different compounds in the gas stream were ionized using an ion source. The mass spectrometer was operated in MS-scan mode and the compounds in biocrude oil were identified using the NIST98 mass spectral library database.

#### 3.2.3. Size exclusion chromatography

The molecular weight distribution of the biocrude fractions were carried out using the gel permeation chromatography (GPC, waters 4214 differential refractometer), with tetrahydrofuran (THF) chromatographically pure as mobile phase solvent at a flow rate of 1 mL/min. Crude samples were dissolved in THF with a concentration of 1% (mass/volume). Once solubilized, the solution was filtered through a 0.22 µm Wathman

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PTFE syringe filter to remove all solid particles. 5  $\mu\text{L}$  of the solution was injected into the chromatographic system. The separations were carried out using a series of two “PL Mixed-D 5  $\mu\text{m}$ ” columns and a “PLgel 5  $\mu\text{m}$ ” pre-column supplied by Polymer Laboratories and sample processing was carried out using AZUR V4.6 software provided by Datalys (Saint Martin d'Hères, France).

### 3.2.4. FTIR analysis

The Fourier transform infrared (FTIR) analysis of feedstock and biocrude oil were recorded at the wavenumber range between 800 and 4000  $\text{cm}^{-1}$  using an Agilent technologies Cary 600 Series FTIR spectrometer (K8006A, Malaysia). Baseline corrections were applied to all the spectra using Agilent Resolutions Pro software (Version 5.3.0). FTIR analysis was carried out using a resolution of 4  $\text{cm}^{-1}$ .

### 3.2.5. Rheological properties of the biocrude oil fractions

The hydrophobic phases obtained after filtration were characterized for its rheological properties in dynamic shear rheometry (Haak MARS III) equipped with a Peltier temperature control system. The fractions were loaded, in the melted state at 80  $^{\circ}\text{C}$ , on a plate-plate geometry (with a diameter of 8 mm) with 1 mm gap. Dynamic shear rheometer takes measures of phase angles  $\delta$  ( $^{\circ}$ ) and of norm of complex modulus ( $|G^*|$ ) at different temperature values at several temperatures (from 0  $^{\circ}\text{C}$  to 80  $^{\circ}\text{C}$ ) in various frequencies (from 0.01 Hz to 10 Hz). The Black diagram consists in plotting the measured phase angles  $\delta$  ( $^{\circ}$ ) as a function of the measure complex modulus  $G^*$  for each temperature-applied shear frequency couple. In practice the lowest values of  $G^*$  for each sample are those measured at the highest temperature (80  $^{\circ}\text{C}$ ) and for the lowest frequency (0.01 Hz). The behavior of hydrothermal conversion products was compared with the pure conventional bitumen.



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### Conclusion

The scheme below summarizes the experimental work, which results will be discussed in chapters 3,4 and 5:

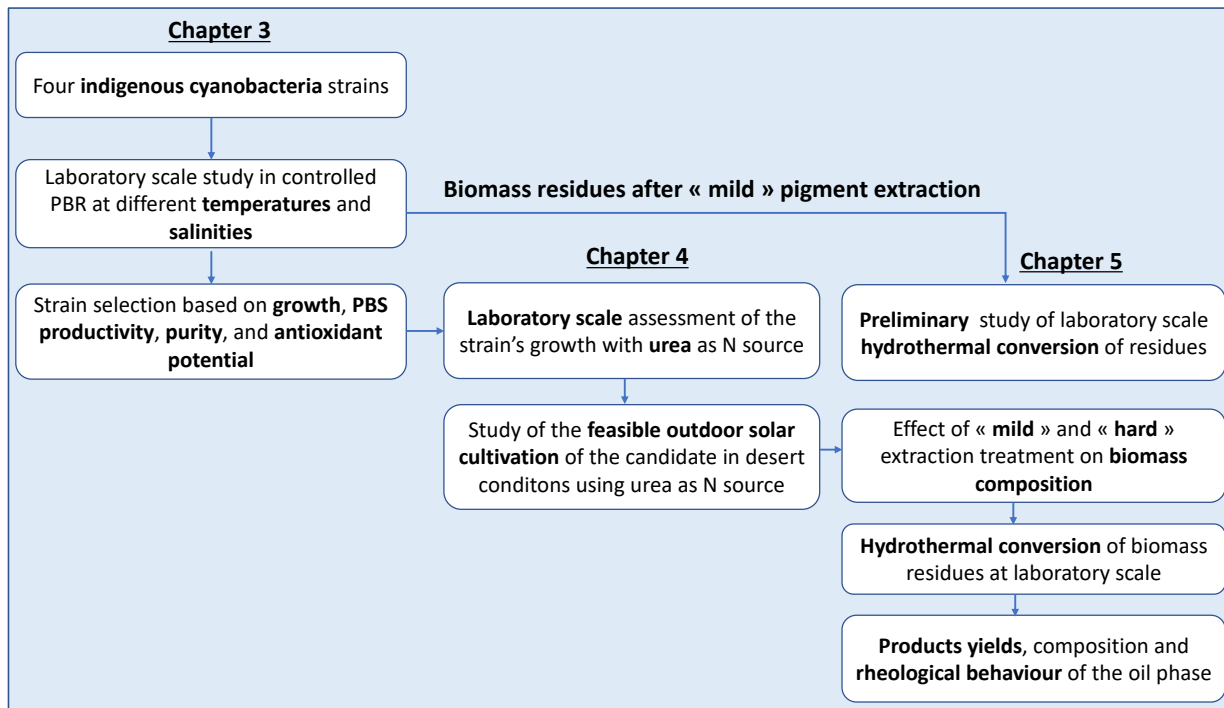


Figure 28: Graphical abstract summarizing the different steps followed during this study

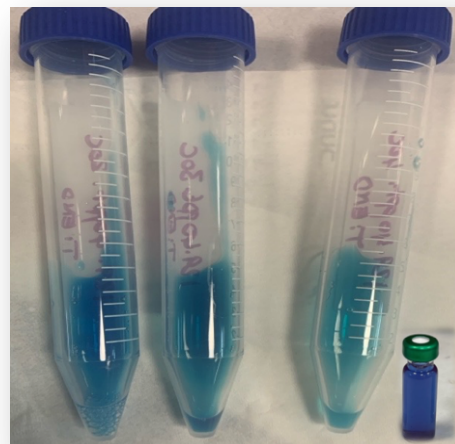
**Chapter 3: Lab scale assessment of  
selected strains' potential for  
outdoor desert environment  
cultivation**

### Chapter 3: Lab scale assessment of selected strains' potential for outdoor desert environment cultivation

### Chapter 3: Lab scale assessment of selected strains' potential for outdoor desert environment cultivation

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Some of the results presented in this chapter were published in the journal of process biochemistry. Title of the publication is: "Assessment of novel halo- and thermotolerant desert cyanobacteria for phycobiliprotein production"  
(<https://doi.org/10.1016/j.procbio.2022.04.017>)



## **Chapter 3: Lab scale assessment of selected strains' potential for outdoor desert environment cultivation**

### **Introduction:**

Desert habitats, like those found in Qatar, are home to a wide variety of cyanobacteria with untapped potential for various applications [233]. However, there is currently limited available information about local cyanobacterial strains and their potential as phycobiliprotein producers as most of the studies were targeting the potential use of green microalgae in bioenergy and feed and food sectors. For a successful biomass production, it is equally important to select strains that can grow well under relevant outdoor conditions in addition to accumulating the desired product.

In view of these considerations, this chapter investigates four different local cyanobacteria species for their ability to produce phycobiliproteins as high valuable product, with a focus on assessing their growth and PBPs production when exposed to temperatures and salinities representative of outdoor cultivation conditions in Qatar. Understanding their interactive effects on the physiological performance of the strains is fundamental to predict their responses to natural environmental conditions throughout the year. The strains were collected from different marine environment, purified to axenic cultures, and characterized through molecular identification. Cultivation experiments were conducted in 1L controlled photobioreactors across different temperature and salinity ranges. The temperatures selected are 20 °C (year average lowest daily temperature), 30 °C (average annual temperature), and 40 °C (year average highest daily temperature), whereas the two salinity levels investigated for each temperature are 40 ppt (part per thousand) and 60 ppt. Their effect on the growth and on phycobiliprotein production are determined, with the objectives: i) to select a strain of future outdoor mass cultivation using open ponds, and ii) to define the best combination of temperature and salinity leading to maximum growth and PBPs production. The effect of variation of these both parameters along with the culture age on the PBPs quality and quantity is monitored to determine the optimum conditions leading to their highest accumulation. Besides, considering their role as sustainable sources of natural antioxidants [234], bioprospecting of the antioxidant potential of the crude PBPs extract is also evaluated. This comprehensive assessment provides a foundation for developing sustainable production strategies for cyanobacterial biomass in Qatar's challenging desert environment

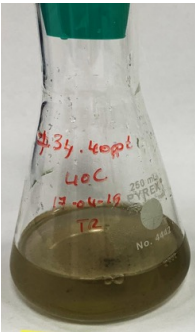

### Chapter 3: Lab scale assessment of selected strains' potential for outdoor desert environment cultivation

#### Results and Discussion:

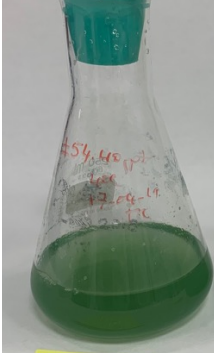
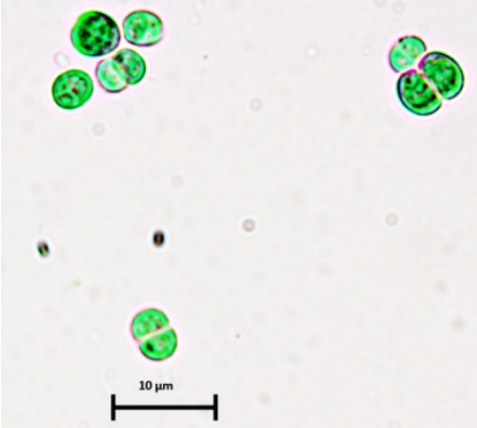
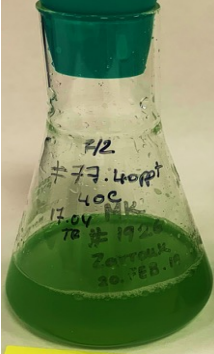
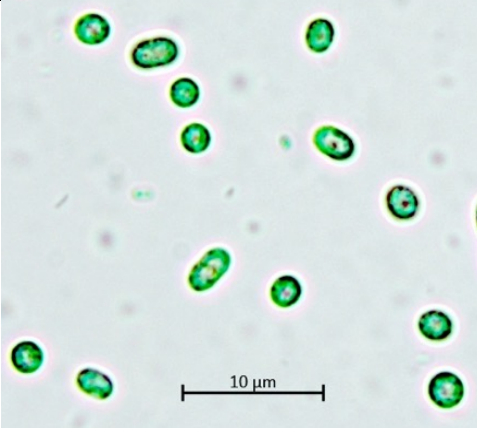
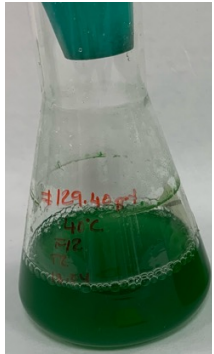

##### 1. Morphological identification of the pre-screened strains

The morphological identification of cyanobacterial cultures by light-microscopy allowed to categorize and classify the strains based on their observable characteristics. The findings indicated the presence of polymorphism, manifested by variations in terms of cell size and structure (Table 6). The visible characteristics of the purified strains permit to divide them into 2 groups: i) A group of big round shaped cells (QUCCCM 34 and QUCCCM 54), respectively colored dark green to black, and green with tendency to clump together into big colonies of spherical aggregates having different sizes and cell numbers; ii) a second group of small size unicellular cells either oval with cylindrical shape (QUCCCM 77) or in pairs and displaying an ellipsoidal shape (QUCCCM 129). None of the above identified groups showed an extension of flagella which might allowed their motility nor existence of heterocyst. This initial taxonomic classification is essential for understanding the diversity within the pre-screened strains and provides a foundation for further investigations. However, Molecular identification is required for more accurate taxonomic classification.

Table 6: Microscopic observation of the 4 selected strains using light microscope at (100X) objective.

Strain	Microscopic picture
<p data-bbox="284 1458 480 1491"><b>QUCCCM 34</b></p> 	

## Chapter 3: Lab scale assessment of selected strains' potential for outdoor desert environment cultivation

<p><b>QUCCCM 54</b></p> 	
<p><b>QUCCCM 77</b></p> 	
<p><b>QUCCCM 129</b></p> 	

### 2. Molecular identification of the 4 shortlisted cyanobacterium strains

The molecular identification was performed the shortlisted strains as different microalgae species can have similar morphologies, and same strains might present

### Chapter 3: Lab scale assessment of selected strains' potential for outdoor desert environment cultivation

different shapes in the different growth stage [235]. Full obtained sequences were blasted into the NCBI BLAST software, and the results were compared with the gene bank. Table 7 gives an overview of the strains, their identified genera according to 16S rDNA analysis and their accession number. Since the similarity % is very high (> 97%) for a full coverage, there was no need sequence the full sequence of the 16sRDNA. The comparison of the 16S rDNA sequences of the four axenic isolates together with the morphological studies confirmed the presence of 4 different genera. From the alignment of the sequences, and the resulting phylogenetic tree (Fig. 39), we concluded that QUCCCM 34 and QUCCCM 54 showed high similarities to the genus *Chroococciopsis* and *Pleurocapsa*, respectively, whilst the other strains QUCCCM 77 and QUCCCM 129 displayed homology with the existing sequences published in the GenBank for *Euhalothece* and *Cyanobacterium* respectively.

Table 7: Molecular details of the cyanobacterial studied strains.

	Similarity	Coverage	Species	Fragment size (bp)	Accession number
<b>QUCCCM34</b>	99.65%	100%	<i>Chroococciopsis sp</i>	855	GenBank: MZ817054
<b>QUCCCM54</b>	97.85%	100%	<i>Pleurocapsa sp</i>	1114	GenBank: MZ817055
<b>QUCCCM77</b>	98.38%	100%	<i>Euhalothece sp</i>	1111	GenBank: MZ817056
<b>QUCCCM129</b>	99.55%	100%	<i>Cyanobacterium aponium</i>	852	GenBank: OK044281

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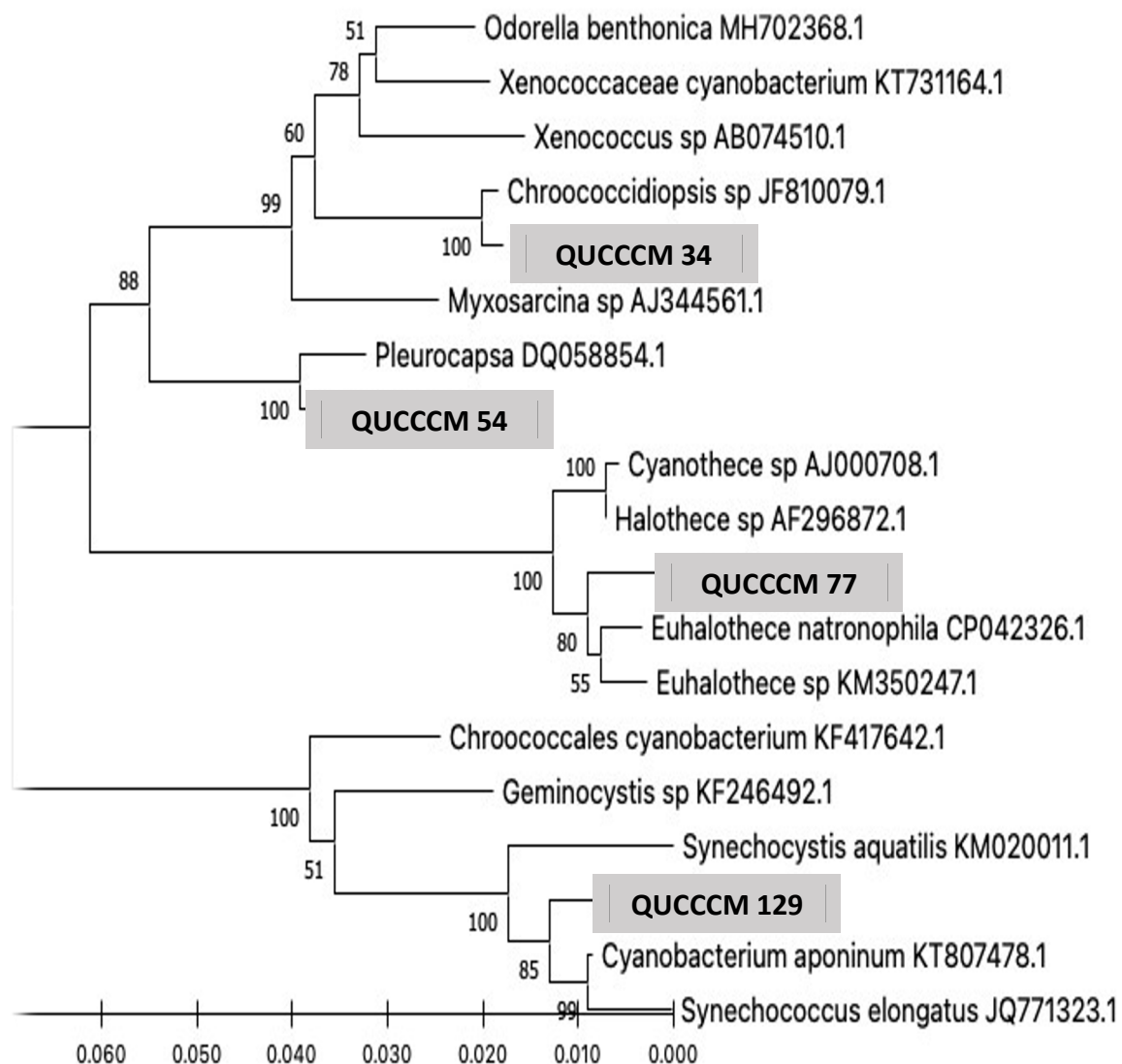


Figure 29: Phylogenetic tree based on the gene sequences of strains QUCCM 34, QUCCM 54, QUCCM 77 and QUCCM 129. Distances within the tree were constructed using the neighbor joining method with MEGA X. Horizontal length are proportional to the evolution

### 3. Elemental and metabolite analysis of the shortlisted cyanobacterial biomass

The biomass produced under “standard” conditions, (30 °C - 40 ppt) was analyzed for Carbon (C), Hydrogen (H), Nitrogen (N) contents, in addition to major metabolites (proteins, carbohydrates, and lipids) and the data are listed in Table 8. The C/N ratio for all the strains varied from 4 to 6. It was lower for *Chroococcidiopsis* (QUCCCM 34) and *Pleurocapsa* (QUCCCM 54) than for *Euhalothece* (QUCCCM 77) and



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*Cyanobacterium* (QUCCCM 129). This trend is in line with the protein content observed, where *Chroococcidiopsis* (QUCCCM 34) and *Pleurocapsa* (QUCCCM 54) presented higher amount (45 and 51%) compared to *Euhalothece* (QUCCCM 77) and *Cyanobacterium* (QUCCCM 129) (32- 35%). Moreover, the protein contents found for (QUCCCM 34) and (QUCCCM 54) were higher than what was reported previously for similar species isolated from arid regions. For example the species *Chroococcidiopsis* sp isolated from *Salar de Atacama* Desert, in northern Chile presented only 36.7% of proteins vs 45% for *Chroococcidiopsis* sp from Qatar [236]. The same findings were noticed for the strain *Pleurocapsa* sp. BERCO6 which is dominating in the wastewater reservoirs of central Punjab [237]. Besides, the protein content of these 2 strains was not only greater than what was found for the same species from different places in the world, but it was within the range of what was reported for *S. platensis*, one of the most protein rich cyanobacteria strains commonly used as a health-food supplement (45–65 %) [238]. The carbohydrates content for all the isolates was however higher compared to other cyanobacteria, such as in *Spirulina platensis* (8–14 %). The reason for such difference can be associated to the protective role the polysaccharides sheath play against desiccation in extreme natural environment [236]. In contrast, results indicated that the lipids amount was relatively low (9 and 11%) for *Chroococcidiopsis* (QUCCCM 34) and *Pleurocapsa* (QUCCCM 54). This is expected as both strains were the highest in terms of growth and proteins quantity. In fact, when the strain metabolism was directed towards protein accumulation lipids will not be synthesized in high amounts. These data were in accordance with what was stated for other *Chroococcidiopsis* species from comparable environmental conditions [236]. However, for *Euhalothece* (QUCCCM 77) and *Cyanobacterium* (QUCCCM 129), the lipids detected were within the range noted for most of cyanobacterial species (29- 21 %) [239].

Table 8: CHN and metabolites data for the 4 cyanobacterial strains (Standard deviation below 2%).

Strains	Proteins (%)	Carbohydrates (%)	Lipids (%)	N%	C%	H%	Other

### Chapter 3: Lab scale assessment of selected strains' potential for outdoor desert environment cultivation

							O, S ... % (* )
<i>Chroococcidiopsis</i> (QUCCCM 34)	45	29	9	10	45	6	39
<i>Pleurocapsa</i> (QUCCCM 54)	51	21	11	11	44	7	38
<i>Euhalothece</i> (QUCCCM 77)	32	29	29	7	44	3	46
<i>Cyanobacterium</i> (QUCCCM 129)	35	28	21	8	40	7	45

\*: Calculated by subtraction

#### 4. Investigation of the selected cyanobacteria strain under different temperature and salinity regimes: PBRs Level

##### 4.1. Influence on cultivation conditions on growth, productivity and CO<sub>2</sub> capture

The 4 shortlisted cyanobacteria strains were further investigated in 1L PBRs under controlled conditions, to elucidate the combined effect of both salinity and temperature variations as witnessed in Qatar climate on the growth and PBPs accumulation.

The increment in biomass concentration for each cyanobacteria strain under different temperature and salinity ranges is plotted in Fig. 30 while Table 9a summarizes the biomass productivities.

The results showed that the impact of culture conditions on growth and biomass productivity varied among the strains. Initially, all isolates presented a lag phase until day 4, reflecting their period of adaptation to the cultivation conditions, followed by a linear growth between day 4 and 8. Subsequently, two scenarios were observed depending on the strains: QUCCCM 77 and QUCCCM 129 reached their stationary phase between day 8 and 10, while the growth of QUCCCM 34 and QUCCCM 54 continued exponentially even after 12 days of culture. Similar trend was reported previously for *Chroococcidiopsis* sp. isolated from comparable weather conditions, where growth persisted even after 17 days of cultivation [240]. Conditions leading to the high productivity were also strain dependent. Among all the strains, QUCCCM 54, *Pleurocapsa* sp., had the highest biomass productivity with 125 mg. L<sup>-1</sup>. d<sup>-1</sup>, followed by QUCCCM 129 (118 mg. L<sup>-1</sup>. d<sup>-1</sup>), QUCCCM 34 (113 mg. L<sup>-1</sup>. d<sup>-1</sup>), while QUCCCM

### Chapter 3: Lab scale assessment of selected strains' potential for outdoor desert environment cultivation

77, *Euhalothece* sp., displayed the lowest growth with very low biomass productivity of ( $45 \text{ mg. L}^{-1} \cdot \text{d}^{-1}$ ). In fact, 3 of the 4 strains exhibited their optimum productivity at growth temperature and salinity of  $30 \text{ }^\circ\text{C}$  and 40 ppt, corresponding to the yearly average annual temperature in Qatar and the dominant salinity of the sea water, except for QUCCCM 129 which growth was slightly favored at  $30 \text{ }^\circ\text{C}$  - 60 ppt. It was also observed that under high temperature of  $40 \text{ }^\circ\text{C}$ , and salinities of 60 ppt the strains did not collapse, highlighting their thermo and halotolerance capacity with potential outdoor cultivation under extreme temperatures recorded in the country.

Indeed, for QUCCCM 34, varying biomass productivities were observed based on temperature and salinity. Highest productivity was at  $30^\circ\text{C}$  and 40 ppt salinity ( $113 \text{ mg. L}^{-1} \cdot \text{d}^{-1}$ ) and slightly decreased at  $40^\circ\text{C}$  but remained relatively consistent across salinity levels except at  $30^\circ\text{C}$  where a drop from  $113 \text{ mg. L}^{-1} \cdot \text{d}^{-1}$  to  $75 \text{ mg. L}^{-1} \cdot \text{d}^{-1}$  was observed.

For QUCCCM 54, at  $20^\circ\text{C}$  and 40 ppt salinity, the strain exhibited a moderate biomass productivity of  $44 \text{ mg. L}^{-1} \cdot \text{d}^{-1}$ , while no growth observed at  $20^\circ\text{C}$  -60 ppt salinity, indicating that the strain may be sensitive or unable to thrive under higher salinity conditions at this temperature. The biomass productivity significantly increased to  $125 \text{ mg. L}^{-1} \cdot \text{d}^{-1}$  at  $30^\circ\text{C}$  - 40 ppt salinity, suggesting that strain 54 preferred higher temperatures for optimal growth under moderate salinity conditions. At  $30^\circ\text{C}$  and 60 ppt salinity, the biomass productivity remains relatively high but slightly lower compared to the lower salinity condition at the same temperature. The growth of the strain remained robust at  $40^\circ\text{C}$  and was maintained at both 40 ppt and 60 ppt salinity, demonstrating the thermo-halotolerant capacity of the strain.

For QUCCCM 129, there was a slow growth at  $20^\circ\text{C}$  - 40ppt, which was inhibited at higher salinity of 60 ppt. Biomass productivity significantly increased to  $91 \text{ mg. L}^{-1} \cdot \text{d}^{-1}$  at  $30^\circ\text{C}$  and 40 ppt salinity and was further enhanced to reach to  $118 \text{ mg. L}^{-1} \cdot \text{d}^{-1}$  at  $30^\circ\text{C}$  -60 ppt, indicating that the strain prefers to thrive at high salinity levels under optimal temperature conditions of  $30^\circ\text{C}$ . Biomass productivity at higher temperature was lower compared to  $30^\circ\text{C}$ . However, salinity increment was observed to enhance productivity.

Among all the strains, QUCCCM 77 was the lowest in terms of biomass productivity, with no growth observed at  $20^\circ\text{C}$  for both salinities. The strain displayed growth at

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higher temperature and salinity, with a biomass productivity of maximum 45 mg/L/day at 30°C and no influence of salinity raise observed regarding the growth. Higher salinity and temperature levels did not largely impact the biomass productivity of QUCCCM77. In fact, as the strain was isolated from sabkha, an area of coastal flats that experiences periodic flooding caused by northwest winds that drive sea water over the sabkha and evaporation resulting in higher salt concentrations of 60 ppt, it became acclimated to both salinities.

Different strategies were developed to explain the growth variations of cyanobacteria above their optimal temperature ranges. Previous research work demonstrated that elevated temperatures result in heat stress, which can affect the functionalities of enzymes (inactivation, denaturation) involved in photosynthetic processes, such as altering the activity of Rubisco, an important enzyme responsible for carbon assimilation, hence impacting the growth [241]. It also influences the membrane fluidity affecting nutrients uptake [242]. Hernando et al. [132] also stated that when temperature rises above the optimum level, an increase in respiration was observed for phytoplankton resulting in lower growth rate. Conversely, the growth under low growth temperature of 20°C was the lowest for all the investigated strains and was inhibited at higher salinity except for QUCCCM 34. These data were expected given the short winter periods in the country, which make these conditions not suitable for growth and biomass production of the strains. Ability of cyanobacteria to thrive under increased salinity is mostly attributed to the synthesis of osmolytic compound such as glucosyl glycerol in species of moderate tolerance and glycine betaine and glutamate betaine in species showing high tolerance [243]. Other researchers related this to the capacity of some strains to adjust their respiration or regulate the intake and discharge of salt [244]. Most of the strains had a slow growth at higher salinity. This can be a consequence of a decrease in photosynthetic efficiency (i.e., the inhibition of the electron transport chain or due the redirection of energy for the active pumping of Na<sup>+</sup> ions and the production of carbohydrates as salt protectors to counterbalance the cell stress [245]. The effect of salinity increase on the biomass productivity at low temperatures was quite interesting. None of the strains that were able to grow at 20 °C- 40 ppt, showed a growth when salinity raised to 60 ppt, except for QUCCCM 34 that presented similar biomass productivity at 20 °C for both salinities. The reason of

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this could be due to the low temperature conditions which were not favoring the metabolic activities required for synthesizing the responsible molecule(s) for salinity resistance.

The co-tolerance to high temperatures and salinities reflected by the present indigenous cyanobacteria strains offers significant advantages for their large-scale cultivation in Qatar or similar desert environments, particularly in open raceway ponds where there is lack of control over temperature and subsequently on salinity due to evaporation. Indeed, considering the local freshwater scarcity, seawater may be added to compensate the evaporation losses for short culture period, leading to a cost-effective production. Furthermore, cultivation of halotolerant strains at high salinities reduces the risk of contamination by unwanted bacteria and predators, which are common causes of culture collapse in long-term open cultivation systems [246].

It was also observed that higher biomass productivities corresponded to greater CO<sub>2</sub> bio fixation, which is evidenced by the data of CO<sub>2</sub> capture rate (Table 9b). Indeed, for all the strains there was a correlation between the data of biomass productivity and CO<sub>2</sub> capture rate. The optimal temperature and salinity for CO<sub>2</sub> capture coincides with optimal growth conditions, suggesting efficient utilization of carbon dioxide for biomass production. QUCCCM 54 had the highest capture rate of 202 mg CO<sub>2</sub> L<sup>-1</sup> d<sup>-1</sup> at 30 °C-40 ppt compared to the other strains. As of such, it presents a promising candidate for CO<sub>2</sub> capture and would be interesting to further investigate its growth ability under enriched CO<sub>2</sub> gas sources, such as industrial flue gasses.

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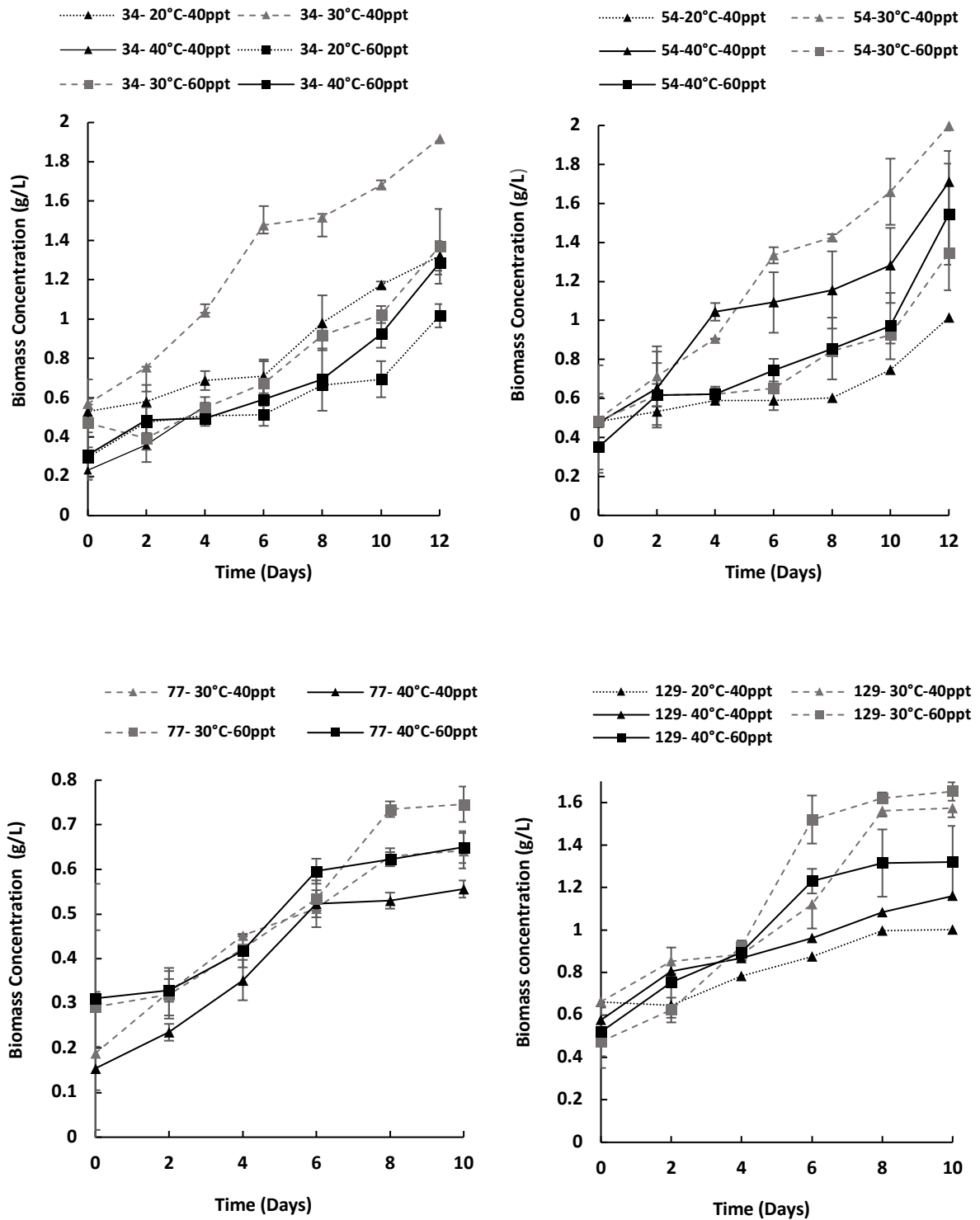


Figure 30: Biomass concentration variation as in function of the culture days and conditions for the 4 studied cyanobacteria strains. Values are means  $\pm$  standard errors ( $n = 2$ ).

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Table 9: Biomass productivities  $\text{mg.L}^{-1}.\text{day}^{-1}$  (a) of the strains under the culture conditions investigated, and (b) carbon capture rate  $\text{mg.L}^{-1}.\text{day}^{-1}$ . The grey colored boxes of the table correspond to conditions leading to the highest productivities of each strain. (Standard deviation below 8%)

(a)	Cultivation conditions					
	40 ppt			60 ppt		
	20°C	30°C	40°C	20°C	30°C	40°C
<b>QUCCCM 34</b>	66	<b>113</b>	82	60	75	81
<b>QUCCCM 54</b>	44	<b>125</b>	103	-	72	100
<b>QUCCCM 77</b>	-	<b>45</b>	40	-	<b>45</b>	34
<b>QUCCCM 129</b>	34	91	58	-	<b>118</b>	80

(b)	Cultivation conditions					
	40 ppt			60 ppt		
	20°C	30°C	40°C	20°C	30°C	40°C
<b>QUCCCM 34</b>	82	<b>186</b>	113	73	105	95
<b>QUCCCM 54</b>	61	<b>202</b>	157	-	105	121
<b>QUCCCM 77</b>	-	<b>67</b>	59	-	66	53
<b>QUCCCM 129</b>	49	133	79	-	<b>173</b>	112

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#### 4.2. Influence of cultivation conditions on phycobiliproteins expression.

The optimization of phycobiliprotein (PBP) yield can be achieved through the control and optimization of nutrient and environmental factors. PBPs accumulation was found to be directly related to biomass, until the organism is exposed to any stress growth conditions [78]. However, this trend is not general for all strains. Despite significant research efforts in the last decade to understand PBP expression, none of the reported studies have explored the impact of culture conditions such as temperature and salinity variations on PBP dynamics across different growth phases. It is particularly important from a production perspective, helping in deciding the time for maximum PBPs production. The earliest synthesis of highest PBPs mainly the target type, the better it is for the economics.

In line with the growth pattern, optimal conditions for total of PBPs (PC+PE) expressions were varying depending on the cultivation conditions and strains, with lower synthesis levels noted at lower temperatures (20 °C) (Table 10). In fact, lower temperature tends to reduce the cellular metabolic activities, which explains the low PBPs production under 20 °C. The highest yield among all the investigated conditions was 105,181, 151, and 197 mg/g<sub>dw</sub> for QUCCCM 34, 54, 77 and 129, under specific cultivation conditions of 40 °C- 60 ppt- day 4, 40 °C- 40 ppt- day 4, 30 °C- 40 ppt- day 10 and 30 °C- 60 ppt- day 8, respectively. Interestingly, QUCCCM 34, 54 had their highest PBPs accumulation in early growth stage which is interesting for a sustainable economic large-scale production. By harvesting the biomass early, the risk of contamination is minimized, and growth expenses are reduced.



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Table 10: PBPs content in the cyanobacteria strains under different culture conditions.

	Yield PBPs (mg/g)						
	Day 0	Day 2	Day4	Day 6	Day 8	Day 10	Day 12
QUCCCM 34- 20°C- 40 ppt	74	17	23	30	58	7	9
QUCCCM 34- 30°C- 40 ppt	74	30	33	34	41	23	27
QUCCCM 34- 40°C- 40 ppt	74	55	53	***	***	***	***
QUCCCM 34- 20°C- 60 ppt	93	38	60	69	78	18	47
QUCCCM 34- 30°C- 60 ppt	93	37	69	67	68	31	43
QUCCCM 34- 40°C- 60 ppt	93	52	105	85	77	73	72
	Day 0	Day 2	Day4	Day 6	Day 8	Day 10	Day 12
QUCCCM 54- 20°C- 40 ppt	35	52	62	32	40	35	46
QUCCCM 54- 30°C- 40 ppt	35	67	118	120	121	116	107
QUCCCM 54- 40°C- 40 ppt	35	108	181	149	144	142	130
QUCCCM 54- 30°C- 60 ppt	21	75	87	94	87	107	66
QUCCCM 54- 40°C- 60 ppt	21	98	121	127	97	97	85
	Day 0	Day 2	Day4	Day 6	Day 8	Day 10	Day 12
QUCCCM 77- 30°C- 40 ppt	64	103	113	130	140	151	***
QUCCCM 77- 40°C- 40 ppt	64	125	115	91	78	62	***
QUCCCM 77- 30°C- 60 ppt	49	93	122	133	137	120	***
QUCCCM 77- 40°C- 60 ppt	49	66	129	127	97	75	***
	Day 0	Day 2	Day4	Day 6	Day 8	Day 10	Day 12
QUCCCM 129- 20°C- 40 ppt	10	9	35	42	57	73	***
QUCCCM 129- 30°C- 40 ppt	10	37	76	97	119	102	***
QUCCCM 129- 40°C- 40 ppt	10	93	108	117	138	93	***
QUCCCM 129- 30°C- 60 ppt	33	13	15	178	197	108	***
QUCCCM 129- 40°C- 60 ppt	33	22	63	155	173	71	***

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The PBPs content was further compared with the total protein content for each strain under different culture conditions and was estimated for the days showing the highest amount of PBPs as mentioned in Fig.31 below. This comparison is important as it indicates whether the cells are actively producing PBPs or if other proteins are dominating and will help in selecting the most favorable strain and condition for producing PBPs.

The analysis revealed varying ratios of PBPs to total protein, ranging from 15.7 to 46.2 depending on the strains, its cultivation conditions, with QUCCCM 34 having the lowest ratio and QUCCCM 54 presenting the highest one. All the conditions leading to the highest PBPs expression presented the highest PBPs: protein ratio except for QUCCCM 129.

For QUCCCM 34, the values ranged between 15.7 and 24.5 with salinity 60 ppt displaying highest ratio than 40 ppt, and the highest value was observed at 40 °C -60 ppt, corresponding to the condition leading to the highest PBPs expression.

QUCCCM 54 presented the highest ratio value of 46.2 % at 40 °C, 40 ppt corresponding to the condition where the PBPs production was promoted.

QUCCCM 77 was displaying the second highest ratio of 41.2 at 30 °C- 40 ppt, while QUCCCM 129 displayed a high ratio of 40.5 at 40 °C - 40 ppt.

Previous research work demonstrated that the PBPs in cyanobacteria constitute 20-40% of total protein [247], which aligns well with most of the ranges observed in our study, with the exception of QUCCCM 54, which displayed a ratio of 46 % higher than what was previously reported.

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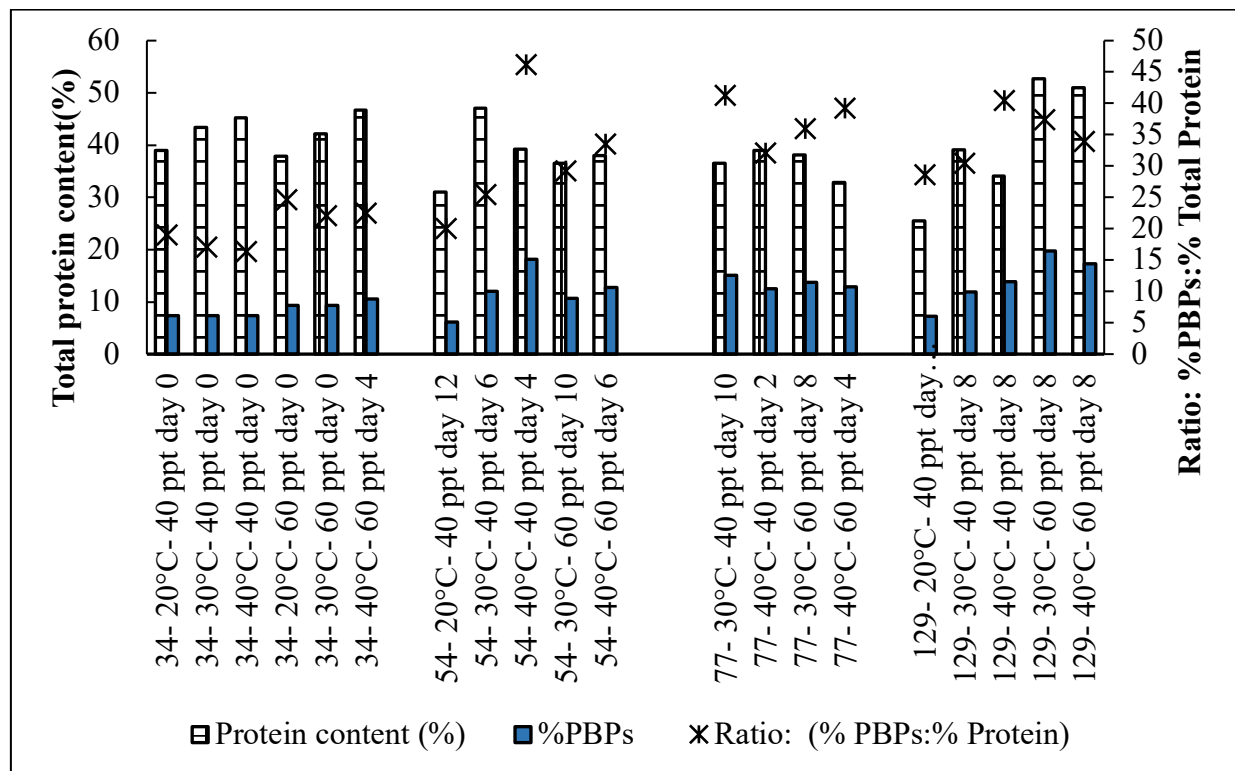


Figure 31 : Ratio of phycobiliproteins to total protein of the 4 investigated strains under the different cultivation condition. For each cultivation conditions the estimation was done for the day showing the highest PBPs expression.

As Different growth conditions are reported to lead to changes in pigment composition as a response to acclimatization [154], we further looked each type of PBPs Phycocyanin content  $\%_{PC} = [ \%_{C-PC} + \%_{APC} ]$ , phycoerythrin content  $\%_{PE}$ , during the different days of growth and how it was affected by temperature and salinity variations. PC in particular C-PC, was the predominant intracellular pigment across the strains accounting up to 88% of the total PBPs. Interestingly. This value was exceeding the normal ranges of PC which are reported to be between 60–75 % of the total PBPs [248]. The maximum production was  $160 \text{ mg}_{PC} \cdot \text{g}^{-1} \text{ d.w.}$ , for QUCCCM 54, followed by QUCCCM 129. This content did not largely differ from the content of the popular species for PC accumulation *A. platensis*, when it was subjected to similar growth conditions (up to  $184 \text{ mg}_{PC} \cdot \text{g}^{-1} \text{ d.w.}$ ) [249]. In contrast, PE synthesis was very low and didn't exceed 5% as a maximum for QUCCCM 34. This value was consistent with what was previously reported for the same species *Chroococciopsis* sp., isolated from similar environment (Desert Chile) [236].

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Different scenarios were observed concerning each PBP's synthesis based on cultivation conditions, and we will be herein discussing their variation with the details of the conditions contributing to their optimal expression separately for each strain.

Regarding PE content (Fig 32): it was observed that QUCCCM 34 had the highest % of 5.2 > QUCCCM 129 (3.4%) > QUCCCM 54 (1.7 %) than QUCCCM 77 (1.4 %)

- QUCCCM 34 had the highest PE content at day 0, followed by a drastic drop to almost reach zero at day 2 and remained low till the end of culture. This initial drop in PE suggests rapid utilization or degradation of Phycoerythrin, leading to a significant decrease in its presence within the culture with no regeneration or synthesis over time. Further investigations into the molecular mechanisms underlying PE expression and regulation are warranted to elucidate why its PE content remains consistently low despite changing environmental conditions. High salinity led to the highest PE expression.
- QUCCCM 54 achieved maximum % PE at 40 °C - 40 ppt at day 4 which did not decrease much and stayed high throughout the culture period of 12 days. Lower temperature led to lower PE content, while increased temperature enhanced the pigment expression. This increment was more pronounced at 40 ppt, while no major effect was detected at high salinity between 30 and 40 °C. We also observed that the peak of PE expression was at day 2 and similar for all the cultivation conditions.
- QUCCCM 77 showed its PE peak at day 2 for both 30°C and 40°C- 40 ppt salinity, followed by a drop during the rest of cultivation days. This suggests an early and rapid accumulation of PE within the culture, particularly at the specified temperature and salinity conditions. Higher salinity (60 ppt) significantly inhibited PE production. Despite not affecting the growth, biosynthesis of PE is sensitive to salinity variations.
- QUCCCM 129 had less than 1% PE at 40 ppt, which was almost similar under temperatures investigated and the cultivation days. This showed that under standard salinity conditions (40 ppt), PE production in QUCCCM129 is minimal and not affected by temperature variations. However, salinity increment led to

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an increase in the PE expression to reach 3- 4 % for 40 °C and 30 °C respectively, highlighting that controlled salinity adjustments could be employed to stimulate PE biosynthesis and maximize pigment content in the strain. we also observed that under all the cultivation conditions, the peak expression of PE was towards the end of cultivation.

Overall, the results indicate that temperature, salinity, cultivation days, and their interactions all have effects on PE expression of the indigenous screened strains. By understanding these factors and their interactions cultivation conditions could be optimized for maximizing PE expression.

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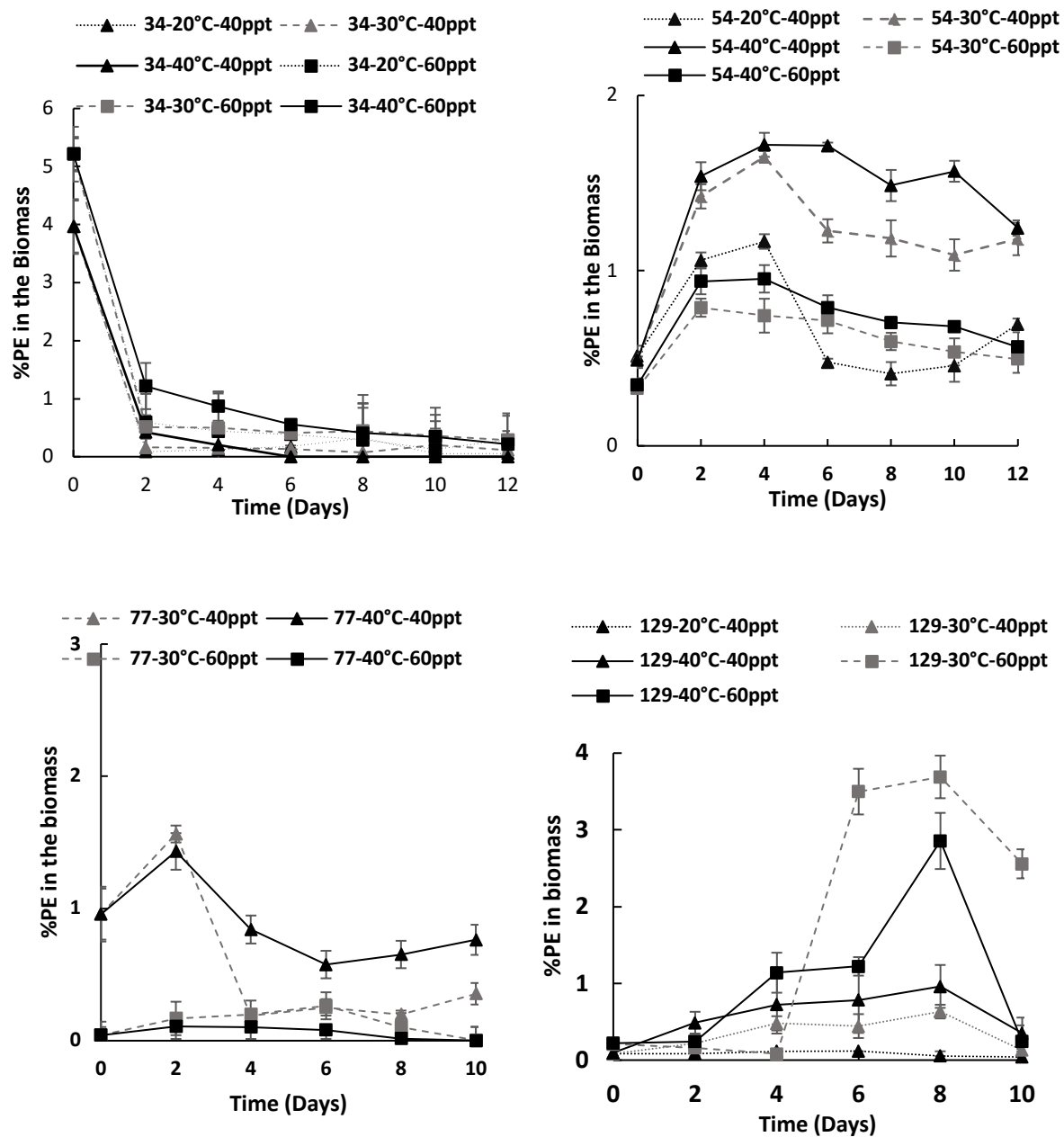


Figure 32: %PE in the crude extract of the 4 screened cyanobacteria strains. Values are means  $\pm$  standard errors (n = 2).

Concerning the PC expression, the results in Fig.33 showed either an increase in the (%) followed by a decrease or stable levels post-maximum expression, indicating the dynamic nature of PC production in response to changing environmental conditions. QUCCCM 54 had the highest % of 16.4 > QUCCCM 129 with 16% > QUCCCM 77 (14.7 %) than QUCCCM 34 (9.6 %). Depending on the strains, the peak of PC expression is usually within the exponential phase (day 4,6) under high temperatures

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of 40 °C, however, at 30 °C the maximum of synthesis is observed later, except QUCCCM 129 where the PC peaked at day 8 for all the investigated conditions.

- QUCCCM 34 had its maximum of PC of 9.6% at day 4 at 40°C- 60 ppt. These conditions likely promote increased enzymatic activity and metabolic processes involved in PC biosynthesis, leading to higher PC yields. Lower temperatures (20°C) resulted in lower peaks and sharper declines, in both salinities indicating less favorable conditions for sustained PC production. It was also observed a synergetic effect with increased temperature and salinity in stimulating the PC synthesis for most of the days.
- QUCCCM 54 expressed the highest PC of 16.4% at day 4 under 40 °C- 40 ppt cultivation conditions. At the optimum cultivation conditions, the PC was around 10% at day 4 and remained almost constant for the rest of the days. Lower temperatures (20°C) resulted in lower peaks and less sustained PC expression, as seen in the 54 -20°C-40ppt condition, however, increased temperatures enhanced PC synthesis. Increase in salinity for the same temperature hindered the pigments expression.
- QUCCCM 77 had the maximum of PC of 14.7 % at day 10 at 30 °C- 40 ppt aligning with the optimum cultivation conditions. We also observed negative correlation between temperature increase and PC content for most of the days, and no major effect of salinity was noted at similar cultivation temperature explaining the halotolerant nature of the strains. Temperature 40°C promoted an early peak in PC expression (day 2 for 40 ppt and day 4 for 60 ppt) but led to a decline over time for both salinities.
- QUCCCM 129 presented the maximum of PC expression of 16% at day 8 at 30 °C- 60 ppt which corresponded to the optimum cultivation condition. Moreover, no major effect of temperature increase was detected on the PC expression between day 6 and 8 for both salinities. However higher salinities had a positive effect on the synthesis of the pigment.

In summary, while the specific effects may vary slightly between strains, the overall trend indicates that both temperature and salinity have a significant impact on PC expression, with higher temperatures generally leading to higher expression levels.

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Additionally, the interaction between temperature and salinity plays a crucial role in modulating PC expression in each strain. Furthermore, it was detected that the maximum of PC production was witnessed at the cultivation conditions of higher temperature for QUCCCM 54 and 34, giving a competitive edge to the strains as PC producers over other commonly ones mainly for cultivation in hot regions, as well as in closed photo bioreactors where cooling is required to reduce the culture temperature. As the key issue in food industry colorant is pigments stability [236], PC extracted from these local strains would be more thermostable as compared to others isolated from strains with lower temperature resistance.

Salt-induced effects have been also investigated and studies showed that nature of PC changed in response to salinity stress [250]. A decrease in PC concentration was examined under salt stress, causing an interruption in the energy transfer between PBPs and photosystem II [133]. This was the case of QUCCCM 54. On the other hand, other research work found that salinity increment could be used as stimulant of PBPs content within the cell [251], which was the case for the rest of strains investigated in the current study, except for QUCCCM 77 where salinity increase was no much inhibiting the PC expression.



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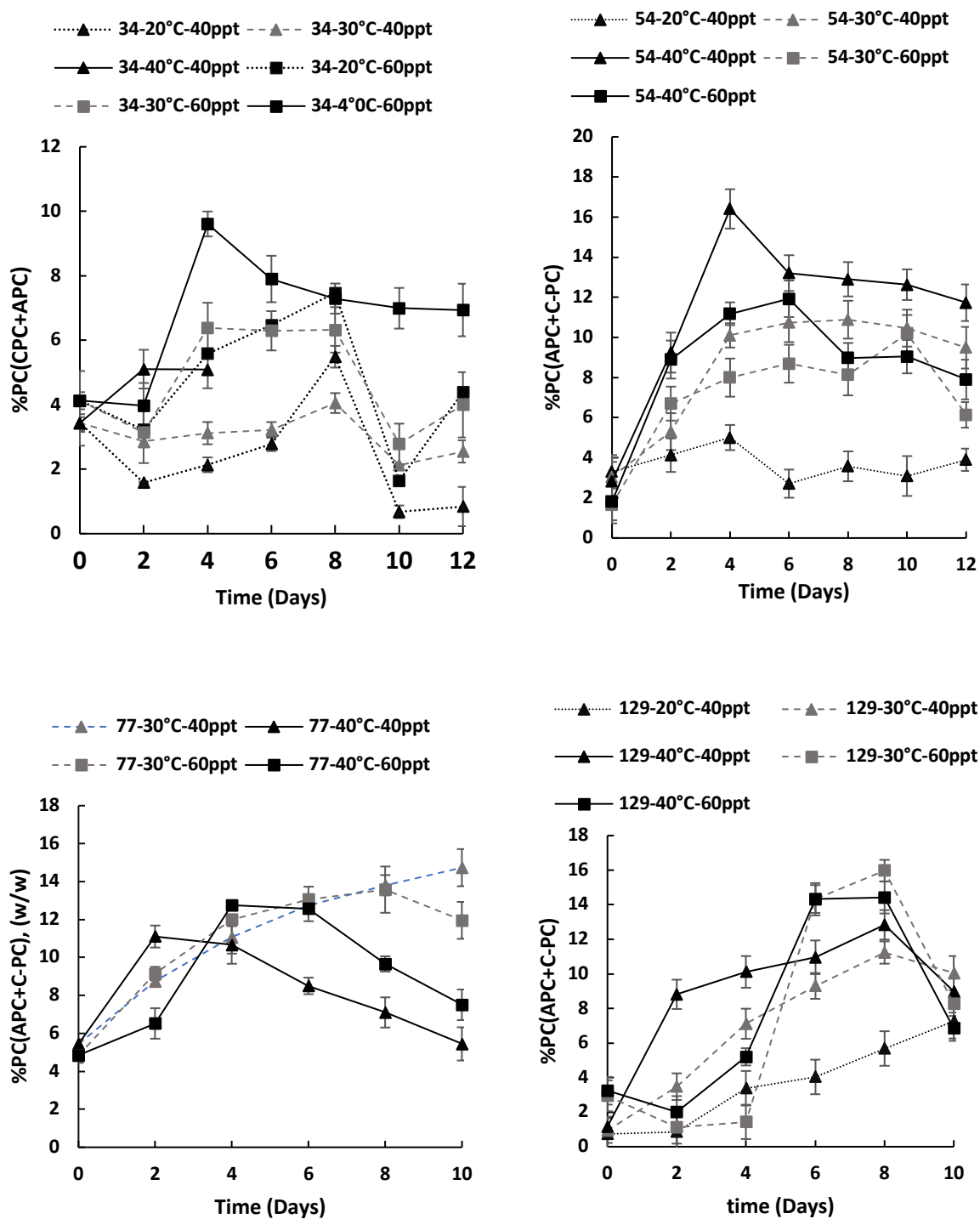


Figure 33: % PC in the crude extract of the 4 screened cyanobacteria strains. Values are means  $\pm$  standard errors ( $n = 2$ ).

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If we now compare the PC productivity, which is a combination of both phycocyanin content and biomass productivity, among the strains for the conditions and day showing the highest expression, we observed that QUCCCM 77 presented the lowest productivity of ~ 7mg/ L/ day < QUCCCM 34 with 8 mg/ L/ day< QUCCCM 54 with 17 mg/L/day< QUCCCM 129 with 18.8 mg/ L/ day (Fig.34).

Our analysis indicates that strains QUCCCM 54 and QUCCCM 129 are particularly promising for high PC production. Notably, QUCCCM 54 demonstrated significant productivity early in the growth stage across various conditions, suggesting that it is a robust strain well-suited for large-scale cultivation with the potential for year-round production. On the other hand, QUCCCM 129 also shows strong potential, particularly under conditions of 30°C and 60 ppt salinity. However, it should be noted that the highest productivity for QUCCCM 129 is achieved if we only consider the amount of PC produced later in the growth cycle. This delayed peak will have economic implications, as it requires more time and additional energy inputs to reach maximum production levels. Furthermore, if we consider the standard annual conditions predominant in Qatar, with a temperature of 30°C and salinity of 40 ppt, a comparison among the strains showed that QUCCCM 54 stands out with the highest productivity of 13.6 mg/L/day> QUCCCM 129 (10.4 mg/L/day)> QUCCCM 77 with 6.7 mg/L/day, and finally QUCCCM 34 with 4.6 mg/L/day. This highlights QUCCCM 54's exceptional suitability for high PC production in Qatar's environmental conditions.

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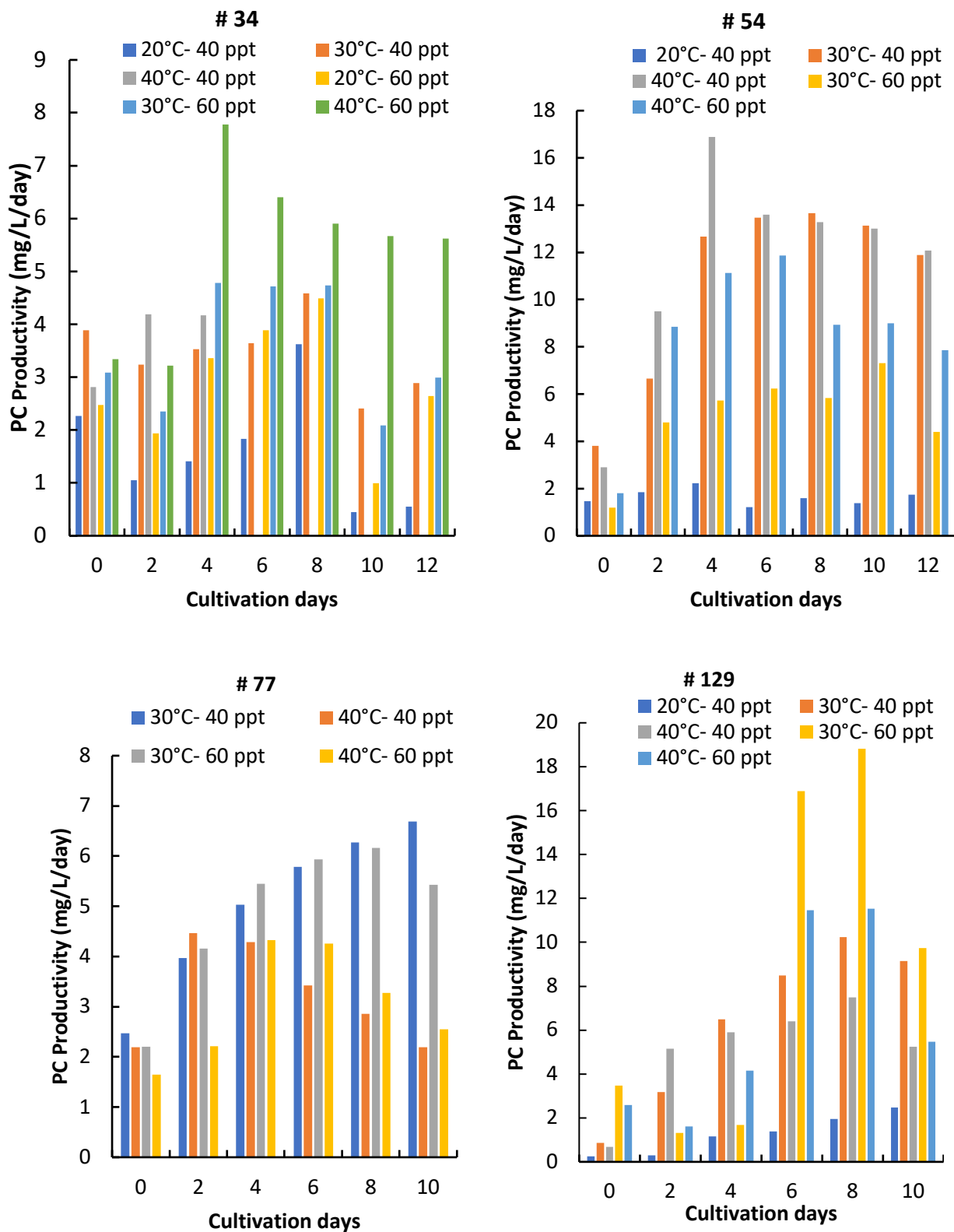


Figure 34: PC Productivity (mg/L/day) for the local isolate under the different investigation conditions

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The purity of the PBPs plays also a vital role in their commercial exploitation. In the present study, C-PC was the major pigment for most strains. Its purity index was in the range of 0.18–1.88, with QUCCCM 54 being the highly efficient in producing high-quality phycocyanin under all tested conditions. (Table. 11). This purity index was greater than the maximum reported by Khatoon et al., for *Spirulina* which was only 0.62 [252]. It is well-known that the purity ratio of PBPs plays a significant role in their commercial applications. Among the main PBPs, phycocyanin is the most valuable pigment used in pharmaceutical and food industry owing to their color, fluorescence and antioxidant properties [252]. Phycocyanin with purity index greater than 0.7 is considered as food grade, where at ratio of 3.9 it is regarded as reactive grade and beyond 4.0 it is counted as analytical grade [143]. Based on the obtained data, both C-PC QUCCCM 54 and 129 exhibited purity index values that meet the criteria for food-grade phycocyanin (greater than 0.7). This indicates that the crude extract from these strains is suitable for food applications based on its purity. In contrast, the purity index values for QUCCCM 34 and 77 fall below the threshold considered food grade (less than 0.7), and the belonging crude extracts does not meet the required purity standards for food applications. Further purification steps using precipitations or flocculation techniques can indeed help selectively separate specific phycobiliproteins, thereby increasing their overall purity. Moreover, refining the extraction protocols and production processes can help in meeting specific purity requirements for diverse applications in food, pharmaceuticals, and biotechnology.

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Table 11: Purity index of C-PC, APC extracted from biomass grown under different culture conditions evaluated as the ratio between absorbance from each PBPs and aromatic amino acids in all proteins at 280 nm. (Standard deviation below 2%)

	Purity index	
	CPC	APC
<b>QUCCCM 34- 20°C- 40 ppt</b>	0.18	0.27
<b>QUCCCM 34- 30°C- 40 ppt</b>	0.13	0.28
<b>QUCCCM 34- 40°C- 40 ppt</b>	0.18	0.2
<b>QUCCCM 34- 20°C- 60 ppt</b>	0.17	0.3
<b>QUCCCM 34- 30°C- 60 ppt</b>	0.14	0.28
<b>QUCCCM 34- 40°C- 60 ppt</b>	0.14	0.21
<b>QUCCCM 54- 20°C- 40 ppt</b>	<b>1.88</b>	0.44
<b>QUCCCM 54- 30°C- 40 ppt</b>	<b>1.89</b>	0.45
<b>QUCCCM 54- 40°C- 40 ppt</b>	<b>1.87</b>	0.46
<b>QUCCCM 54- 30°C- 60 ppt</b>	<b>1.87</b>	0.48
<b>QUCCCM 54- 40°C- 60 ppt</b>	<b>1.89</b>	0.49
<b>QUCCCM 77- 30°C- 40 ppt</b>	0.4	0.12
<b>QUCCCM 77- 40°C- 40 ppt</b>	0.42	0.18
<b>QUCCCM 77- 30°C- 60 ppt</b>	0.37	0.14
<b>QUCCCM 77- 40°C- 60 ppt</b>	0.39	0.17
<b>QUCCCM 129- 20°C- 40 ppt</b>	0.88	0.28
<b>QUCCCM 129- 30°C- 40 ppt</b>	0.91	0.3
<b>QUCCCM 129- 40°C- 40 ppt</b>	0.88	0.32
<b>QUCCCM 129- 30°C- 60 ppt</b>	0.9	0.2
<b>QUCCCM 129- 40°C- 60 ppt</b>	0.88	0.29

#### 4.3. Antioxidant ability of the pigment extracts

The demand for biobased antioxidants has surged across various industries in recent years. Phycobiliproteins extract from cyanobacteria have emerged as particularly promising sources of sustainable natural antioxidants [75]. In this sense, the antioxidant potential of the crude PBPS extract from the local investigated strains was evaluated. It is advised that the total antioxidant activity is evaluated by different methods as it can be manifested in a wide range of actions and different antioxidant mechanisms should be taken into account [253]. Thus, two antioxidant activity assays using Trolox equivalent antioxidant capacity (TEAC) determination TEAC and DPPH radical scavenging activity using were performed in this study to provide a

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comprehensive assessment of the antioxidant properties of the crude extract. Ideally, a high TEAC value should correspond to a high DPPH % inhibition, indicating strong antioxidant activity across both assays. For each strain and culture conditions, both assays were carried out for the day exhibiting the highest PBPs content. The results of the 2 different assays and their correlation with the PBPs content are given in Table 12.

As we can see from the results, the crude extracts from the local investigated strains displayed antioxidant capacity with the maximum of activity varying depending on both the cultivation conditions and the specific strain tested. The ranking of antioxidant activity estimated as Trolox activity across the strains was observed as follows: QUCCCM 54 > QUCCCM 34 > QUCCCM 129 > QUCCCM 77 at cultivation conditions of 40 °C - 40ppt, 40 °C - 60 ppt, 30 °C - 60ppt and 30 °C - 40 ppt, respectively.

Interestingly, within each strain, the conditions that led to the highest antioxidant activity consistently corresponded to those resulting in the maximum production of phycobiliproteins. This suggests a potential correlation between PBPs production and antioxidant activity, indicating their possible involvement in providing antioxidant effects and suggesting that the most abundant type of PBPs is probably influencing this antioxidant potential. The specific contributions of PC and PE vary between strains and conditions, with PC seems to have more contribution to antioxidant activity compared to PE. However, further purification of each PBPs and antioxidant studies of their individual pure forms are required to support this hypothesis and precisely determine the type of PBPs responsible of the observed antioxidant capacities. Moreover, it was observed that salinity increase enhanced the antioxidant activity of QUCCCM 34 and QUCCCM 129, while it was improved by temperature for QUCCCM 54. Hence optimizing cultivation parameters could potentially lead to extracts with higher antioxidant capacity, offering promising opportunities for the development of natural antioxidant products. For strain 77, salinity and temperature increase were found to have negative effect on its antioxidant ability.

In the case of Trolox assay, the reducing power of the extract, determined as Trolox equivalent antioxidant capacity (TEAC) was ranging between a minimum of 10 mM TEAC. g<sup>-1</sup> d.w and a maximum of 59 mM TEAC. g<sup>-1</sup> d.w. The most potent extract of PBPs was issued from QUCCCM 54 cultivated at 40 °C -40 ppt with 59 mM TEAC. g<sup>-1</sup>

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dw, followed by the extract from QUCCCM 34 cultivated at 40 °C- 60 ppt with 55 mM TEAC.  $\text{g}^{-1}$  dw. Previous research work stated that samples with TEAC activity more than 10  $\mu\text{mol}$  Trolox equivalents.  $\text{g}^{-1}$  d.w (eq.  $\text{g}^{-1}$  d.w) are considered important in antioxidant content [254]. Consequently, all the PBPs extracts investigated during this study are promising in their antioxidant capacity as their TEAC values exceeded this threshold, with strain and cultivation conditions being an important factor for maximal antioxidant ability. Furthermore, the observed TEAC values exceeded those reported in various common sources of antioxidants, which are widely used in cooking, cosmetics and several therapeutic preparations, such as turmeric with a TEAC value of 13.5  $\mu\text{mol}$  Trolox equivalent/g extract [255] and kokam (*Garcinia indica*) with a TEAC value of 29.3 nmol of Trolox equivalent/g fresh weight [256].

The results of DPPH assay were in correlation with the data of the Trolox assay as it followed the same trend observed with regards to cultivation conditions effect. Accordingly, the highest scavenging percentage 49 % was also found to be maximal for QUCCCM 54 and the lowest was 9 % for QUCCCM 77, reinforcing the highest potential of our local isolates, QUCCCM 54, as candidates for high antioxidant content observed using the Trolox assay. Moreover, for most strains and conditions, there is a positive correlation between TEAC values and DPPH % inhibition with higher TEAC values corresponding to higher DPPH % inhibition, indicating strong antioxidant. For example, for QUCCCM 34 at 40°C - 60 ppt, both TEAC (55 mM) and DPPH % inhibition (33%) are high. In some cases, this correlation is not perfect. For instance, the DPPH scavenging activity of crude extract from QUCCCM 129-30°C- 60 ppt was slightly higher than the one of QUCCCM 34- 40°C- 60 ppt with 40% compared to 33%, respectively while the Trolox data were similar. This discrepancy may arise from differences in the mechanisms underlying the two assays or variations in the specific antioxidants present in the extracts that interact differently with the DPPH radicals. In the DPPH assay, the antioxidant effect was likely to be due to the hydrogen donating ability of the extract, while the ABTS assay is a measure of the activity of the antioxidant in scavenging proton radicals through donation of electrons [257].

Earlier studies reported that phycocyanin from *Spirulina.sp*, one of the best producers for phycocyanin, is only displaying 25 % DPPH scavenging activity [258, 259]. In our study the DPPH scavenging ability of PBPs extracts from QUCCCM 34 at 40 °C- 60

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ppt, QUCCCM 54 at most conditions and QUCCCM 129 at 30 °C- 60 ppt and 40°C- 60 ppt, presented higher DPPH scavenging ability qualifying these extracts as potential candidates for antioxidant applications. Another study which investigating the pure PC extracts of *Spirulina platensis* reported 40 % of DPPH radical scavenging activity of C-PC at the concentration of 1.25 mg/ mL [260]. In our current work, the crude extracts were tested for their antioxidant activity at a concentration of 1 mg/ mL without purification and displayed higher DPPH scavenging ability of 49 % for QUCCCM 54- 40 °C- 40ppt. This significant antioxidant activity of the crude extract underlines the potential for further development and application.

The obtained data highlights the remarkable potential of our local isolates, particularly QUCCCM 54, as candidates for high antioxidant content. This opens up exciting possibilities for various applications in biotechnology and beyond. The antioxidant properties of our isolates position them as potential candidates for treating diseases caused by the excess of reactive oxygen species (ROS), which are implicated in various health disorders. For instance, PC with high antioxidant capacity is able to inhibit cell proliferation of some cancers such as human leukemia K562 cells [261]. Additionally, the biotechnological applications of these isolates are vast, ranging from health sectors to industries such as food, cosmetics, and pharmaceuticals.



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Table 12: Trolox activity and DPPH inhibition (%) of the pigment extracts from the 4 investigated cyanobacteria strains under different culture conditions. Values of PC and PE at the highest PBPs expression for each culture condition are also mentioned.

Strains	mM TEAC. g <sup>-1</sup> d. w	DPPH % inhibition	PC (mg. g <sup>-1</sup> d.w)	PE (mg. g <sup>-1</sup> d.w)
QUCCCM 34- 20°C - 40 ppt	40	11	34	40
QUCCCM 34- 30°C - 40 ppt	43	14	34	40
QUCCCM 34- 40°C- 40 ppt	38	17	34	40
QUCCCM 34- 20°C- 60 ppt	48	16	41	52
QUCCCM 34- 30°C- 60 ppt	50	25	41	52
QUCCCM 34- 40°C- 60 ppt	55	33	96	9
QUCCCM 54- 20°C- 40 ppt	41	18	50	12
QUCCCM 54- 30°C- 40 ppt	43	35	109	12
QUCCCM 54- 40°C- 40 ppt	59	49	164	17
QUCCCM 54- 30°C- 60 ppt	22	23	102	5
QUCCCM 54- 40°C- 60 ppt	36	31	119	8
QUCCCM 77- 30°C- 40 ppt	17	17	147	4
QUCCCM 77- 40°C- 40 ppt	13	14	111	14
QUCCCM 77- 30°C- 60 ppt	10	13	136	1
QUCCCM 77- 40°C- 60 ppt	10	9	128	1
QUCCCM 129- 20°C- 40 ppt	10	10	73	0
QUCCCM 129- 30°C- 40 ppt	11	20	112	6
QUCCCM 129- 40°C- 40 ppt	13	17	128	10
QUCCCM 129- 30°C- 60 ppt	38	40	160	18
QUCCCM 129- 40°C- 60 ppt	36	30	144	29

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#### **Conclusion**

In this chapter, four indigenous cyanobacteria strains were screened indoor in 1L photobioreactors (PBR) with a primary focus on their growth and PBPs production under combined effects of salinity and temperature in conditions close to the real outdoor conditions in Qatar.

The strains were first identified based on their 16s rDNA sequence analysis and biochemical composition and the results showed higher similarities with *Chroococciopsis* (QUCCCM 34), *Pleurocapsa* (QUCCCM 54), *Euhalothece* (QUCCCM 77) and *Cyanobacterium* (QUCCCM 129).

The investigation of the growth and PBPs production at various water temperatures (up to 40 °C) and salinities (up to 60 ppt) allowed assessing their suitability as viable candidates for yearlong production under deserted outdoor cultivation conditions. Indeed, the study underscores their resilience to temperature and salinity variations, highlighting their thermo and halo tolerance. Additionally, it emphasizes their ability to capture carbon associated with simultaneous PBPs expression, which is crucial for environmental sustainability. The findings also reveal that PBPs synthesis was affected by salinity, temperature in addition to the culture age, with PC being the most dominant PBPs. Thus, close adjustment of environmental abiotic stress and the harvesting time is required to optimize the pigment production.

Furthermore, bioprospecting of antioxidant potential for the crude PBPs extracts using DPPH and Trolox assays reported remarkable antioxidant properties, with strain and cultivation conditions being an important factor for maximal antioxidant ability. Interestingly, the observed values exceeded those reported in various common sources of antioxidants, which are widely used in cooking, cosmetics and several therapeutic preparations. These results may qualify the strains as potential candidates for treating several diseases caused by the excess of Reactive oxygen species (ROS). It also opens doors for several biotechnological uses and may be directly or indirectly used for industrial production such as natural colorant, in cosmetics, pharmaceutical and health food industries.

The optimum conditions leading to highest PC expression, and antioxidant ability data gathered in Table 13, lead us to the selection of strain *Pleurocapsa* sp., QUCCCM 54,

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for the outdoor cultivation study presented in the next chapter. In addition to its increased biomass productivity and CO<sub>2</sub> capture, it produces high-quality phycobiliproteins, particularly phycocyanin (PC), which is a highly valuable product due to its antioxidant properties and commercial applications in food, cosmetics, and pharmaceuticals. Remarkably, the PC production was observed to be at an early growth stage, which is advantageous for maximizing output over shorter cultivation periods. Another critical advantage observed during the study was the natural self-settling ability of QUCCCM 54. This characteristic simplifies the harvesting process on a large scale, reducing the need for additional mechanical or chemical separation methods. The ease of harvesting enhances the strain's overall economic viability for industrial applications. Taking all this into account, QUCCCM 54 stands out as promising strain for sustainable and efficient PC- rich biomass production.

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Table 13: Summary of the conditions leading to the highest PC expression for the 4 shortlisted strains along with the corresponding purity and observed antioxidant activity of the extract.

Strain	Conditions leading to the highest PC expression	Time of the highest PC production	PC yield (mg. g <sup>-1</sup> dw)	PC productivity (mg. L <sup>-1</sup> . day <sup>-1</sup> )	CPC purity	APC purity	mM TEAC. g <sup>-1</sup> dw	% DPPH inhibition
QUCCCM 34	40 °C – 60 ppt	Day 4	96	8	0.14	0.21	55	33
QUCCCM 54	40 °C – 40 ppt	Day 4	<b>164</b>	<b>17</b>	<b>1.87</b>	<b>0.46</b>	<b>59</b>	<b>49</b>
QUCCCM 77	30 °C - 40 ppt	Day 10	147	7	0.40	0.12	17	17
QUCCCM 129	30 °C – 60 ppt	Day 8	159	18.8	0.9	0.2	38	40

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## **Chapter 4: Towards outdoor cultivation of the selected local isolate *Pleurocapsa* Sp.**

### **Introduction**

This chapter explores the feasibility of large-scale cultivation under real outdoor environmental conditions, by evaluating the growth and PBP production of the strain QUCCCM 54. This marine strain selected from the results of chapter 3 belongs to the *Pleurocapsa* group of cyanobacteria, which represent a phylogenetically well-framed group, but are still weakly investigated on several levels [262]. Marine *Pleurocapsa* species are known for their ability to tolerate high levels of salinity and desiccation, making them well-suited to living in intertidal zones where they are frequently exposed to fluctuating conditions [263]. Beyond their ecological roles, the species has drawn attention as PBPs producers which are essential for their photosynthetic efficiency and play an important role in their ecological adaptations highlighting their potential for various biotechnological applications [264]

The results of chapter 3 showed the resilience of the strain QUCCCM 54 to high temperatures and salinities while producing high-quality PBPs, particularly phycocyanin one of the most commercially valuable cyanobacteria pigments. However, transition from indoor to outdoor cultivation for algae culture marks a significant shift in the growth and production process. While indoor cultivation occurs in controlled conditions allowing optimal growth, outdoor systems face limitations due to the variable environmental conditions, nutrient management challenges, contamination risks, and physical disturbances impacting algae growth and productivity. With optimized cultivation practices, and cost-effective solutions for both upstream and downstream processing, it is possible to achieve sustainable large-scale biomass production to meet the growing demand for phycocyanin and other valuable cyanobacterial products.

Nitrogen is an essential macronutrient which plays a vital role in the growth and PC production. One of the concerns regarding large scale cultivation and PBPs is the high requirement of nitrogen which increases production costs [74]. To address this, the study explores the potential of using urea, a low-cost nitrogen source, as an alternative to sodium nitrate in the culture media. Qatar has one of the largest urea manufacturing plants named Qatar Fertilizer Company (QAFCO), which annually generates 14-15 tons of urea waste disposed of in landfills due to its differences in morphology, form, and color from commercially desired products. This nitrogen waste presents an

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opportunity for use in algae cultivation, potentially reducing production costs and environmental impact.

Within this framework, the initial part of this study evaluates the feasibility of using urea for cultivating *Pleurocapsa* sp. QUCCCM 54, prior to implementing it in mass production. Following this, the upscaling of the selected QUCCCM 54 under outdoor conditions was examined. The study was performed in 200 L open raceway ponds using F/2 media with urea as nitrogen source. Two different cultivation media were tested: one rich amended with 10X nitrogen concentration, and one with low nitrogen concentration (2X nitrogen) as potential solution to reduce the nitrogen input without inhibiting the PBPs expression. The study further explored how varying urea concentrations and environmental factors affected growth, PBPs production, and biomass composition.

### **Results and discussion**

#### **1. Urea assimilation: Impact on growth and PBPs expression**

As shown in Fig. 35, the growth was promoted in the medium with urea compared to the control 10X F/2 with the commonly used nitrogen source, sodium nitrate. Indeed, for an equivalent nitrogen concentration, the biomass productivity was 224 mg/ L/ Day for urea vs 125 mg/L/day for sodium nitrate, with an enhanced biomass productivity around 1.7 -fold higher than that obtained in photoautotrophic control cultures. Previous work showed that urea can freely diffuse through the phospholipidic bilayer of cyanobacteria [265-267]. It is then broken down by an intracellular urease, which hydrolyzes urea to form ammonium ( $\text{NH}_4^+$ ) the reduced form of nitrogen and bicarbonate ( $\text{HCO}_3^-$ ). Cells incorporate ammonia directly into the nitrogen [268, 269], whereas nitrate, unlike urea, needs to cross two cellular membranes before being completely reduced to  $\text{NH}_4^+$  in chloroplasts for assimilation [270]. Due to low energetic demands for  $\text{NH}_4^+$  utilization, It is considered the preferred N form among cyanobacteria [268]. Researchers have found that *Spirulina platensis* utilizes urea better than nitrate in fed-batch and batch cultures [116]. Further research was conducted to determine whether such a consumption preference existed. This was accomplished by simultaneously adding nitrate and urea and tracking their



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consumption over time. The results showed that when urea and nitrate were combined, urea was consumed first as the sole nitrogen source, while nitrate started to be consumed only later in the exponential growth phase [111, 271]. All these findings support the use of urea for improved nitrogen assimilation and uptake. It was inferred that the conversion efficacy of nitrogen sources into algal biomass is as follows: nitrate < ammonium < urea [272], which supported the results of our present study as urea lowers the energetic constraints of N acquisition and can offer the benefit of additional nutrient building blocks (urea supplying both N and C to the cell).

While the current study emphasized on the use of urea as the preferred nitrogen source by the local isolate *Pleurocapsa* sp., the results also demonstrated the ability of QUCCCM 54 to grow in mixotrophy by processing the carbon obtained after urea hydrolysis leading to an increase in the percentage of total organic carbon in the urea-based culture (Fig.35). Some microalgae strains are able to combine autotrophic photosynthesis with mixotrophic assimilation of the released CO<sub>2</sub> from HCO<sub>3</sub><sup>-</sup>, or directly utilize HCO<sub>3</sub><sup>-</sup>, after urea hydrolysis [117, 273], hence growth on urea could support up to 7.6% of carbon cell requirements, assuming a "Redfield ratio" of C:N of 7:1 as a conserved quota for cyanobacteria cells [274]. This may be cost effective for cells in carbon-limited environments, particularly when light energy is a limiting factor, such as in dense culture where cellular self-shading reduces light availability. Experiments with the filamentous cyanobacteria *A. platensis* showed that the biomass and photosynthetic pigment produced increased by 1.5 - 2 fold when growing in mixotrophy [275, 276]. Chojnacka and Noworyta [277] compared *Spirulina* sp. growth in photoautotrophic, heterotrophic and mixotrophic cultures. They found that mixotrophic cultures reduced photo-inhibition and improved growth rates over both autotrophic and heterotrophic cultures. It was also reported by previous studies that some microalgae as *Haematococcus* sp., *Chlorella* sp., *H. pluvialis*, *M. pusillum*, *Anacystis nidulans* and *A. variabilis* could also utilize sodium acetate, a low-cost chemical byproduct from industrial production, as organic carbon to improve their growths [278-281]. Moreover, earlier experiments have also identified greater cell numbers in response to reduced nitrogen forms such as urea which upon hydrolysis in water produces ammonium, compared to oxidized inputs like nitrate (NO<sub>3</sub><sup>-</sup>) and nitrite (NO<sub>2</sub><sup>-</sup>) [282, 283].

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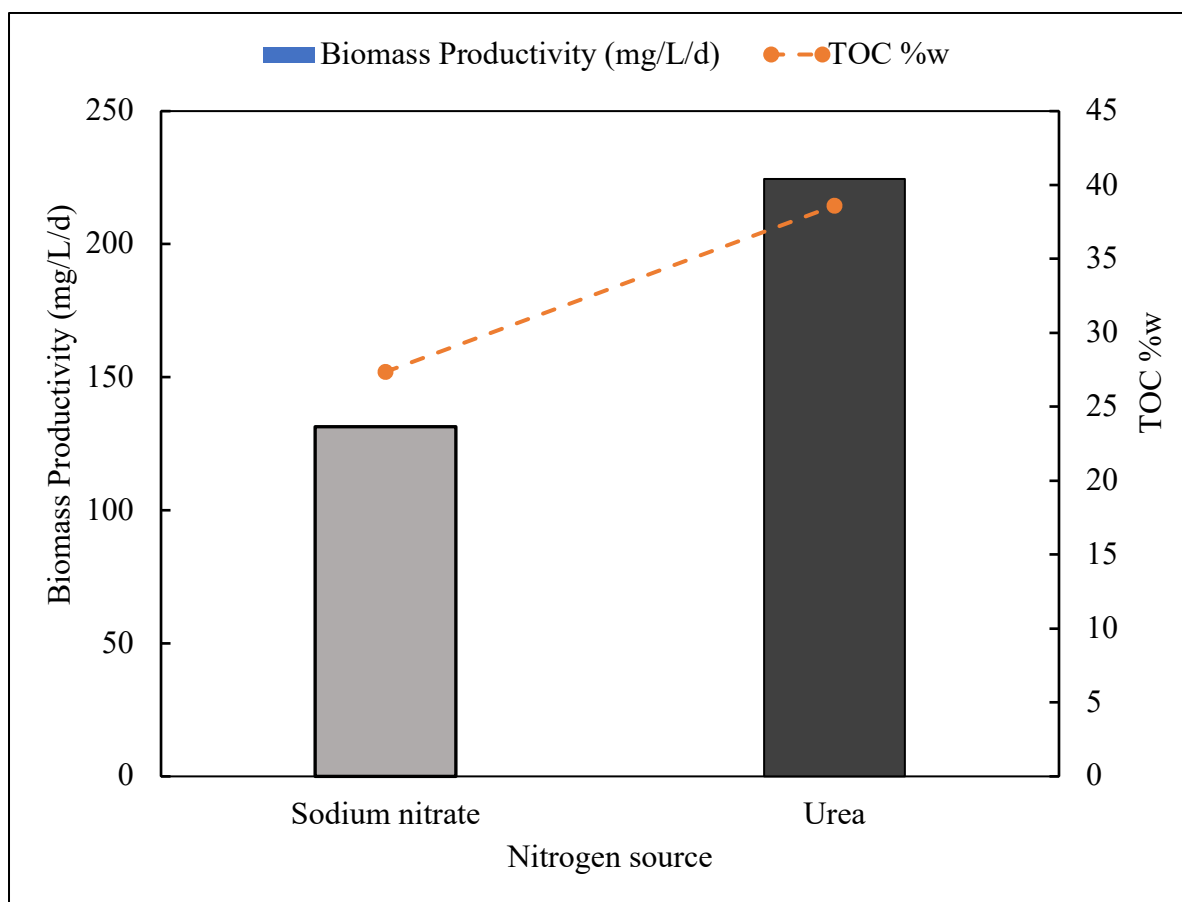


Figure 35: Biomass productivity and TOC % of *Pleurocapsa* sp. under 2 different nitrogen sources.

We further evaluated the effects of using urea as nitrogen source on PBPs accumulation (Fig.36). The results aligned with what was observed for the growth and showed that the nitrogen source also impacted the pigment synthesis with an increase in the PBPs yield of 1.5-fold at the end of the cultivation period. Indeed, *Pleurocapsa* sp., cultured in F/2 medium with urea exhibited higher PBP content of  $15.3 \pm 0.14\%$  with CPC ( $10.2 \% \pm 0.15$ ), APC ( $3.7\% \pm 0.16$ ), and PE ( $1.3 \% \pm 0.21$ ) than other culture with sodium nitrate as nitrogen source which produced only  $10 \pm 0.09\%$  of PBPs (CPC:  $6.2\% \pm 0.19$ ; APC:  $2.7\% \pm 0.26$  APC and PE:  $1\% \pm 0.19$ ). It was also noticed that the increase varied depending on the type of pigments with PC being the main PBPs affected by the change in the nitrogen source. Previous studies also reported that the use of urea enhanced the phycocyanin content in *Spirulina platensis* [116]. Ajayan et al. [66] found similar findings, where the use of urea improved the PC values of *A. platensis* from  $9.92 \pm 0.13$  to  $14.81 \pm 0.2 \%$ . These findings are consistent with previous

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research on how urea as organic nitrogen form is easily assimilated by cyanobacteria as opposed to other sources, and enhanced the production of N-rich pigments due to its spontaneous hydrolysis to ammonia [284].

The physiological capacity of cyanobacteria to incorporate urea into pigment synthesis may offer a competitive edge by enhancing light absorption capabilities and N storage. Furthermore, these nitrogen stores are potentially for sustaining cyanobacterial growth. In addition to providing nitrogen and carbon, urea has also been shown to enhance the growth under salt stress conditions by acting as an osmo-protectant [285]. Based on the outcomes of this study, urea will be later adopted for both biomass production at large-scale.

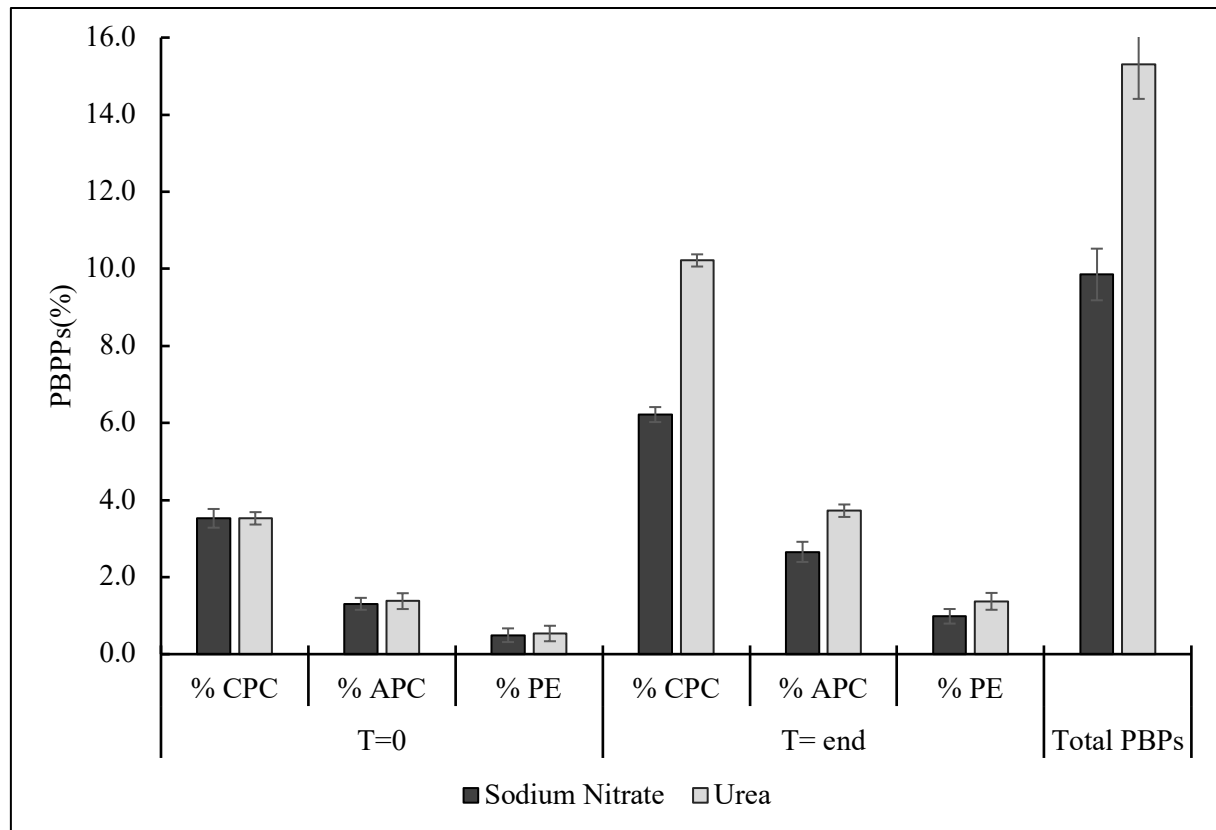


Figure 36: PBPPs (%) in the biomass cultivated under 2 different nitrogen sources after 12 days of cultivation.

### 2. Outdoor cultivation under different urea concentrations

#### 2.1. Influence of climatic conditions and media composition on growth, PBPPs

The outdoor trials were initiated during the mid of April 2021. First trial was unsuccessful, and the culture died after 3 days. Inoculation was performed in the mid

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of the day, and cultures were initially scaled up using 10 % inoculum volumes (vol/vol). During this period, the collected data of light intensities showed peaks up to 2500  $\mu\text{mol photons/m}^2/\text{s}$ , and a peak average of 2325.5  $\mu\text{mol photons/m}^2/\text{s}$  (Fig 37). OD<sub>750nm</sub> increased during the first 48 h. Subsequently, reduction in the readings was observed, and visual observations showed changes in culture color from green to yellow. due to chlorophyll degradation, followed by a culture crash after 72 h of inoculation (Fig. 37 and Fig.38).

In chapter 3, *Pleurocapsa* sp. QUCCCM 54 was investigated indoor in 1L PBRs. The strain demonstrated ability to grow and produce PBPs under different temperature and salinity ranges. The light intensity was set to of 240-300  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . It was clear from the obtained data that the transition from low-light/high-density inoculum indoor culture settings to high-light/low-density outdoor culture conditions caused the collapse of outdoor cultivation. Therefore, it is hypothesized that in addition to the strain's ability to grow under high irradiance levels, the light: biomass ratio at the moment of inoculation is also crucial for outdoor scale-up to avoid photooxidation/photoinhibition.

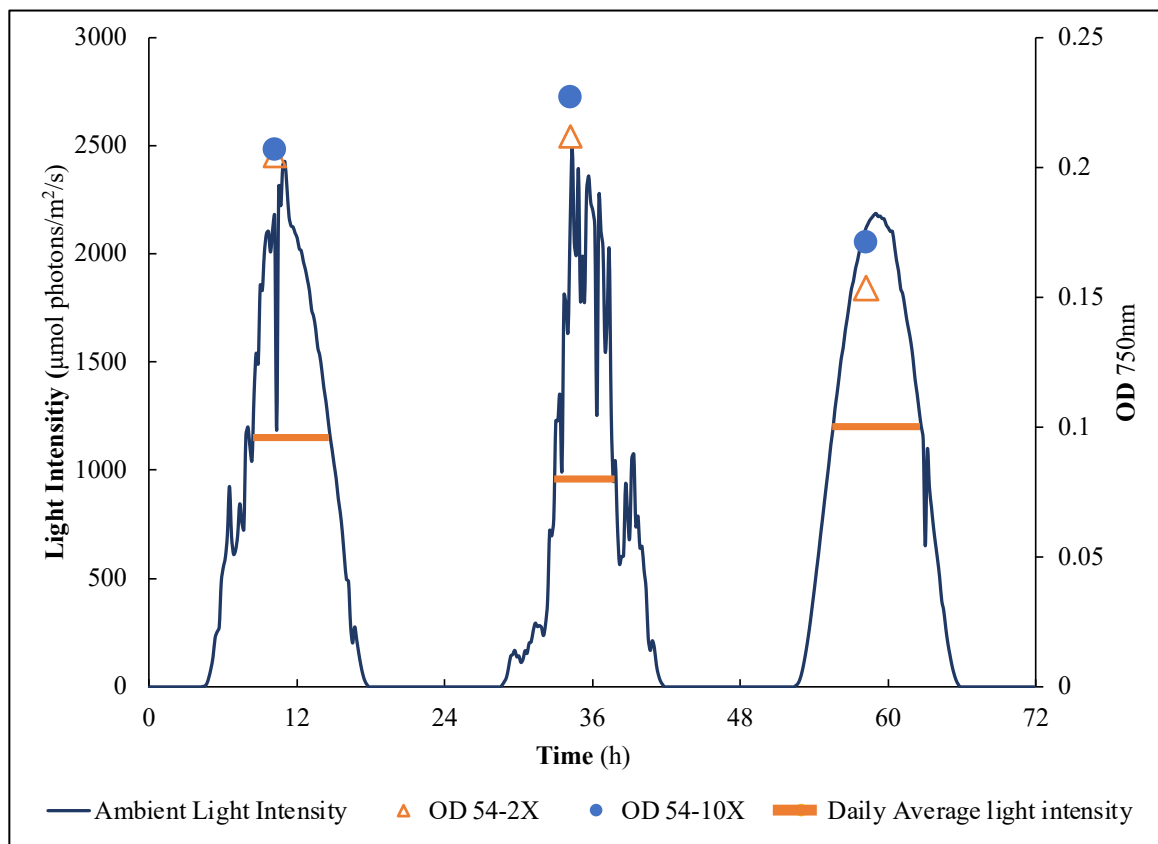


Figure 37: PAR light intensity ( $\mu\text{mol photon/m}^2/\text{s}$ ) and Culture turbidity (OD750) for the first outdoor growth trial of *Pleurocapsa* sp. QUCCCM 54 inoculated at 10 % (vol/vol) inoculum volumes.

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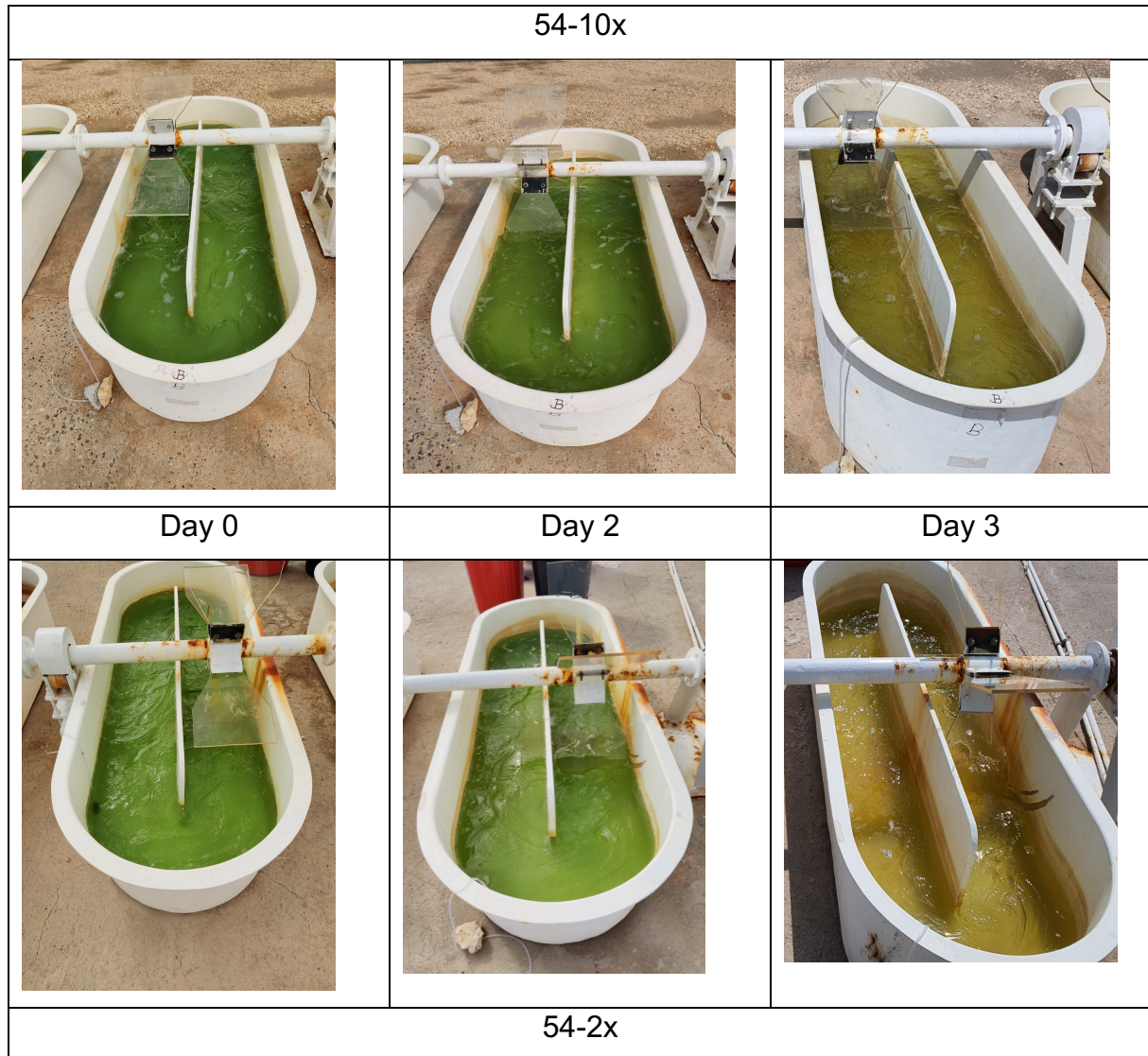


Figure 38: Outdoor first growth trials of *Pleurocapsa* sp, inoculated at 10% (vol/vol) inoculum volumes in 200 L raceway tanks.

The experiment was further repeated with higher culture inoculum of 20 % (vol/vol) as strategy to avoid culture crash. Moreover, to prevent direct exposure of cells to high light intensities at the time of inoculation, the scale-up was done at 4 pm, two hours before sunset. Partial shading using a net was also applied during the period of cultivation to reduce the received irradiance and protect the culture to some extent from photooxidative effects. Cultivation occurred between April 26<sup>th</sup>- May 4<sup>th</sup>, 2021. Throughout this period, the highest measured light intensity peak was 3425  $\mu\text{mol}/\text{m}^2/\text{s}$  on day 2, with a maximum daily average of 779  $\mu\text{mol}/\text{m}^2/\text{s}$ , as indicated in Table 14.

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The average high and low culture temperature was 30 °C and 21 °C and was 6 -10 °C lower than the maximum ambient temperature depending on the days (Fig. 39).

Table 14: Peak light intensities and their daily averages during the second trial outdoor culture in open ponds during cultivation period.

Day	Light intensity ( $\mu\text{mol}/\text{m}^2/\text{s}$ )		Day/ light duration
	Peak	Average	Duration (hours)
1	1901	622	13,3
2	2948	779	13,4
3	3425	586	13,4
4	1318	464	13,4
5	1553	530	13,5
6	1662	517	13,5
7	996	346	13,5
8	1029	161	13,5

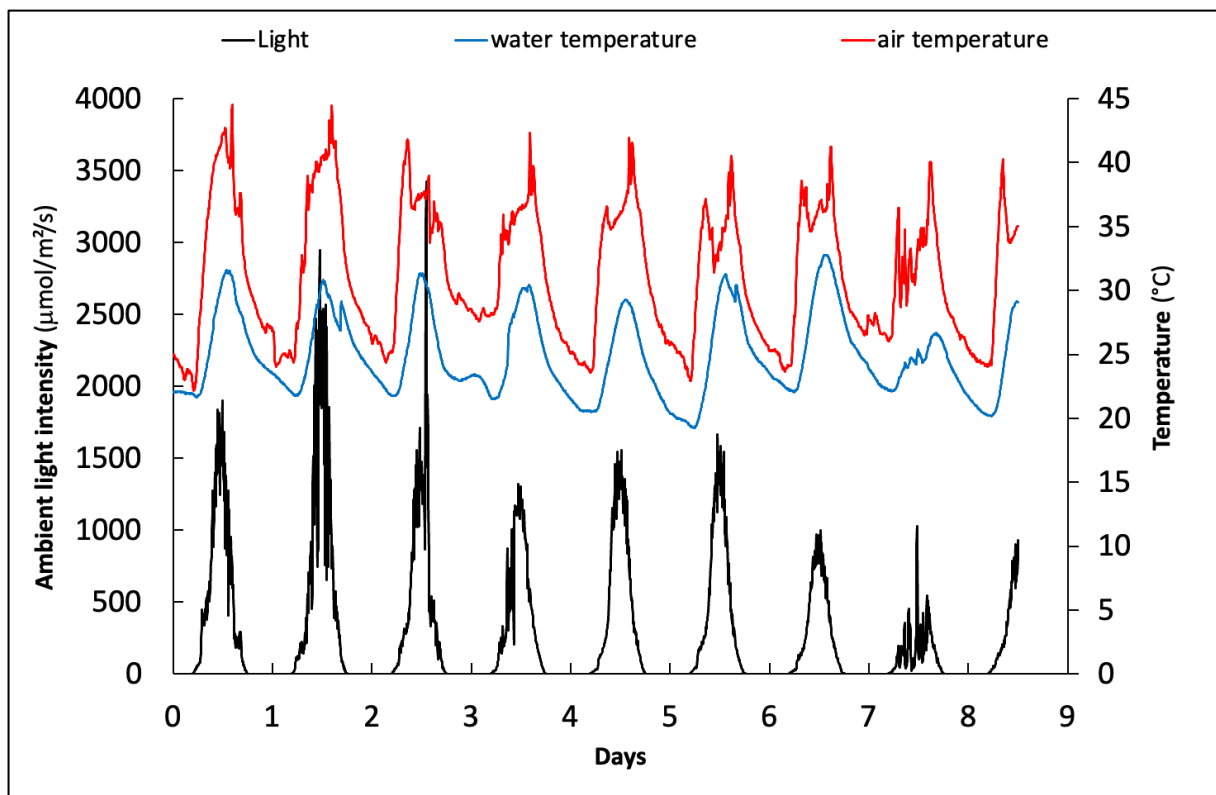


Figure 39: Outdoor data of light intensity, water and air temperature for the *Pleurocapsa* outdoor culture in open ponds during the cultivation period.

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We also observed that the cultures remained homogenous and well-mixed throughout the experiment time despite the strains' tendency to settle, indicating that the raceways had good agitation while they were in use, allowing a uniform distribution of the light, temperature, and nutrients throughout the pond (Fig. 40a and 40b). It is also worth to note that microscopic observations were performed each alternative day and showed that the culture remained monoculture and free of contamination until the end of experiment.

The second large-scale trial was successful and demonstrated the strain ability to survive in the outdoor conditions as shown in Fig. 40 suggesting that the culture crash outdoor in the first trial was more likely due to the suboptimal inoculum volumes vs the high light intensities observed, and the use of higher inoculum is proved to be beneficial.

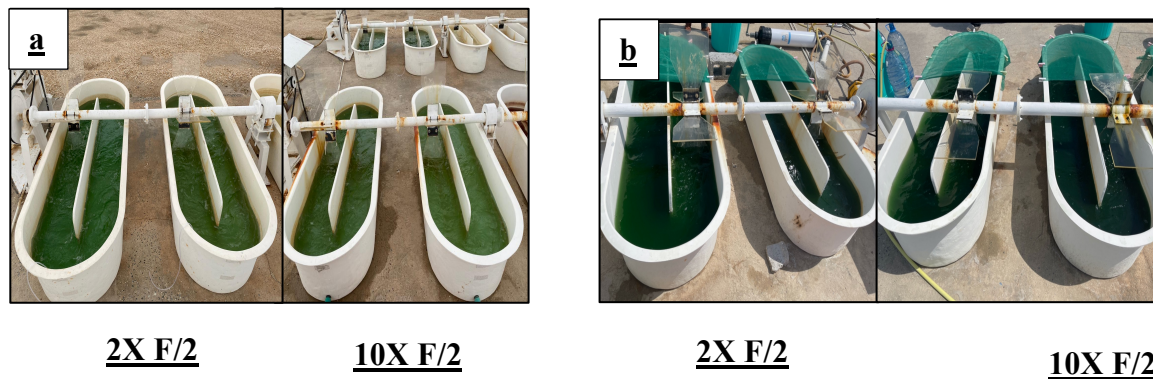


Figure 40: Outdoor growth of *Pleurocapsa*, QUCCCM 54 inoculated at 20% (vol/vol) inoculum volumes in 200L open raceway ponds at the time of inoculation (a), and at the end of culture (b) in both nitrogen concentrations.

Initially, the light intensities were extremely high during the 3 days post inoculation with 1901, 2948, and 3425  $\mu\text{mol}/\text{m}^2/\text{s}$ , however the daily averages were of 622, 779 and 586  $\mu\text{mol}/\text{m}^2/\text{s}$ , which was lower than what was recorded during the previous trial (daily average of 1000  $\mu\text{mol}/\text{m}^2/\text{s}$  as minimum). Over this period, the biomass concentrations were quite low under both cultivation conditions and no increase was seen post inoculation probably due to a cellular acclimation of the cells (Fig.41). The PBPs production also supported these findings, with the first three days only showing a low average daily increase of 15.5  $\text{mg}_{\text{PBPs}}/\text{g}_X$  for 2X F/2 and 16.5  $\text{mg}_{\text{PBPs}}/\text{g}_X$  for 10X F/2 (Fig.43). It was until the fourth day of cultivation that the biomass concentration started to rise, reaching a peak of 0.819 g/L for 10X F/2 and 0.850 g/L for 2X F/2. This was also linked to an increase in the concentrations of PBPs which is shown to occur in the following days. Notably, for both cultivation conditions, the PBPs content raised by 3% from day 4 to day 6 to reach a maximum of 113.1  $\text{mg}_{\text{PBPs}}/\text{g}_X$  on day 6 for biomass

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cultivated in 10X F/2, and 119.6 mg<sub>PBP<sub>s</sub></sub>/g<sub>X</sub> on 8<sup>th</sup> day for biomass grown in 2X F/2 media. We also observed that both culture biomass remained predominantly characterized by the high expression of phycocyanin with a yield of 110.8 mg/g and 106 mg/g and a corresponding productivity of 7 mg/L/day and 7.3 mg/L/day for biomass of 54 2X and 10X, respectively.

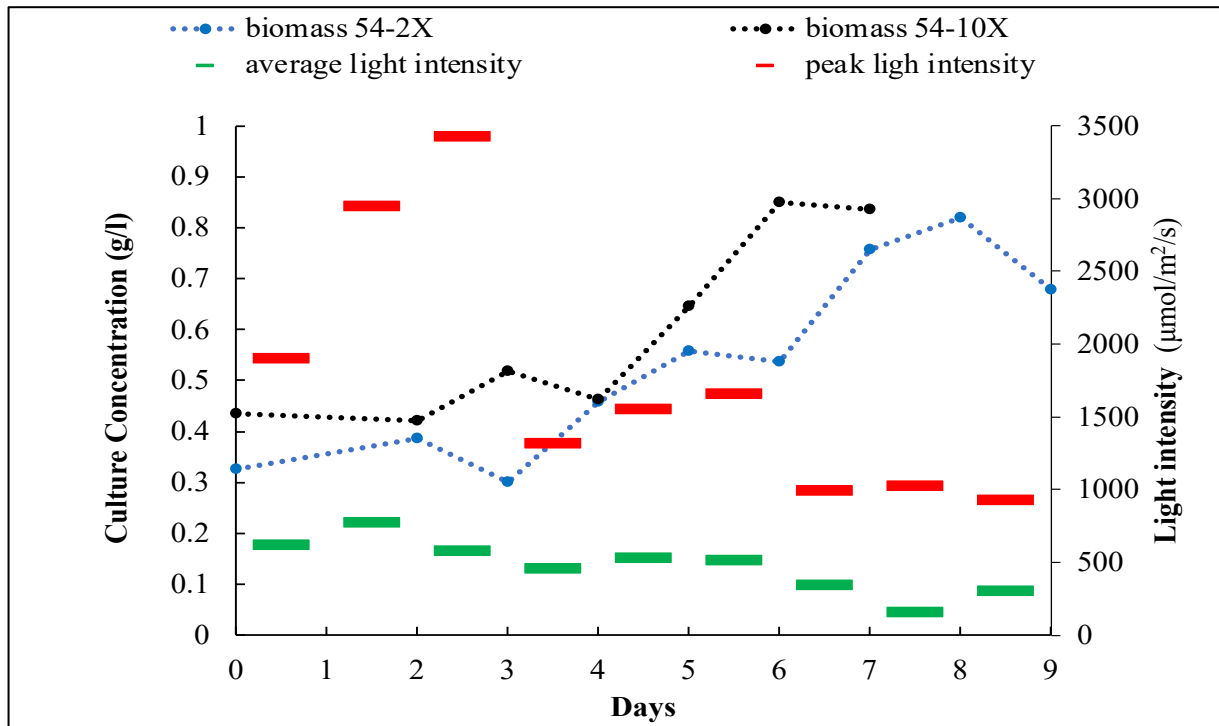


Figure 41: Biomass density (g<sub>X</sub>/L) of *Pleurocapsa* inoculated at 20% (vol/vol) inoculum volumes in 2X and 10X F/2 media, and light intensity variations during the period of outdoor cultivation. Red bars represent the peak light intensities (μmol/m<sup>2</sup>/s.), and the green bars indicates the daily average light intensity (μmol/m<sup>2</sup>/s).



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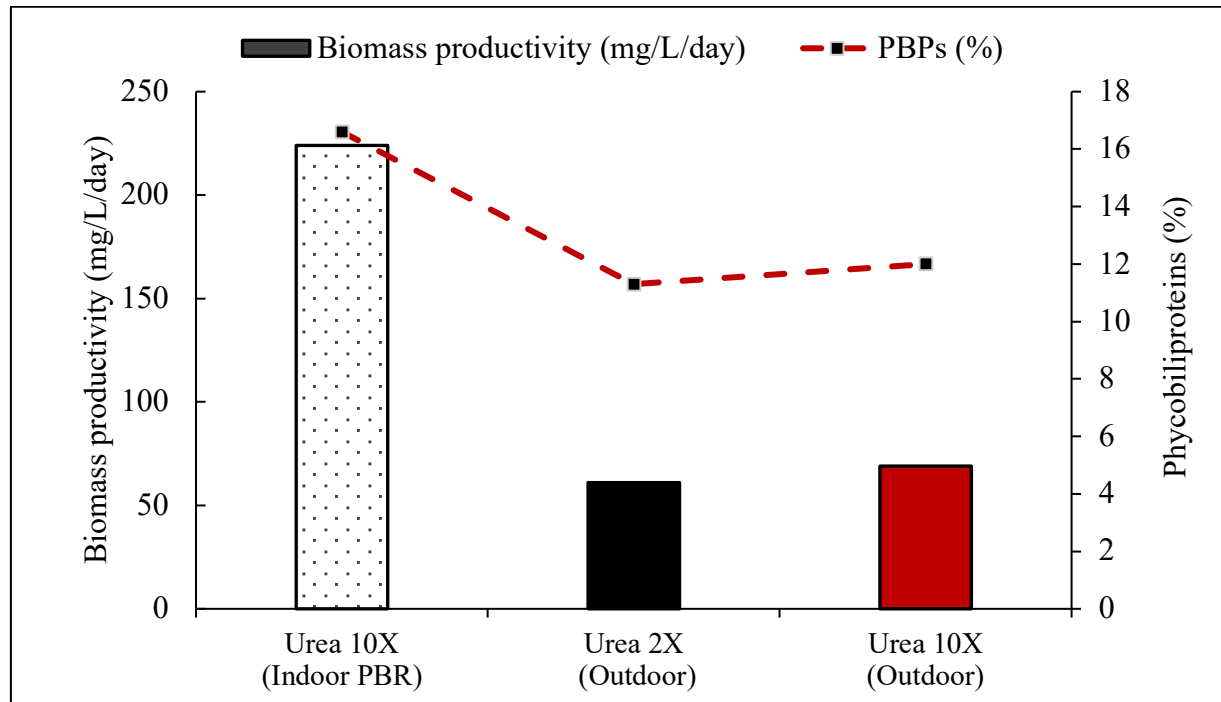


Figure 42 : Biomass productivity and PBP (%) variation at T=end between indoor and outdoor production using two different cultivation system: photobioreactors vs open ponds and urea as nitrogen source.

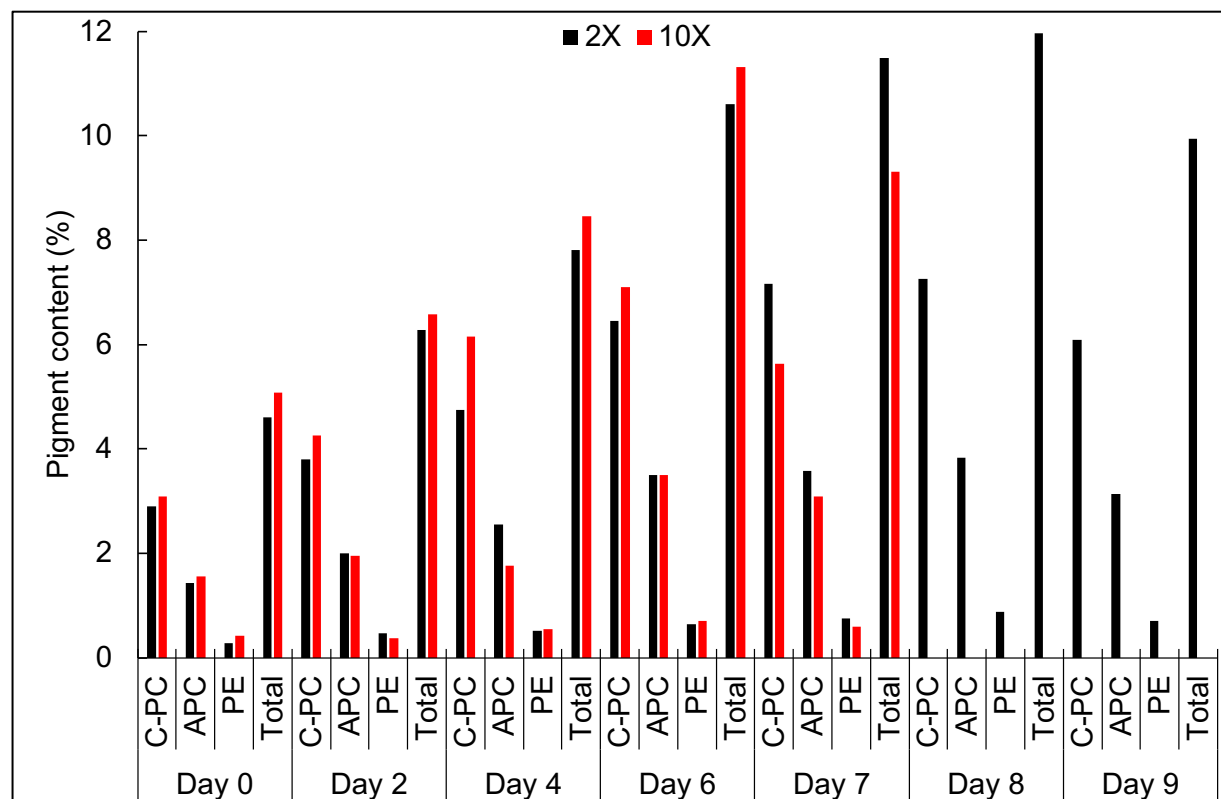


Figure 43: Variation in the phycobiliproteins (%) over time in QUCCCM54 biomass cultivated outdoor at different culture conditions: 2X F/2 and 10X F/2 media.

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However, despite the observed growth, the productivity was much lower than for indoor tests (Fig.42). Indeed, in outdoor cultivation the data showed that the real environmental conditions impeded the growth and significantly reduced the biomass productivity, which decreased from 224 mg/L/day to 69 mg/L/day between indoor and outdoor cultures, respectively. Nonetheless, if we don't consider the 3 days of acclimatation, the productivity will be more, around 110 mg/L/day for 10X F/2 and 104 for 2X f/2, yet still lower than the indoor productivity. At the end of the experiment, the areal biomass productivity of QUCCCM 54 was  $14 \pm 0.16$  g<sub>DW</sub>/m<sup>2</sup>/day and  $12 \pm 0.11$  g<sub>DW</sub>/m<sup>2</sup>/day in 10X and 2X concentration of urea, respectively.

Similar to the growth patterns, PBPs expression was negatively affected by outdoor cultivation conditions when compared to data obtained indoor using urea as nitrogen source (Fig.43). The maximum PBPs content observed was  $11.31 \pm 0.71\%$  for 2X F/2 and  $11.96 \pm 0.2\%$  in 10X F/2 media compared to  $16.6 \pm 0.89\%$  for indoor investigation. In general, exposure to high light intensities often leads to a decrease in the synthesis of phycobiliproteins. This is because high light conditions can induce photoinhibition and oxidative stress, prompting cells to downregulate the production of these proteins to avoid damage from excess energy absorption.

More investigations are necessary to further understand the effects of fluctuation of light intensity on the strain, as well as to determine whether a considerable adaptation period is required to avoid the culture collapse under outdoor conditions, for example under (semi-) continuous conditions.

Discrepancies between laboratory and outdoor cultivation are unfortunately very common to happen, as it is exceedingly difficult to replicate the environmental conditions under laboratory settings. Light is a fundamental driver of photosynthesis, the process by which microalgae convert light energy into chemical energy, leading to their growth and biomass production. From the outdoor data, it is evident that the strain could not withstand the high light intensities seen during the first three days post outdoor inoculation leading to low biomass productivity. Variations in the photoperiod duration could be responsible of the low outdoor biomass productivity. In fact, the day/night duration ratio observed outdoor was  $13.5:10.5 = 1.28$ , however, if we consider the duration where light intensity exceeded  $100 \mu\text{mol}/\text{m}^2/\text{s}$ , the daytime will be only from 2 to 12 = 10 hours, as mentioned in the graph, Fig 41, which further reduces the day night ratio to  $10:12 = 0.83$ , which is a bit lower compared to the one

#### **Chapter 4: Towards outdoor cultivation of the selected local isolate *Pleurocapsa* Sp.**

applied in photobioreactor 12:12 = 1. Another possible reason could be the presence of damaging UVR which generally difficult to be mimicked indoor. During the month where the experiments were conducted, the UV-index in Qatar is usually very high and ranges between 10–12. Exposure of cyanobacteria to UVR can cause DNA damage, and negatively affect photosynthesis and growth. Finally, variations in operating systems can also alter the availability of dissolved CO<sub>2</sub>, which in turn reduces the biomass productivity. In fact, closed photobioreactors lower CO<sub>2</sub> losses compared to open raceway ponds because they can increase mass transfer efficiency and retention duration, which promotes the algal growth.

The outdoor data also highlighted that variation in nitrogen concentrations had no significant impact on the growth and pigments of QUCCCM 54. Both parameters increased up to a certain threshold, after which there was no further growth or PBP's expression observed in nitrogen rich media 10X F/2 when compared to 2X F/2.

While higher urea concentrations generally support increased productivity, the data also suggest that their production reaches a maximum at specific points. The 2X nitrogen concentration is more conducive to balanced and sustainable biomass production, with efficient nitrogen use and minimal waste. The 10X nitrogen concentration, while promoting rapid early growth, is less efficient in the long term due to nitrogen wastage and potential environmental impact such as risk of eutrophication from unused nutrients.

As the species *Pleurocapsa* was not largely investigated, no much data were found to compare with, nonetheless, a study conducted by Canizales et al. [286] investigated the effect of different urea concentrations (ranging from 1 to 4 mM) on the growth of *Synechocystis* and similar trend was reported, where the highest biomass was achieved at a urea concentration of 2 mM, while higher urea concentrations (3 and 4 mM) did not significantly improve biomass production. Similar results were reported by Chen et al. [287], where the maximum C-phycoerythrin (C-PE) content of *Arthrospira platensis* reached 12% despite increasing NaNO<sub>3</sub> concentration from 0.03 to 0.045 M. It is stated that the microalgal nitrogen requirement dominates the total energy requirements for microalgal cultivation [288]. For instance, an increase of 1% in cellular nitrogen content requires an additional 0.23 GJ per tonne of biomass production. Thus, the low nitrogen requirements proved during this study through the optimized supply

## **Chapter 4: Towards outdoor cultivation of the selected local isolate *Pleurocapsa* Sp.**

of urea can be adapted for maximizing the economic production of QUCCCM 54 biomass at large scale

### **2.2. Biochemical composition of the harvested biomass**

The final biomass issued from outdoor cultivation was further characterized in terms of ash content, HHV and primary metabolites and the properties of the biomass feedstock are listed in Table 15. The biochemical composition was not largely affected by the variations in urea concentrations, same as the growth and PBP's expression. Proteins were the dominant metabolite for both conditions and were varying between 25 % and 29 %. Carbohydrates were the second high fraction. They varied between 17 % and 21% while the lipids content was the lowest among the metabolites expressed and were ranging between 12- 15 % for QUCCCM 54 2X and 10X, respectively. In general, under stress exposure such nutrients deficiency, etc, the growth is often inhibited, while the lipid content tend to increase as storage molecules [289]. However, the observed percentages during this study were similar across both cultivation conditions, highlighting that the cultivation conditions were not stressful for the strain. This supports the previous hypothesis suggesting that the strain has an assimilation threshold, and using a cultivation media with only 2X concentration of nitrogen is not limiting the growth nor the biosynthesis of metabolites. Moreover, the HHVs of the ash-free dry biomass of QUCCCM 54 was almost similar between both cultivation conditions with a value of 18.8 and 20 MJ/kg for 2X and 10X F/2 respectively. This similarity indicates that the overall energy content of the biomass is relatively consistent across different urea concentrations.

We also note that the strains presented high ash content, with 26% for the biomass grown in 10X urea concentration and 24% for the biomass from 2X urea concentration media. These values are significantly higher compared to *Spirulina*, which has an ash content of only 8%. Indeed, as the strain was grown in outdoor open ponds, it was exposed to dust and sand contamination. Moreover, marine strains are known by the accumulation of inorganic compounds inside their cells as facet of their cellular mechanism adaption to the salinity stress. These include ion compartmentation that involve the accumulation of excess saline ions ( $\text{Na}^+$ ) in the vacuoles as metabolic response [290]. The ability of numerous cyanobacteria to perform the calcite or magnesium precipitation could also be linked to the high observed ash content.

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Table 15: Composition of the QUCCCM 54 raw biomass cultivated outdoor at both culture conditions.

ALGAL SPECIES	QUCCM 54-2X	QUCCM 54-10X
<b><u>ASH CONTENT (%)</u></b>	24	26
<b><u>HHV ASH FREE BASIS (MJ/KG)</u></b>	18.8	20
<b><u>BIOCHEMICAL ANALYSIS WT% DRY BASIS</u></b>		
Lipids	15 ± 1.4	12 ± 0.9
Proteins	25 ± 2.4	29 ± 1.8
Carbohydrates	21 ± 1.3	17 ± 0.6

To understand the composition of the QUCCCM 54, SEM-EDX analysis were performed, and the data showed that the major inorganic elements were mainly Ca, Cl, Fe, P, Na, Mg and Si (Fig. 44). The source of the detected minerals could be attributed to the dust coming from the outdoor cultivation using an open raceway pond system, as well as the nutrients and salts from the cultivation media F2 that were added during the growing phase, trapped in the wet initial biomass, and then concentrated in the dry residue.

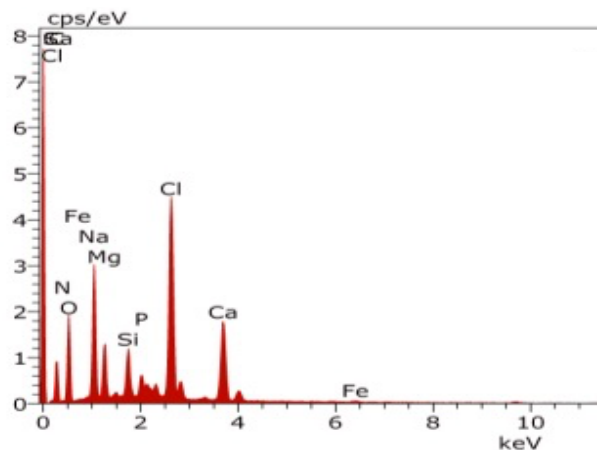


Figure 44: SEM-EDX Analysis of QUCCCM 54 raw biomass

## **Chapter 4: Towards outdoor cultivation of the selected local isolate *Pleurocapsa* Sp.**

### **Conclusion**

In this chapter, we initially evaluated the ability of the selected strain *Pleurocapsa sp* to grow indoor with urea as source of nitrogen. The obtained results demonstrated its ability to grow in mixotrophic conditions, utilizing urea not only for nitrogen but also as a secondary carbon source. This dual assimilation positively impacted both growth rates and PBPs accumulation, highlighting a promising pathway for achieving sustainable biomass production. Accordingly, urea was adopted as nitrogen source for the large-scale studies and its optimized supply were considered outdoor.

We further proceeded with upscaling the strain under real environment conditions. Two different concentrations of nitrogen 2X and 10X were adopted to assess the feasibility of reducing nitrogen input in large-scale production. The first results of the transition from controlled indoor to outdoor conditions underscored the critical importance of inoculum density in achieving successful biomass production. Indeed, in the initial attempt of upscaling in open ponds the transition from a low-light/high-density inoculum to a high-light/low-density culture caused crash probably due to photooxidative cell death. However, when cultivation is repeated with higher inoculum volume of 20%, the culture was able to grow, indicating successful acclimation to the outdoor high light intensities and showed the strain resilience under challenging environmental conditions.

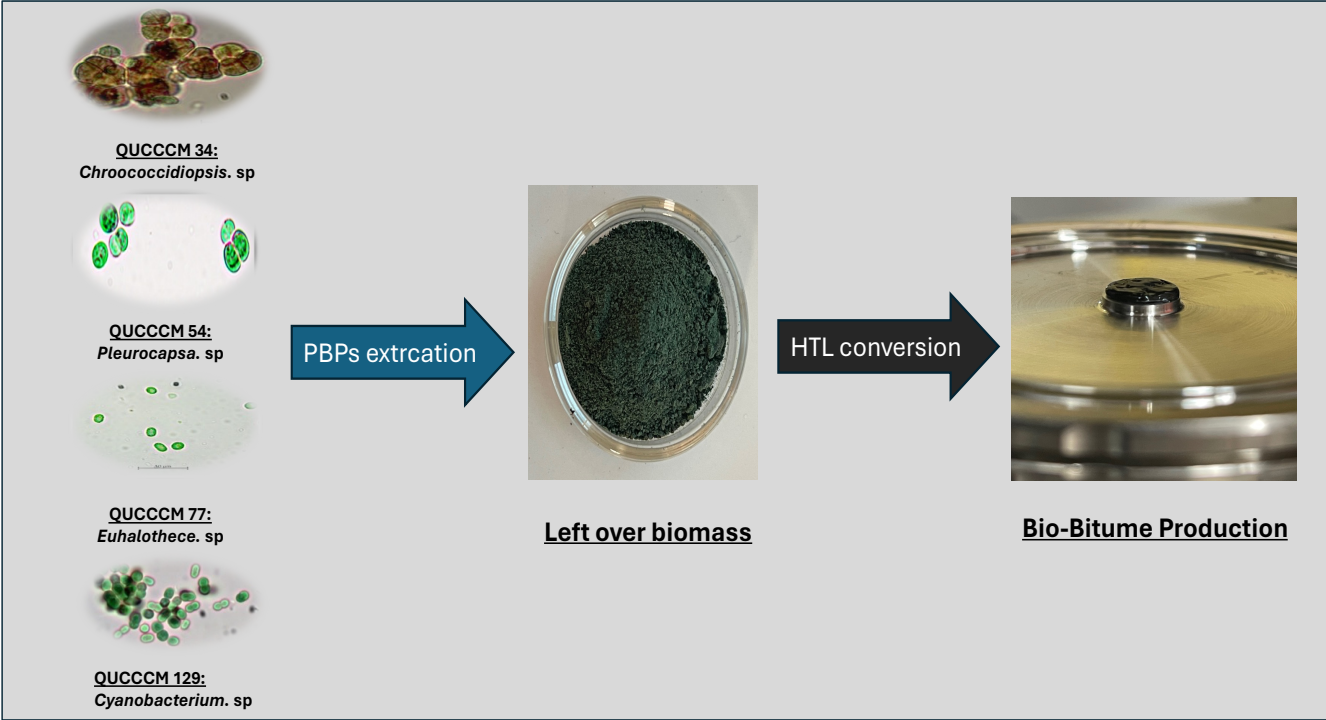
Additionally, the large-scale study revealed that variations in nitrogen concentrations (2X and 10X) had minimal impact on both growth and PBPs biosynthesis. These findings were promising, suggesting potential reductions in nitrogen inputs without compromising productivity, offering both economic and environmental benefits. However, when compared to indoor photobioreactor studies, biomass productivities were notably lower. This discrepancy highlights the challenges imposed by real environmental conditions on growth rates and underscores the need for further optimization of outdoor cultivation practices.

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## Chapter 5: Feasibility of a biorefinery approach targeting bio bitumen as secondary product

### Introduction

Bitumen, a viscous material derived from crude oil, is a non-renewable resource with decreasing availability. Its production and use contribute significantly to carbon emissions and environmental pollution, presenting ecological challenges. Therefore, transitioning to renewable alternatives is crucial for mitigating these adverse effects and ensuring a sustainable and environmentally friendly supply of bitumen-like materials in the future. As discussed in Chapter 1, recently, a proof of concept was established to transform algae-biomass residues into a material mimicking the rheological behaviour of a bitumen through the process of hydrothermal conversion [205, 232, 291]. While previous studies focused on industrially cultivated freshwater species, specifically *Scenedesmus* sp. and *Spirulina* sp., this last chapter aims to explore the possible extension of these findings to selected Qatar marine cyanobacteria species. Therefore, the pre-extracted biomass samples issued from the 4 local strains produced indoor in PBRs (chapter 3), as well as from the outdoor large-scale cultivation of the selected QUCCCM 54 (chapter 4) were used as feedstock for this study.

Due to the limited quantity of available biomass, a small scale 30 mL hydrothermal conversion reactor requiring only 3 grams of biomass per batch was used, while the previous studies on industrial spirulina residues were performed on larger reactors. This industrial biomass was used as a reference in order to study the effect of this downscaling of the hydrothermal conversion process.

In addition, for the outdoor cultivation biomass, available in higher quantities, two types of pigment extraction treatment were performed before hydrothermal conversion:

- i) A « **mild** » extraction process, consisting in using a tissue Lyser machine (Qiagen, Hilden Germany) as for the monitoring of PBPs during indoor cultivation in chapter 3.
- ii) A « **hard** » extraction process, simulated by further treating the biomass by High Pressure Homogenization (HPH)

This allowed investigating the influence of such treatment on biomass residues composition, and on the subsequent hydrothermal conversion products yields, as well as on the biocrude oil phase composition and rheological properties.

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### Results and discussion

#### 1. Preliminary study of Laboratory scale hydrothermal conversion of biomass residues

In chapter 3, 4 indigenous strains were investigated for their potential to grow and produce PBPs under different temperature and salinity regimes as observed in outdoor real environmental conditions.

After PBPs extraction, the leftover biomass from the conditions yielding the highest PBPs production was subjected to HTL conversion, following the procedures detailed in Chapter 2, "Materials and Methods," section on Hydrothermal Liquefaction experiments.

Table 16 showed the yield of the hydrophobic phase obtained after HTL conversion, the other products being, the gas phase and the aqueous phase, will not be discussed here. These yields were calculated using the equation in material and methods, paragraph 1.2. and was important to prove the feasibility of HTL system that will be adopted for the further HTL conversion. In fact, given the small quantity of biomass produced from PBR studies, it was not possible to use the same instrumented and temperature controlled 300 mL HTL reactor previously used to convert the *Spirulina* biomass. A 30 mL Swagelok reactor was proposed instead.

As can be seen on Table 16, this "downscaling" of the process does not affect the hydrophobic phase yield from *Spirulina* biomass: 50 % for the 300 mL reactor vs 52 % for the 30 mL reactor. However, the oil/solid ratio is significantly reduced from  $38/12 = 3.2$ , to  $30/22 = 1.33$ . This may result from the lower loading ratio of 50% for the 30 mL reactor, compared to 75% for the 300 mL reactor in previous studies [172]. Indeed, The early works of Audo et al [173] showed that this parameter strongly affects the viscosity of the hydrophobic phase (unfiltered mixture of biocrude oil and solid residues), which decreased when the loading ratio increased. Such viscosity decrease suggests a decrease of the oil/solid ratio with the loading ratio, which seems confirmed by our results. Another reason could be possibly the lower effective duration of the isotherm at 260°C in the 30 mL reactor. Rolland et al. [172] showed in his work using

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300 mL instrumented reactor that during the process the *Spirulina* biomass is first converted into a solid biochar during the heating step from 25°C to 260°C through hydrothermal carbonization, where the biocrude oil phase is obtained further during the isotherm at 260°C. In our case, no thermocouple was present inside the 30 mL reactor, only the steel temperature is measured, and the 60 minutes isotherm starts when it reaches 260°C. It is possible that the reaction temperature inside actually takes a few minutes more to reach 260°C.

Overall, the preliminary conversion results indicate the HTL in the 30 mL reactor is not optimal. However, it allows to compare the yields obtained for the extracted biomass from the local isolates which presented a total hydrophobic phase yields in the range of 47- 52 %, mostly identical for all the strains and comparable to that obtained for *Spirulina* (54%). However, after separation of the water insoluble phase into biocrude oil and solid fraction through filtration, it was observed that the oil/solid ratio is highly dependent on the strain and decreasing with the following ranking:

*Spirulina* > *Euhalothece* > 1 > *Cyanobacterium* > *Chroococcidiopsis* > *Pleurocapsa*.

This is probably due to the high ash content in the raw biomass. In fact, from estimation of the maximum theoretical ash content in the hydrophobic phase by dividing the value of ash content of raw biomass by the hydrophobic phase yield (example 21 /48 » 43% for *Pleurocapsa*. sp.), we can understand that all the ash material present in the biomass was transferred to the hydrophobic phase. This was also the case for *Chroococcidiopsis* (QUCCCM 34) and *Cyanobacterium* (QUCCCM 129) with an oil/solid ratio below 1. As opposition, both *Euhalothece* (QUCCCM 77) and the *Spirulina* data showed simultaneously a lower than theoretical maximum ash content in the hydrophobic phase, with oil/solid ratio higher than 1. These results highlighted the possible relationship between the amount of ash in the biomass feedstock and the strain's capacity to produce more/ less oil after HTL conversion. This very high ash/mineral ratio in most local strains may be explained by the presence of inorganic compounds coming from the microalga media. Indeed, as the strains were isolated from saline environment, one facet of their cellular mechanism adapted to resist to salinity stress is the accumulation of inorganic compounds inside the cells. These include ion compartmentation that involve the accumulation of excess saline ions (Na<sup>+</sup>)

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in the vacuoles as metabolic response to cope with the extreme salt conditions [290]. In addition, the ability of numerous cyanobacteria to perform the calcite or magnesium precipitation could be linked to the high observed ash content.

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Table 16: Details of the hydrophobic fraction obtained after HTL conversion of the pre extracted biomass of the different local isolated species in comparison to the reference one.

HTL Reactor volume		30 mL					300 mL
Biomass Yields and compositions		<i>Chroococidiopsis</i> QUCCCM 34	<i>Euhalothece</i> QUCCCM 77	<i>Pleurocapsa</i> QUCCCM 54	<i>Cyanobacterium</i> QUCCCM 129	<i>Spirulina</i>	<i>Spirulina</i>
Hydrophobic Phase Yield (%)		52	48	48	47	52.5	50
Components yields	Biocrude oil (%)	16	28	9	22	30	38
	Solid (%)	36	20	39	25	22.5	12
	Oil/Solid ratio	0.4	1.4	0.2	0.9	1.33	3.2
	Ash (%)	30	38	43	29	11	5
Ash content of raw biomass (%)		15	22	21	13	8	8
Theoretical maximum ash content in hydrophobic phase (%)		29	46	44	28	15	16

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If we now consider the rheology of the biocrude oil samples, it appears that only those from *Chroococcidiopsis* (QUCCCM 34) and *Cyanobacterium* (QUCCCM 129) have a bitumen like behavior. In Fig. 45, the phase angle ( $\delta$ ) was plotted as a function of the complex modulus for each biocrude oil fraction. This graphical representation called “black diagram” allows detecting a rheological signature similar to bitumen: that is to say fluid at high temperature ( $\delta \gg 90^\circ$  with a very low modulus), solid at low temperature ( $\delta \gg 0^\circ$  with a very high modulus), and with a continuous evolution. As shown the data, the biocrude oil produced from *Cyanobacterium* and *Chroococcidiopsis* species exhibited a continuous black curve with a signature similar to the conventional bitumen and the oil obtained from *Spirulina* using 30 mL reactor. This behavior is comparable as well to the one reported for *Spirulina* oil obtained with a 300 mL reactor [231], and for the oil from the green microalgae strain *Scenedesmus* sp, obtained through hydrothermal conversion under the same working conditions [173]. Thus, both this phase oily could be a good alternative to bituminous binders.

On the other hand, the rheological behavior for *Euhalothece* (QUCCCM 77) is not at all comparable with that of bitumen and the visco-elastic measurements do not show any continuity between the isotherms in the black diagram, referring to an unstable thermo-rheological behavior of its biocrude oil phase.

Finally, for *Pleurocapsa* sp. (QUCCCM 54) a more stable thermo-rheological behavior is observed, with a continuity between the isotherms between  $0^\circ\text{C}$  and  $50^\circ\text{C}$ . At higher temperatures, where the oil is essentially viscous, the signal becomes unstable because the viscosity is too low. At low temperature, the “solid” behavior of bitumen was not observed, and the phase angle remained higher than  $60^\circ$  even at  $0^\circ\text{C}$ .

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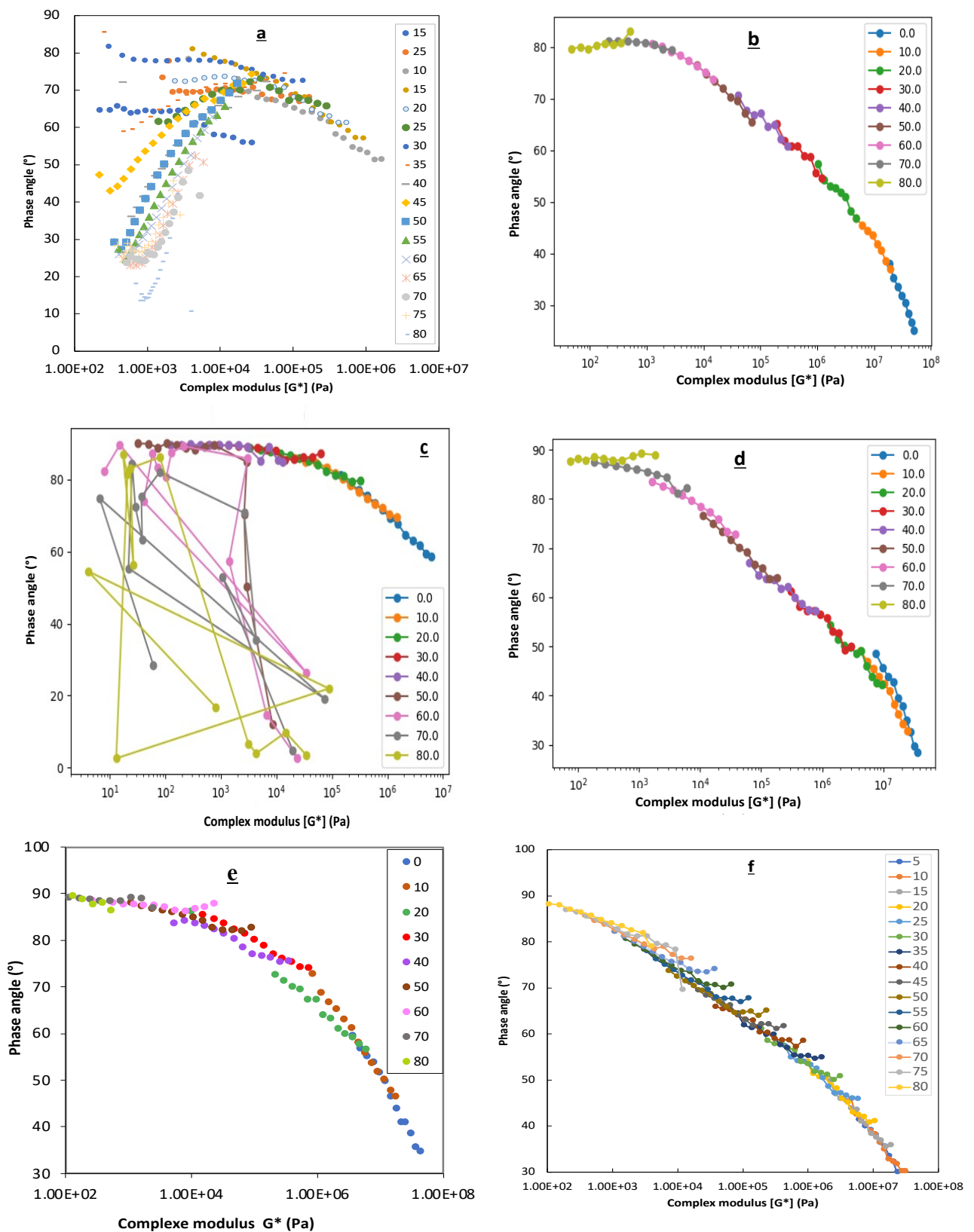


Figure 45: Rheological characterization of the biocrude oil phases: Phase angle versus norm of the complex modulus for the hydrophobic fractions obtained after HTL conversion of the biomass of: **(a)** *Euhalothece* sp. **(b)** *Chroococcidiopsis*.sp, **(c)** *Pleurocapsa*. sp, and **(d)** *Cyanobacterium*, by comparison with **(e)** *Spirulina* sp and **(f)** reference conventional bitumen. All the biocrude oil phase were obtained using a 30mL reactor.

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Unfortunately, the low oil yield, high ash content and the obtained rheological properties further question the feasibility of the HTL conversion of biomass residues to bio bitumen after PBP extraction for *Pleurocapsa*. sp, (QUCCCM 54) the best strain for PBPs production. Hence further investigations were carried out on the biomass obtained from outdoor large-scale production and the feasibility of producing bio-bitumen.

### 2. Effect of pigment extraction process on *Pleurocapsa* biomass residues composition

The strain *Pleurocapsa* sp. grows in small colonies, surrounded by a well- developed rigid cell wall and membrane that serves as a protecting boundary which makes the extraction of intra-molecules challenging. Hence, efficient disrupting of the cell wall is essential. PBPs being the target high valuable products were in obtained in chapters 3 and 4 following the “mild” extraction process using tissue lyser, as cell lysis techniques. Generally, this method suited our routine lab analysis, as it is less time and labor consuming, plus it showed efficiency for other strains cell wall disruption.

However, in the case of the 4 native strains studied, and in particular QUCCCM 54, *Pleurocapsa* strain, a very high ash content is observed in the residues of chapter 3. Therefore, for the biomass produced outdoor in chapter 4 with two different urea concentrations, we studied two different pigment extraction processes, leading to 6 different batches:

- The raw biomasses, after harvesting, referred as **2X raw** and **10X raw** in the following.
- The biomass residues after a “mild” pigment extraction process with the Tissue Lyser machine, referred as **2X mild** and **10X mild** in the following.
- The biomass residues after a simulated “hard” pigment extraction process by further processing a fraction of the 2X mild and 10X mild batches, by High Pressure cell disruption, referred as **2X hard** and **10X hard** in the following.

This approach aims to reduce the high inorganic content and enhance the thermal decomposition characteristics of the biomass. This second treatment resulted in a mass yield of 75%, indicating that a significantly higher water-soluble fraction is



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liberated due to a more efficient cell lysis and biomass destruction. All the QUCCCM 54 feedstocks from mild and hard PBPs extraction process were later characterized then further subjected to HTL conversion. A comparison between these biomass types and *Spirulina* was conducted to evaluate their relative performance and suitability for producing a biocrude displaying a rheology similar to conventional bitumen.

### 2.1. Biochemical composition of the biomass feedstocks

Fig. 46a and 46b illustrate the metabolite composition and ash content in the 2X and 10X biomass batches, respectively. Compared to *Spirulina* biomass, the *Pleurocapsa* harvested biomass contains more lipids and carbohydrates and less proteins. However, the most striking result concerns the ash contents of 24 % and 26% for 2X raw and 10X raw, respectively, which are about 3-fold higher than that of *Spirulina* (8%). This composition is minimally affected by the mild pigment extraction, with 2X mild and 10X mild biomass residues still represent an ash content of 23 % and 22 %, respectively suggesting that the minerals contributing to the ash content are tightly bound to the biomass and require stronger cell destruction to be released in the water. Indeed, the hard pigment extraction, simulated by the additional HPH treatment leads to a significant reduction of 50 % in the ash contents to reach 12% and 13 % for 2X hard and 10X hard, respectively. This demonstrates the positive effect of pretreatment in weakening the cell wall and releasing water-soluble molecules that were previously locked within the biomass. Meanwhile, it was observed that the levels of the three key metabolites—lipids, carbohydrates, and proteins—are only slightly reduced. It is worth noting that the yield of the HPH process was approximately 75%. A large part of the 25% loss may be ascribed to minerals.

The comparative analysis of the biochemical composition of *Pleurocapsa* and *Spirulina* highlights differences in their metabolic profiles and elemental compositions, which can be attributed to their biological characteristics, growth conditions and the effect of treatment. The biomass of *Pleurocapsa* was rich in lipids and carbohydrates, while proteins were more expressed in *Spirulina*. This was expected as the pre-extracted biomass of *Spirulina*.sp is one of the most cyanobacteria strains known for their high protein content.

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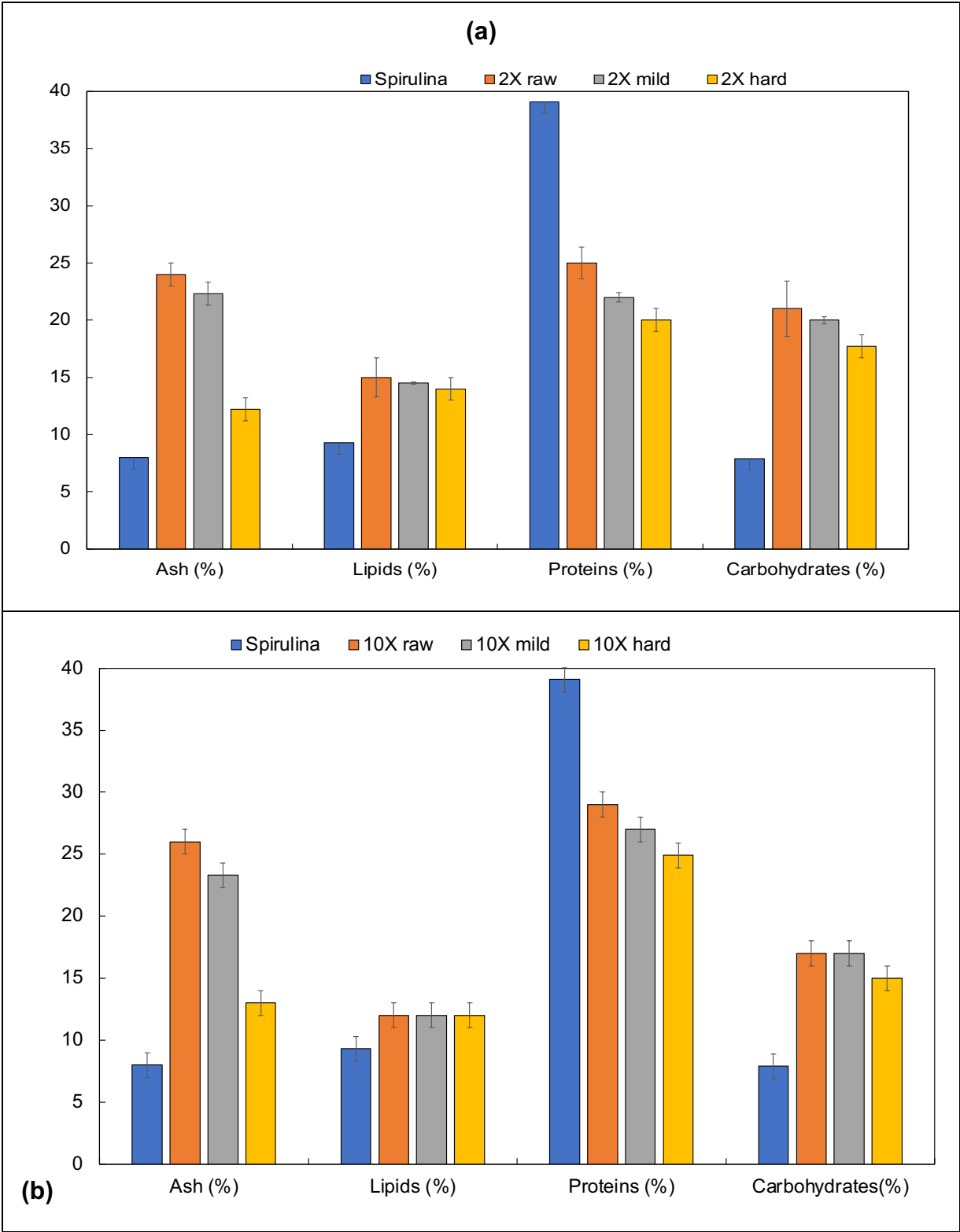
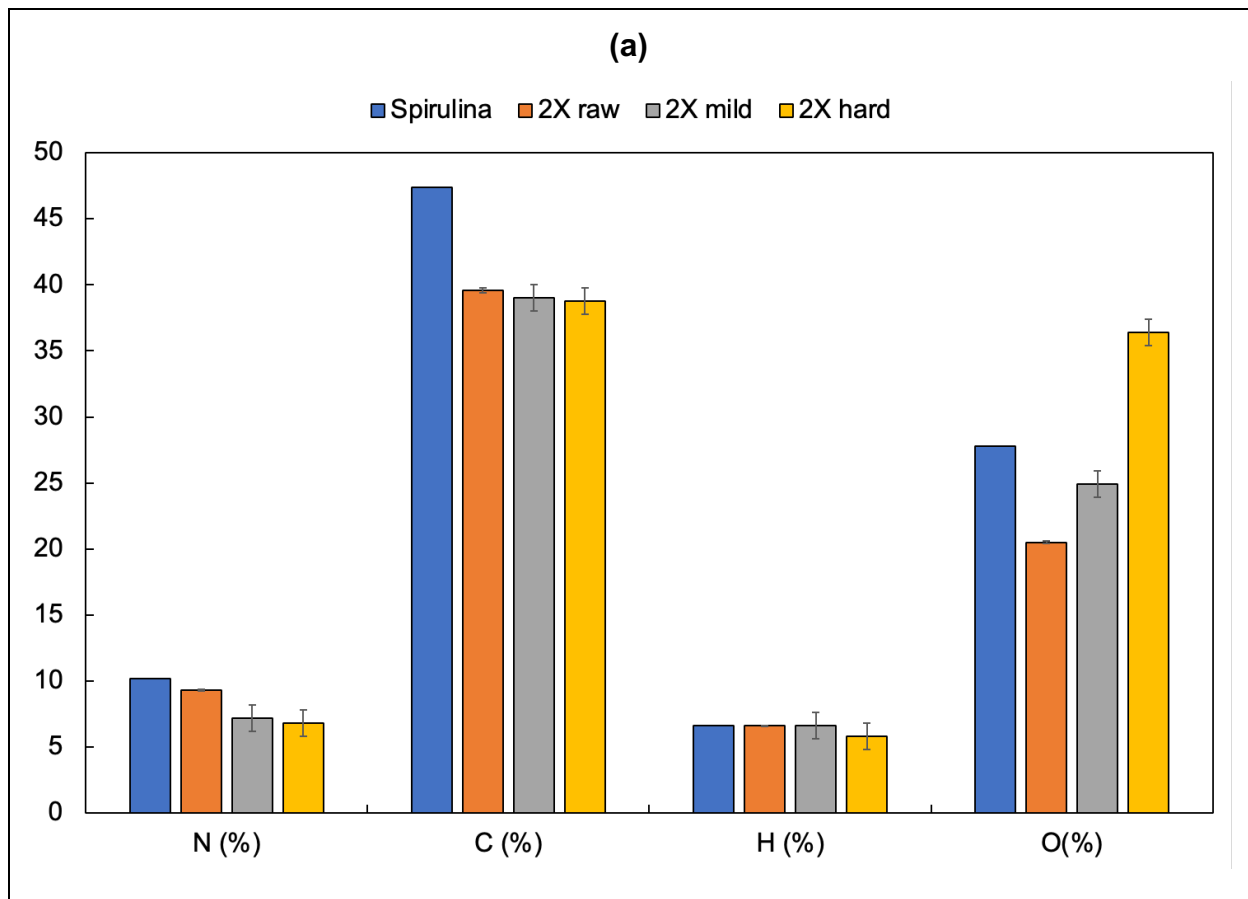


Figure 46 : Biomass composition between the raw, mild and hard extracted QUCCCM 54 feedstock cultivated outdoor at 2X (a) and 10X (b) urea in comparison to Spirulina obtained from Algosource.

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Moreover, the evolution of the CHNO compositions is presented on Fig. 47a and 47b. It was observed that the *Spirulina* had higher contents of C, and N, and lower O content than that of QUCCCM 54 at both cultivation conditions and the simulated “hard” extraction process with 47.4% vs. 39 %, 10.2% vs. 6- 7% and 26.2 vs. 35.8%, respectively.



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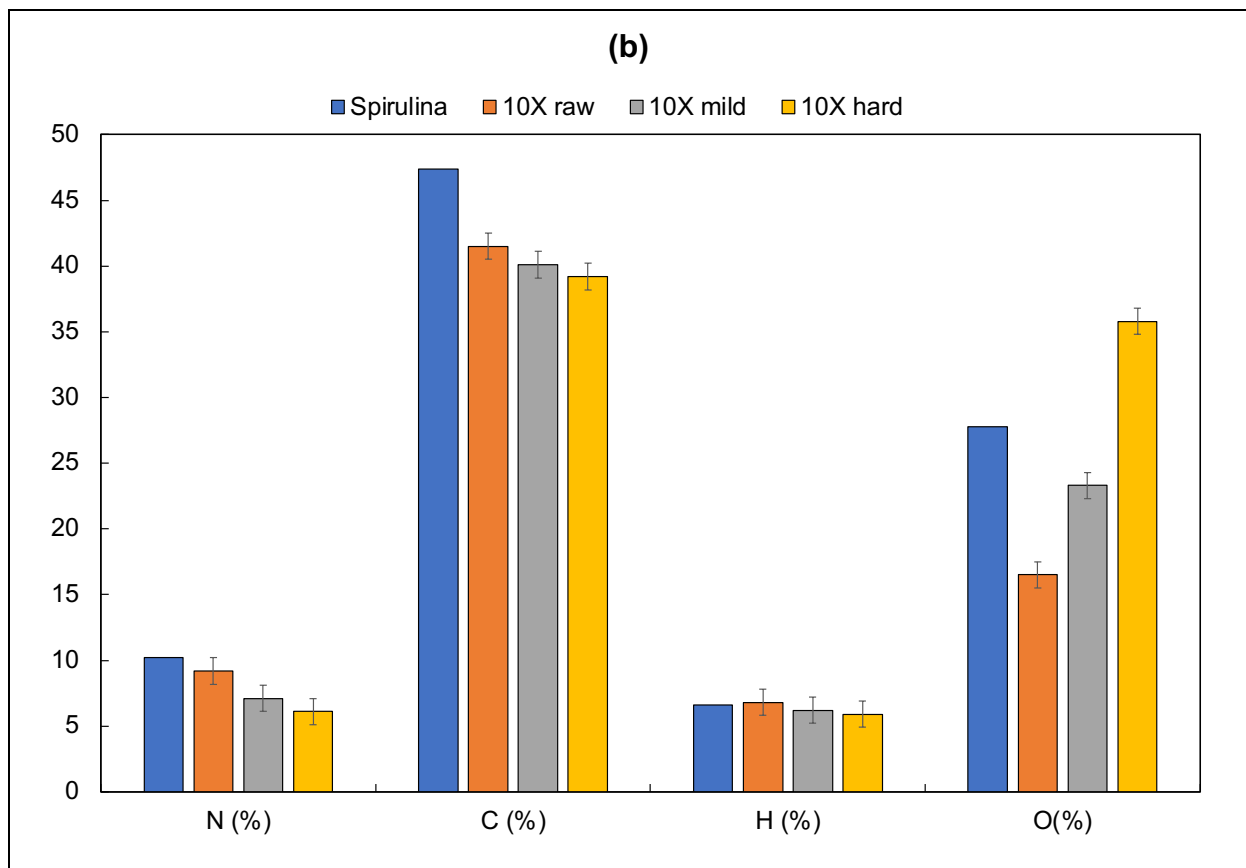


Figure 47 : CHNO compositions of the raw, mild and hard extracted QUCCCM 54 feedstock cultivated outdoor at 2X (a) and 10X (b) urea, in comparison to Spirulina obtained from Algosource.

### 2.2. Electronic Microscopy and EDX analysis of the biomass residues before and after HPH pretreatment

SEM was used to observe the surface morphology of the different QUCCCM 54 biomass samples raw, mild and hard. Figure 49 shows an example of QUCCM 54-2X. The results pointed that the morphology of the 2X raw QUCCCM 54 sample was found intact, relatively smooth, with the presence of a large amount of debris on and between the cells (Fig. 48a). As an opposition, for 2X hard (Fig 48c), a complete disintegration of the cell wall in addition to cell shrinkage in size and separation was observed due to the strong shear forces generated during the process, while for the 2X mild treatment most cells maintained their membrane integrity and only few presented formations of holes (Fig. 48b). It was also detected that the treatment led to an importance reduction in the amount of the debris on and between the cells.

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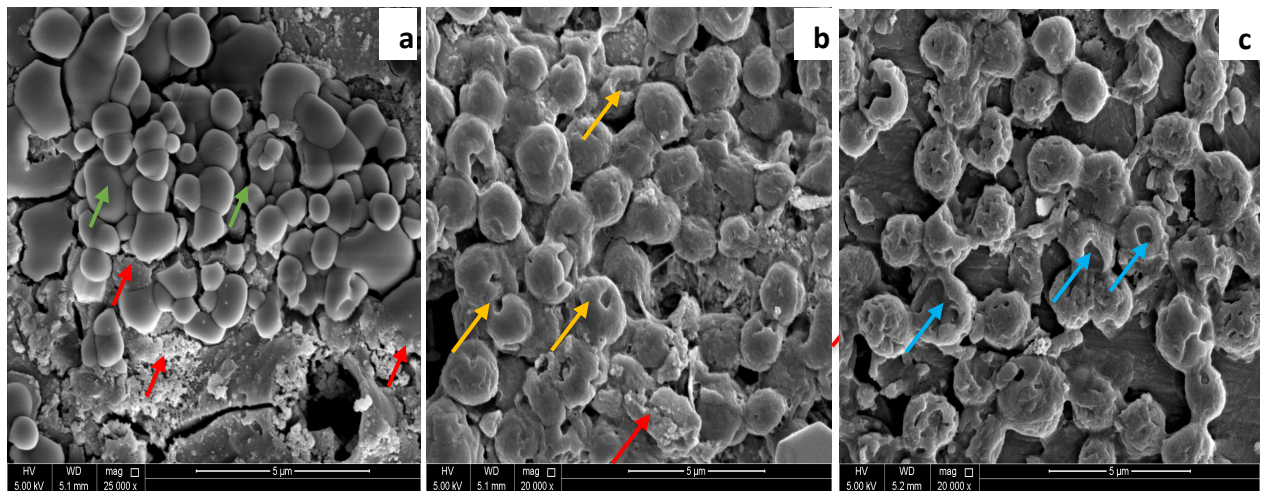


Figure 48: Figure 4. Scanning electron microscopy (SEM) of *Pleurocapsa* cells; **(a)** the image shows a uniform surface of algal cells that were not yet extracted (green arrows); **(b)** the image shows a partially fractured algal cell wall (yellow arrows), after mild treatment for PBPs extraction and **(c)** *Pleurocapsa* cells showing large cell wall fractures after hard mechanical treatment (blue arrows).

Concurrently, EDX analysis revealed a decrease in the **peak** intensity of most inorganic compounds in particular the content in Ca, Cl, Na and Mg as shown in Fig. 49. This suggests that the additional high pressure cell disruption allows the liberation of minerals present not only as extracellular particles, but also inside the cells. The presence of relatively high amounts of Ca may be related to the presence of calcium carbonate.

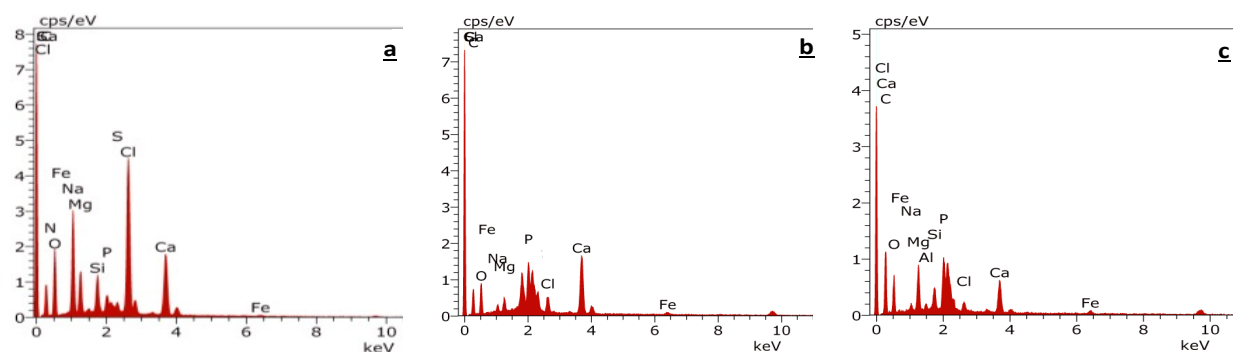


Figure 49: EDX of QUCCCM 54 2X: raw biomass **(a)** Biomass extracted using mild treatment **(b)** and biomass extracted using hard mechanical treatment **(c)**.

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### 3. Effect of pigment extraction process on Hydrothermal conversion products

#### 3.1. Yields, elemental composition and energetic content of the hydrophobic phases

##### 3.1.1. Yield of the different HTL fractions:

The leftover biomass after PBPs mild and hard extraction was converted to biocrude oil through HTL process to examine if: (1) the cultivation conditions and/or (2) the hard extraction treatment could effectively improve the yield and characteristics of the resulting bio-crude oil. Fig. 50a allows comparing the yields of each phase obtained after HTL conversion for the different *Pleurocapsa* biomass residues. The hydrophobic fraction (biocrude oil+ residues) was the most dominant followed by aqueous than gaseous phases. The % of the aqueous fraction was ranging between 9 to 12%. Nevertheless, it should be noted that there was some loss during the recovery process, either by outflowing while opening the reactor due to the high pressure, and/ or wasted during the collection which makes the comparison between different biomass not representative. The gaseous phase was not collected nor analyzed, however based on previous studies under the same HTL conditions it was estimated to be in the range of 2 % [231].

As for spirulina, the total yield amounts of hydrophobic phase (biocrude oil + solid residues) are always close to 50%. However, the oil/solid ratio is strongly dependent on the biomass and its downstream processing: the highest values are observed for 2X hard ( $27/31 \approx 0.87$ ) and 10X hard ( $21/31 \approx 0.68$ ), while 2X mild and 10X mild lead to oil/solid ratios close to 0.36. This suggest that the HPH treatment has an influence on the subsequent biomass hydrothermal conversion. However, after dissolving the hydrophobic phase in dichloromethane and separation through filtration into biocrude oil and solid fraction, we observed that the solid residues (biochar) were more prominent. The temperature applied during this study is a crossover region between hydrothermal carbonization process (HTC) and HTL, resulting initially to a partial transformation of the biomass through carbonization reactions, which can explain the high solid residues content observed. Besides, the high carbohydrates content

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observed in the strain can also lead to an increase in solid residue as reported by Biller et al. [292].

The biocrude oil of QUCCCM 54, which is the target of this study, was found to be notably low, with negligible differences observed between the two urea culture conditions (16 wt% and 14 wt% for QUCCCM 54-2X mild and QUCCCM 54-10X mild, respectively). This is consistent with the previous findings of growth and biomass composition, suggesting that despite the varying nitrogen content in the media, the resulting biomass did not largely differ in composition. In contrast, the application of hard treatment led to a substantial increase of ~ 1.5 folds in the biocrude oil yields, which raised from 16 wt% and 14 wt% to reach 27 wt% and 21 wt%, for QUCCCM 54-2X hard and QUCCCM 54-10X hard, respectively. This improvement can be explained by the positive effect of the treatment in effectively weakening QUCCCM 54 cells, facilitating the further excretion of their cellular components, and thus promoting their better thermal decomposition and subsequent conversion into biocrude. These findings highlight the detrimental impact of high ash content and poorly lysed biomass on the HTL process, which resulted in lower biocrude oil yields.

The maximum biocrude oil obtained during this study (27% wt for QUCCCM 54-2X hard) was observed to be closer to the oil fraction of *Spirulina* » 30 wt%, yet, both yields are found to be low compared to other biocrudes from different algae feedstocks as the composition of the feedstock affect their yields. For instance, Li et al., [293] obtained 55 wt% biocrude from *Nannochloropsis* sp. at 260°C, 60 min.

The solid residues from the biomass of QUCCCM 54-2X hard and QUCCCM 54-10X hard were 10% lower compared to those obtained without high-pressure homogenization extraction treatment. This yield reduction after HPH treatment is likely due to the decreased ash content in the biomass feedstock prior to hydrothermal liquefaction conversion and highlighting the importance of hard treatment step in reducing the non-organic components in the biomass, leading to a lower solid residue fraction post-conversion. However, despite this decrease, the solid residue yields are still higher than those observed for *Spirulina*.

As shown in Fig. 50b and 50c, which present the CHNO composition and ash content (%) in the biomass and HTL products, the oil phases contain very low ash content (ranging from 0.6% to 2.1%, depending on the starting biomass). In contrast, the solid

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residues from HTL contain significantly higher ash fractions. For example, in the 2X mild and 10X mild samples, the solid residues contain 45.2% and 47.8% ash, respectively. By multiplying these ash values by the solid residue yields and dividing by the initial biomass ash content, we can estimate the fraction of minerals retained in the solid residues. Indeed, for 2X mild, the calculation is  $45.2\% \times 0.43 / 22.3\% = 87\%$ , and for 10X mild, it is  $47.8\% \times 0.40 / 23.3\% = 82\%$ . Similarly, for 2X hard and 10X hard samples, the recovery of ash in solid residues is calculated as  $39\% \times 0.31 / 12.2\% = 99\%$  for 2X hard, and  $37.6\% \times 0.31 / 13\% = 90\%$  for 10X hard. These calculations suggest that the amount of solid residue after HTL is strongly correlated with the initial ash content of the biomass. Therefore, the HPH treatment, by reducing the ash content in the biomass, indirectly increases the biocrude oil-to-solid ratio observed in the HTL process.

The elemental analysis of the biochar revealed also an increase in the carbon compared to the raw biomass. As previously mentioned, increased carbon content occurs due to a higher degree of polymerization observed during HTL process following the low temperature applied in this study, leading to a more condensed carbon structure in the biochar [294, 295]. No oxygen was detected in the biochar, however, N% was present in the range of 5- 6%. The presence of these renewable essential elements such as carbon, nitrogen, in the solid residues suggests their possible use as a soil amendment for agricultural applications. Although, there are limited studies on reuse of HTL solid residues, Leng et al. [296] reported that biochar obtained through the HTL process have higher absorption capacity compared with activated carbon. Also, solid residues were found useful in removing the dye in the effluent discharged from textile industry [297], which may be beneficial to the textile producing companies.

The generated biocrude oil presented a carbon, hydrogen, nitrogen and oxygen content between 60 -72 %, 8.5 - 9 %, 5 - 6 % and 13 - 34 % respectively, which was comparable to the range of values reported in literature for the liquefaction of most algae [298]. The abundance of these elements was dependent on the initial composition of the feedstock. However, differences between the mild and hard biomass treatment were noted. Indeed, in presence of HPH treatment, an increase in the carbon and hydrogen amounts, the two abundant elements in bitumen, was noticed with a raise in carbon from 62% in the biocrude oil from the mild treated QUCCCM 54-

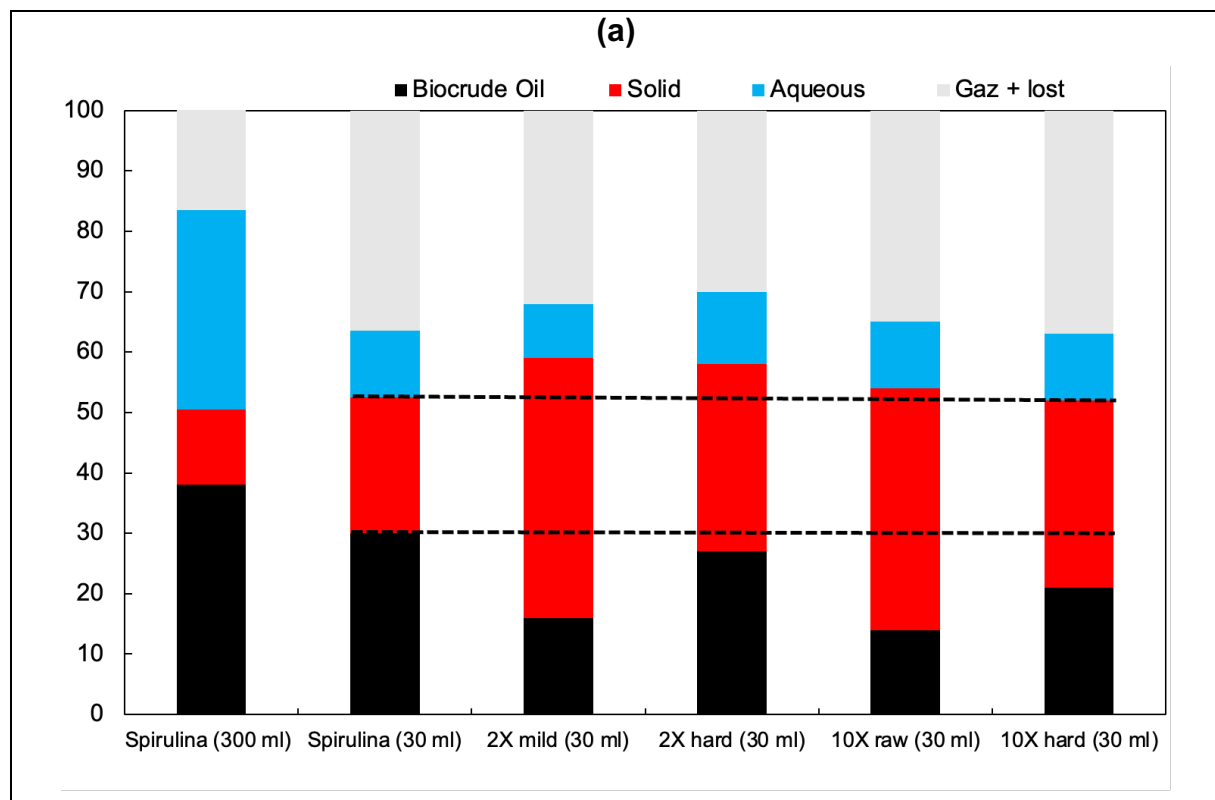


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2X biomass to reach 72.8 % in the hard treated one. Hydrogen content was not largely affected by the treatment. On the other hand, there was a 10% oxygen removal in the oil fraction, while only around 1% difference for the nitrogen content suggesting that the proteins are most solubilized in the water phase during the heating step. Similar trend was observed for the elemental results of the biocrude oil of 54-10X between the mild and hard treatment.

Conventional bitumen, on the other hand, presented absence of ash and oxygen and lower concentrations of N (~ 1%) which was different compared to algal biocrude oil samples. The detected N and O in the biocrude oil are likely to make it more sensitive to moisture and oxidation than petroleum bitumen [232]. Therefore, removal of these heteroatoms by further processing would have to be considered to upgrade the oil to a conventional hydrocarbon fuel and make it suitable for blending with existing refinery petrocrude or for direct use.

All these findings indicate the positive impact of the treatment on the “quality” of the biocrude oil phase obtained from QUCCCM 54 to make it closer in composition to the one of *Spirulina* and the petroleum bitumen.



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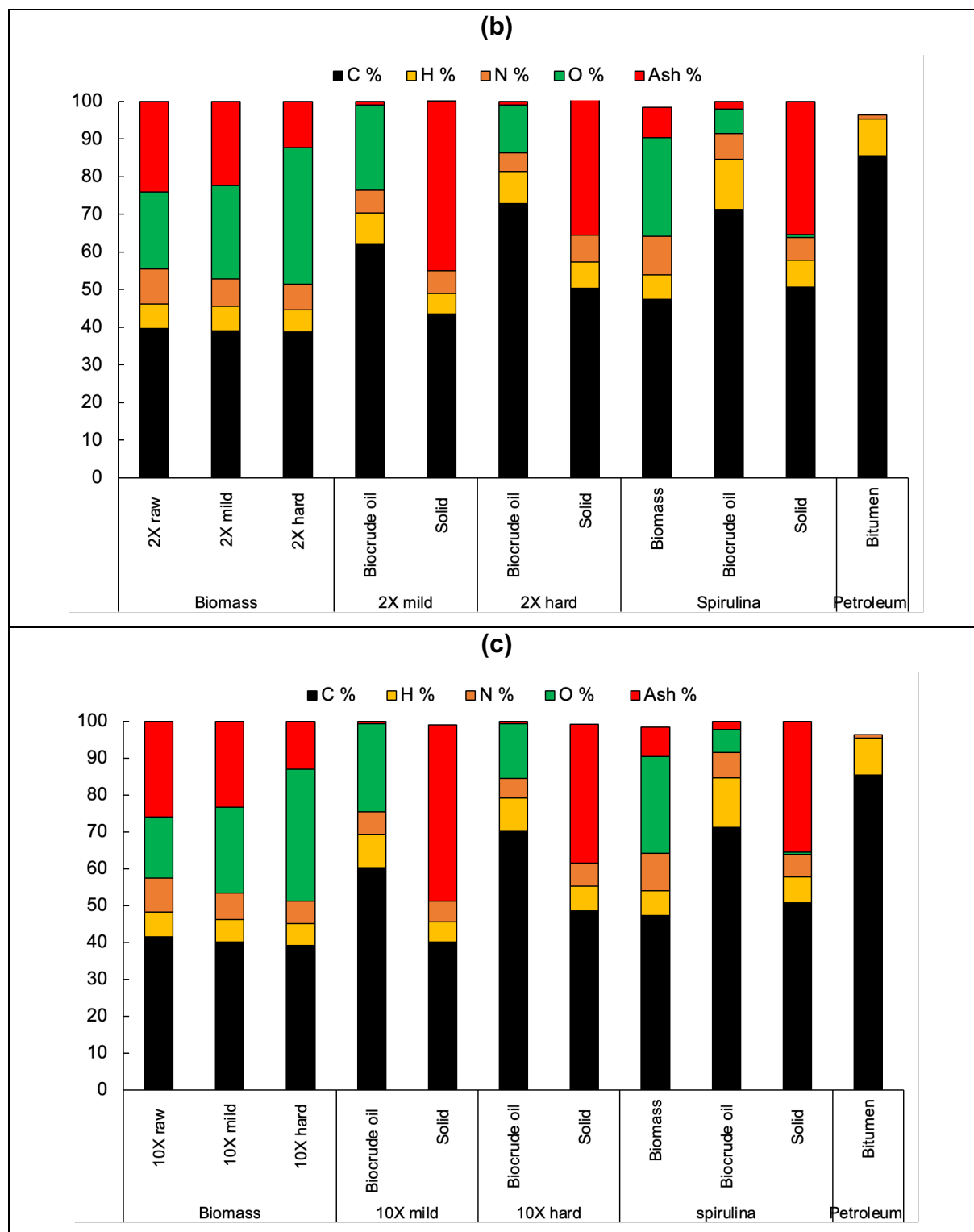


Figure 50: (a) Yield of different fractions obtained after HTL Process at 260 °C using sawagelok reactor for strain QUCCCM 54 biomasses with mild and hard mechanical treatment, in comparison to Spirulina obtained from Algosource. Chemical composition of the biomass and hydrophobic products: inorganic ash content and elemental analysis of the organic phase of QUCCCM 54 biomass cultivated outdoor at 2X (b) and 10X F2 (c), and Spirulina in comparison to the petroleum bitumen grade 50/70.

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### 3.1.2. Energetic content of the hydrophobic phases

The HHVs of the raw biomass and biocrude oil samples obtained were estimated as shown in Fig. 51. It should be mentioned here that the initial HHVs of the both QUCCCM 54 biomass feedstock was very low (16-18 MJ/kg) suggesting that the material is less energy-dense probably due to the high ash and oxygen content in the strain which are known to adversely affect the calorific value of biomass. After undergoing hydrothermal liquefaction conversion, the HHV of the biocrude oil from mild biomass issued from both cultivation conditions increased to reach 29.1 MJ/kg, and further rise was observed for the hard biomass to reach a maximum of 34 MJ/kg for 54-2X hard. similar trend was noted for the HHVs of 54-10X biomass. This improvement can be linked to the reduction of oxygenated compounds and the concentration of more energy-dense hydrocarbons in the biocrude. the HHV values produced from this study aligned well with the previously reported ranges for biocrudes from various algal species such as *Chlorella*, *Spirulina*, and *Tetraselmis* (27.7–36.2 MJ/kg). However, it is still below the reported value for the high-hydrocarbon algal species *B. brauna* with 45.9 MJ/kg, and for the bitumen grade 50/70, which has a HHV value of 41.6 MJ/kg [172].

Furthermore, the energy recovery (ER) of the different HTL fraction with mild and hard treatment was estimated. Energy recovered from initial feedstock to biocrude is one of the important indicators of the efficiency and effectiveness of HTL process. Potential feedstocks and treatment leading to maximum recoveries of energy in biocrude are highly recommended. The results demonstrated that despite the biomass loss post treatment (Recovery yield of 75%), the energy recovery of the oil fraction after hard extraction treatment displayed higher rate. In point of fact, there was a 17 % improvement in the ER values of oil after HPH treatment for QUCCCM 54-2X hard, going from 26 % to 43 %, and a 10 % improvement for QUCCCM 10X hard, going from 22 % to 32 % indicating that the biocrude oils fraction from the QUCCCM 54 hard biomass recovered more energy than that from the mild one. The data also highlighted that the oil from QUCCCM 54-2X displayed higher ER compared to the one from *Spirulina*. These findings align with the trend in HHVs and biocrude yields emphasizing the importance of mechanical treatments in breaking down biomass structures, facilitating better conversion and energy recovery during the HTL process.

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Overall, the above results confirm that the hard treated QUCCCM 54 biomass, in particular 54- 2X, can be regarded as better feedstock for the production of HTL biocrude offering enhanced yield, heating value, and energy recovery.

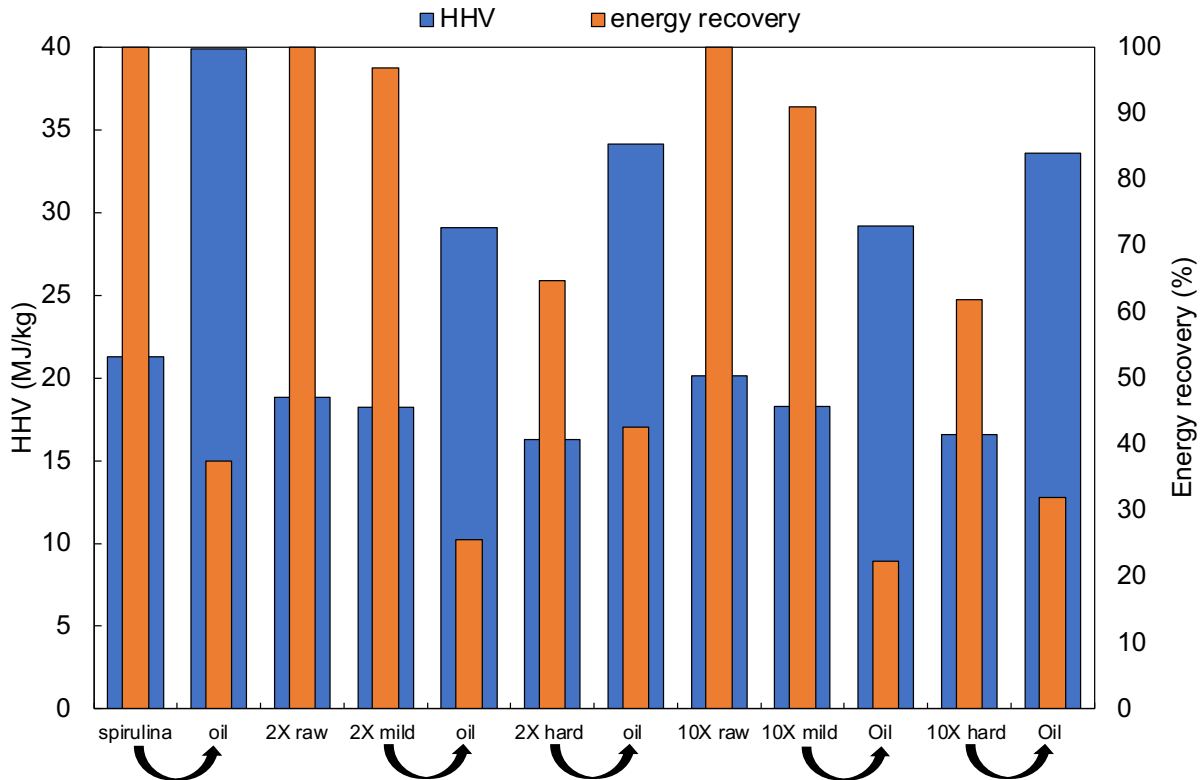


Figure 51: HHV, Yields and ER values of the biomass and Biocrude oils obtained after HTL conversion of QUCCCM 54 biomass cultivated outdoor at 2X and 10X F2, with mild and hard extraction, and Spirulina biomass residues.

### 3.1.3. Molecular composition of the biocrude oil phase

To better understand the factors contributing to the improvement of biocrude oil fraction observed between mild and hard treated QUCCCM 54 biomass, further analysis was performed in particular FTIR, SEC and GC-MS, and the obtained results are discussed in the following paragraphs.

#### 3.1.3.1. FTIR analysis

FTIR analysis were performed for the biomass from both cultivation conditions, with and without treatment and the biocrude oil after HTL conversion, to determine the chemical bonds present and elucidate the effect of the treatment. The spectrograms

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obtained by FT-IR are shown on Fig. 52. The curves were normalized to the highest absorption peak intensity. Some curves were shifted for the sake of clarity. Generally, the FTIR spectrum is divided into three regions: (i) hydroxyl (O-H), amine (N-H) and hydrocarbon (C-H) bonds region ( $2500-4000\text{ cm}^{-1}$ ), (ii) characteristic absorption bands of bonds ( $1500-2000\text{ cm}^{-1}$ ), and (iii) the fingerprint region (below  $1500\text{ cm}^{-1}$ ).

The strongest peaks for conventional bitumen are observed at  $2917\text{ cm}^{-1}$ ,  $2848\text{ cm}^{-1}$ ,  $1468\text{ cm}^{-1}$  and  $1374\text{ cm}^{-1}$  and correspond to the C-H stretching and bending vibrations aliphatic hydrocarbon chains. Concurrently the two other peaks observed at  $1468\text{ cm}^{-1}$  and  $1374\text{ cm}^{-1}$  correspond to C-H bending modes. Mainly alkane C-H are present underlining its aliphatic characteristics as expected for a fossil carbon material.

On the other hand, the biomass samples presented strongest bands at  $\approx 1636\text{ cm}^{-1}$ ;  $\approx 1538\text{ cm}^{-1}$ ;  $\approx 1020\text{ cm}^{-1}$ ;  $\approx 1147\text{ cm}^{-1}$ ; and in the  $3000\text{ to }3500\text{ cm}^{-1}$  range. This indicates the presence of aliphatic oxygenated or nitrogenated compounds and was in agreement with the elemental analysis. Likewise, all the biocrude oil samples had similar profile indicating the presence of aliphatic oxygenated or nitrogenated compounds after HTL.

Conversely, the peak at  $\approx 1636-1640\text{ cm}^{-1}$  remains strong indicating the presence of C=O bonds, probably due to carboxylic acids, esters and amides identified previously in GC-MS. Alongside, the C-H peaks at  $2917\text{ cm}^{-1}$ ,  $2848\text{ cm}^{-1}$ ,  $1468\text{ cm}^{-1}$  and  $1374\text{ cm}^{-1}$  are much stronger than in the biomass, due to the high carbon and hydrogen contents observed in elemental analysis of the biocrude fractions, which produce a strongly aliphatic character of the oils (In agreement with elemental analysis decrease of C/O and C/N ratios).

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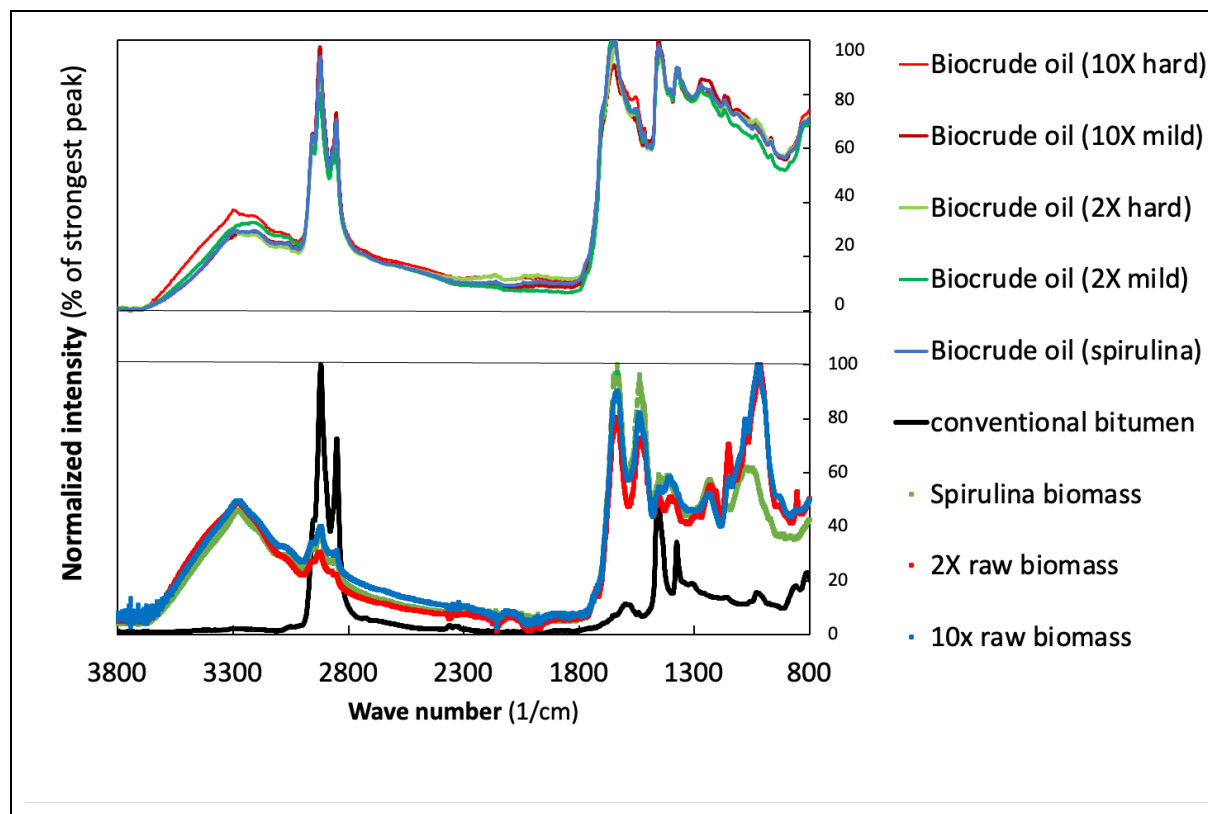


Figure 52: FTIR Spectrograms of Spirulina biomass, QUCCCM 54 raw biomasses from both cultivation conditions and the corresponding biocrude oils in comparison to the conventional bitumen.

### 3.1.3.2. Molecular weight distribution

Gel permeation chromatography (GPC) was performed to assess the impact of HTL-converted biomass residues on the molecular mass distribution of the resulting biocrude oil fraction. Fig. 53a shows the size exclusion chromatography elution curves obtained. For all samples, the elution times corresponding to molecular mass distribution are between 14 and 19.7 min (at higher elution times, the signal is perturbed by the contribution of the THF solvent). According to the calibration performed, this corresponds to molecular masses ranging from 53 to 10000 g/mol.

It is notable that all the curves exhibit a peak around 17.5 min ( $\gg$  400g/mol). However, they also display varying shoulder peaks at different elution times, indicating the presence of different populations. To qualitatively assess the main populations in each oil sample, MagicPlot™ software was used to deconvolute the peaks. The results of

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this mathematical analysis should be interpreted with caution, as they provide only qualitative comparisons between the different samples. An example is shown in Fig. 53b, illustrating the five peaks identified in the biocrude oil derived from Spirulina biomass.

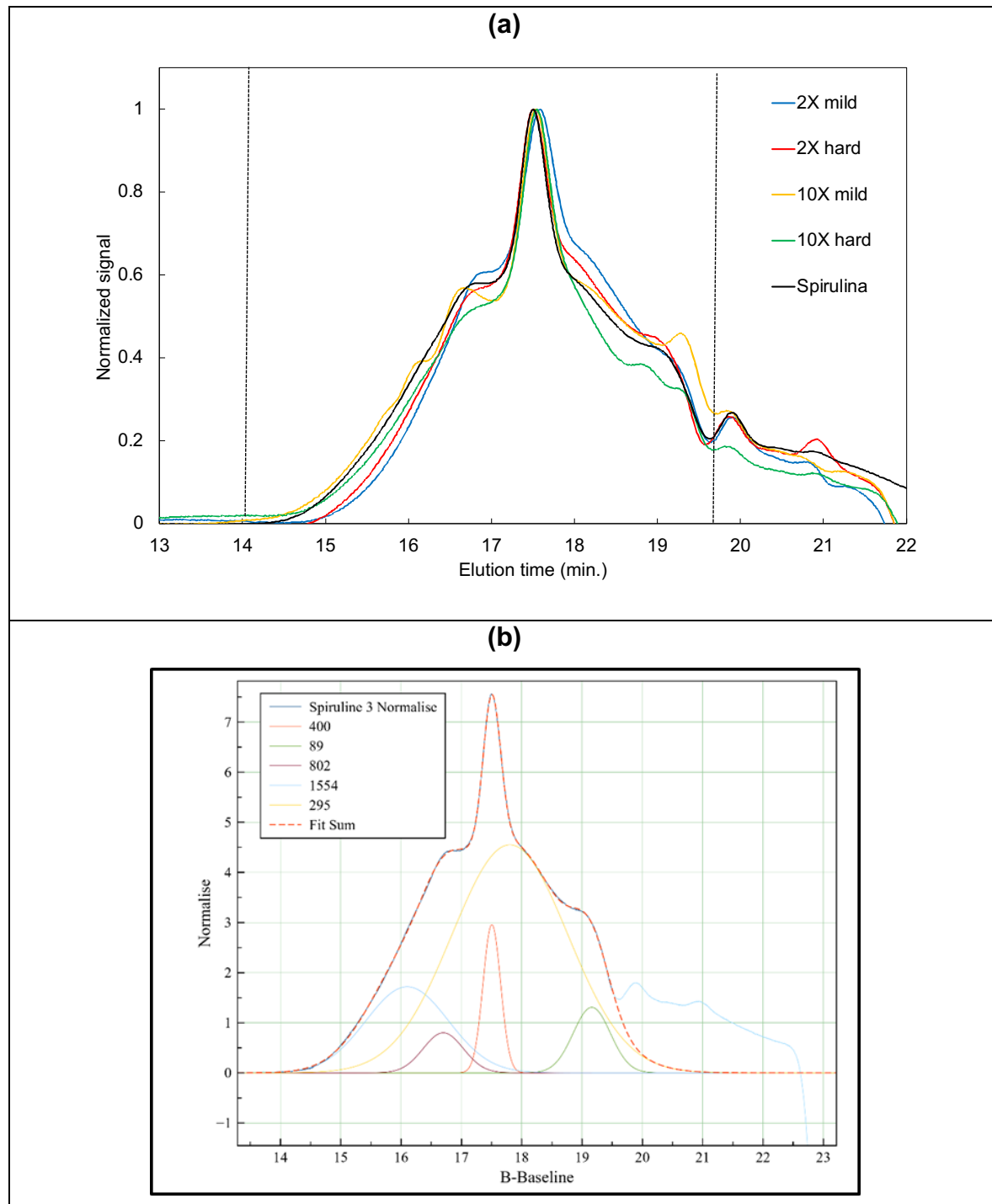


Figure 53: (a) Normalized elution curves obtained for the biocrude oil samples by SEC, and (b) fitting example for the biocrude oil from spirulina biomass.

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Similar deconvolutions were conducted for the other elution curves for *Pleurocapsa* biomass, with the curve fitting results provided in the supplementary data. In the following discussion, we only focus on the apex mass values and fractional areas of the peaks identified in oils produced from different biomass sources (Fig. 54a, 54b, and 54c).

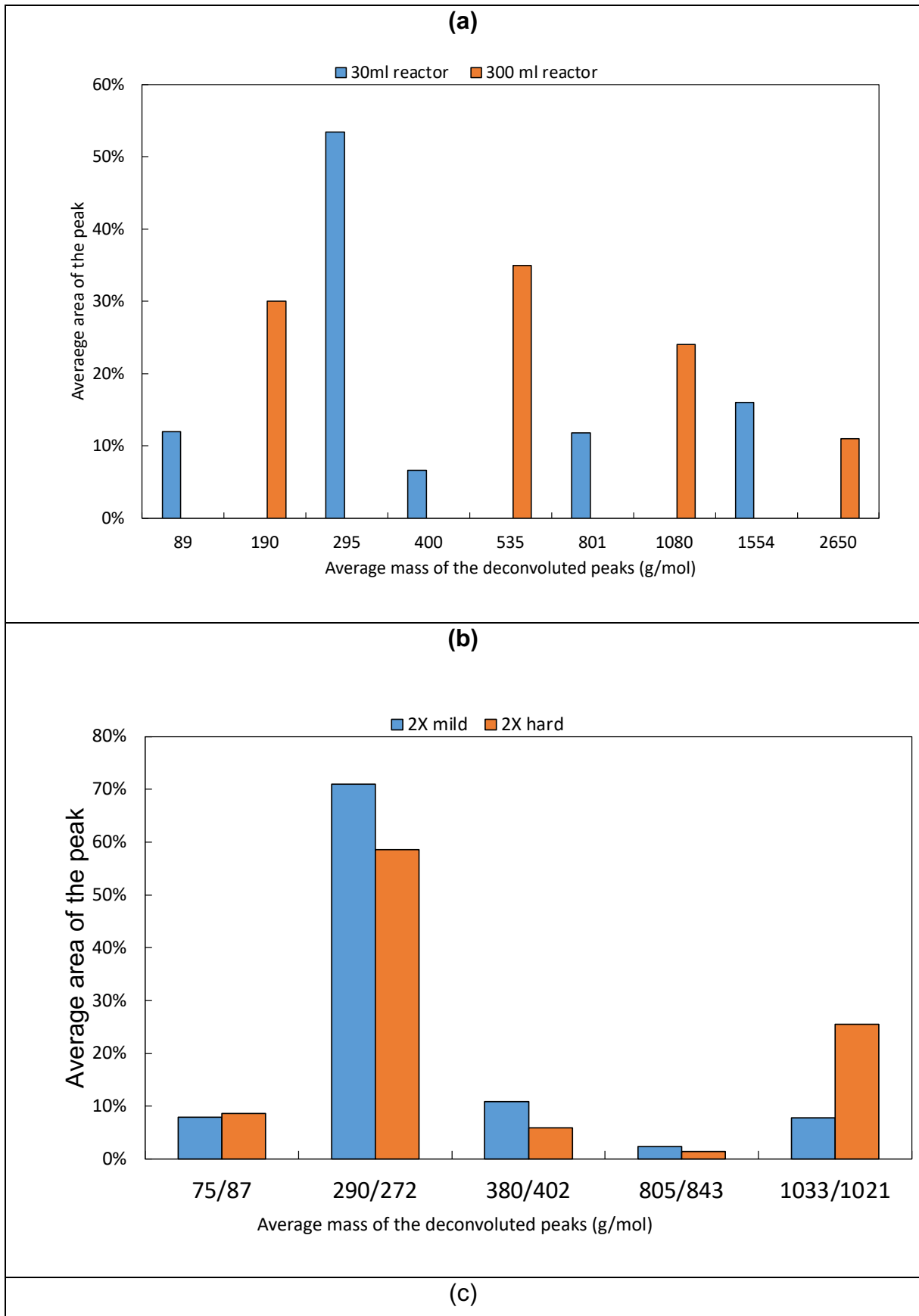
In fig. 54a, we plotted the peaks identified for the oil obtained from the HTL conversion of the same *Spirulina* biomass residue, using the 30 mL reactor in this study, and a controlled 300 mL reactor from a previous study (Rolland et al., 2020). In our case, five distinct molecular populations were identified. Notably, the peak with an apex at 400 g/mol had the smallest fractional area, representing only 7% of the total area below the elution curve. The peak with the largest fractional area (53%) had an apex at 295 g/mol, while the other peaks were centered at 89, 801, and 1554 g/mol. In contrast, the 300 mL reactor (Rolland et al., 2020) revealed only four populations with apexes at 2650, 1080, 535, and 190 g/mol. Qualitatively, the populations observed in our study using the 30 mL reactor are shifted toward lower molecular masses and more concentrated around 295 g/mol. This highlights the significant sensitivity of hydrothermal conversion to reaction conditions and emphasizes the challenges in reproducing results when scaling down the reactor size, as observed in this case.

Fig. 54b illustrates the distribution of the deconvoluted peaks for oils derived from 2X mild and hard biomasses, both showing five distinct molecular populations. The largest peak, with an apex around 280 g/mol, accounts for 70% of the total area for the oil obtained from 2X mild biomass, while the highest molecular mass peak, with an apex at 1033 g/mol, represents less than 8%. In contrast, for the oil obtained from 2X hard biomass, the area of the higher molecular mass peak significantly increases to 25%.

Fig. 54c presents the results for oils obtained from 10X mild and hard biomasses. It is noticed that the apexes of the deconvoluted peaks do not align perfectly. However, similar qualitative observations can be made: a primary peak around 320 g/mol accounts for 80% of the area in the oil from biomass with mild treatment, while the higher molecular weight peaks remain relatively weak (below 5%). In the oil from hard treated biomass, the area of the 320 g/mol peak is slightly reduced, but a significant peak emerges at 1264 g/mol, representing 21% of the area. This shift in molecular mass distribution may help explain the differences in the rheological behaviors observed between the biocrude oils.



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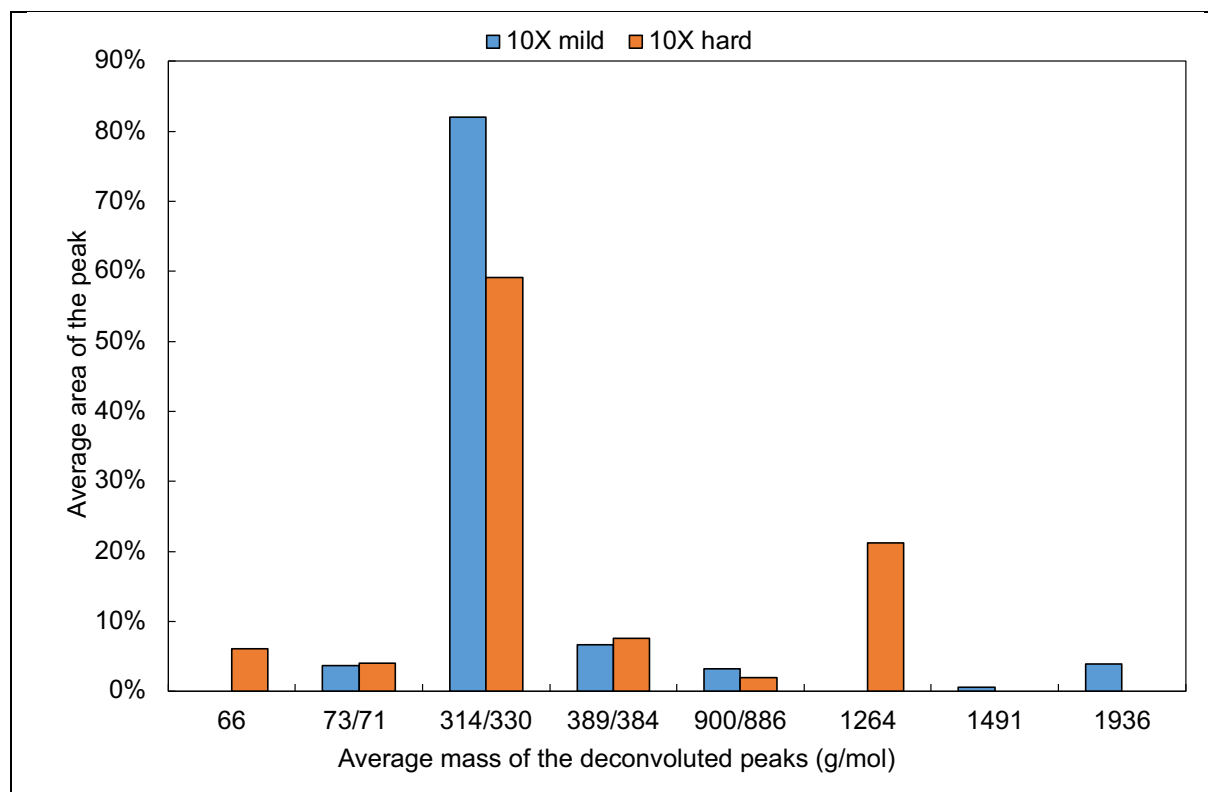


Figure 54 : Molecular mass of the main peaks for biocrude oils from *Spirulina* (a) and *Pleurocapsa* biomass: 2X mild and 2X hard (b); and 10X mild and 10X hard (c).

### 3.1.3.3. Low molecular weight compounds

GC-MS analyses of biocrude oil converted from 54-2X mild, 54-2X hard, 54-10X mild and 54-10X hard and from the *Spirulina* biomass were conducted to verify the organic compounds in the biocrude oil and elucidate the changes in the main volatile components of the biocrude post HPH treatment. The identification of specific compounds in biocrude is critical for assessing its suitability for various applications. The chemical structure of the compounds was identified by the highest similarity in MS library. This technique only provides partial information on the products composition and only the fraction with molecular weight < 300 g/mol were identifiable (Table 17). Results obtained by GC-MS were consistent with the SEC data confirming presence of molecules with low molecular weights between 84 g/mol as minimum and 280 g/mol as maximum, with abundance of the pics between 140- 200 and pics corresponding to N-heterocyclic compounds. The residual complex components of the biocrude with

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greater molecular weights than this value could not be detected due to the detection limit of GC-MS technique.

Analysis of the results revealed that the biocrude is a complex mixture, and a number of peaks were displayed in the total ion chromatograms. To ease the discussion, the major chemical compounds were classified into seven chemical families based on their relevant functional groups: hydrocarbons, fatty acids, carboxylic acids, phenols, esters, cyclic compounds and the N- heterocyclic compounds. These compounds were derived from carbohydrates, proteins and lipid components of the feedstock via different mechanisms. The lipid fraction produces hydrocarbons such as alkanes and alkynes after decarboxylation process, where the fatty acids are obtained after hydrolysis. On the other hand, deamination and decarboxylation of proteins lead to the formation of carboxylic acid and amine, respectively. Cyclic compounds are the products of the dehydration of the algal carbohydrates while phenols are the results of their ring opening dehydration, or from the reaction of fatty acids with ammonia. The esters are resulting from the reaction of carboxylic acids with alcohols. Comparison between the GC-MS profiles of both biomass from QUCCM 54 and *Spirulina* revealed that they presented similarity in the composition with varying percentages except for esters, which were not detected in the *Spirulina*'s biocrude oil. This composition was dominated by N- heterocyclic compounds which sum up to 59%, depending on the cultivation conditions, species and treatment applied, followed by hydrocarbons, fatty acids > phenols, carboxylic acids > esters, cyclic compounds. Tetradecanamide was the major N-heterocyclic compounds with 13.11%, followed by octanamide in addition to pyrroles, pyridines, indole and their derivatives (such as 1-methyl-2-pyrrolidinone, 1-methylpiperidine, methylindole). This is consistent with the previous literature about liquefaction of the low lipid, high starch and protein algae [299]. The dominance of nitrogenated compounds explains the high nitrogen content in the crude, however, literature shows that might negatively affect the final biocrude quality and subsequent upgrading are required [300]. Hydrocarbons were in the range of C<sub>6</sub>-C<sub>20</sub> and were dominated by 1-Methylnaphthalene (9.12%). Fatty acids were mainly hexanedioic (6.53%), tetradecanoic, pentadecanoic hexadecanoic, which is in consistent with the most commonly dominant fatty acids in microalgal biomass. Phenols were largely the phenyl phenols and 3,5-dimethoxy Phenol with 5.36%. Benzene-1,2-dicarboxylic acid was the most expressed carboxylic acids (4.16%), while Butanoic acid, 3-methyl-, 2-

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phenylethyl ester, and 1,3-2H-Isobenzofuranone were the sole ester and cyclic compound detected respectively. A noticeable change in the chemical composition following HPH treatment was witnessed (Fig. 55). Indeed, a reduction in the N-heterocyclic compounds was noted, which decreased from 54% to 41% and from 59% to 53% between the mild and hard treatment for 54-2X and 10X biomass, respectively, followed by % phenols. Additionally, we observed that the treatment promoted the formation of fatty acid fractions, as evidenced by their content, which increased under HTL processes from 10% to 15% and from 12% to 20%, between the mild and hard treated biomass of 54-2X and 54-10X, respectively, while the amount of hydrocarbons showed no discernible variations.

These aforementioned data obtained by GC-MS were highlighting the efficacy of the treatment in denitrogenating the biocrude and improving its quality and were consistent with the previously reported findings during this work regarding the positive effect of the HPH treatment applied.

Table 17: Example of the different groups detected in the volatile fraction of the biocrude analyzed through GC-MS technique.

<b><u>Hydrocarbons: Alkanes/alkenes/alkynes</u></b>	<b>Molecular weight (g/mol)</b>
Cyclohexacane	84
4-Decanone: C <sub>10</sub> H <sub>20</sub> O	156
1-Methylnaphthalene: C <sub>11</sub> H <sub>10</sub>	142
Benzene, (4-methyl-4-pentenyl): C <sub>12</sub> H <sub>16</sub>	160
3,7,11,15-tetramethyl-2-hexadecene: C <sub>20</sub> H <sub>40</sub>	280
<b><u>Fatty acids</u></b>	<b>Molecular weight (g/mol)</b>
Tetradecanoic acid: C <sub>14</sub> H <sub>28</sub> O <sub>2</sub>	228
Pentadecanoic acid: C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>	242
Hexadecanoic acid: C <sub>16</sub> H <sub>32</sub> O	256
Hexanedioic acid: C <sub>18</sub> H <sub>30</sub> O <sub>4</sub>	146

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<b><u>Carboxylic, dicarboxylic acids</u></b>	<b>Molecular weight (g/mol)</b>
Succinic acid: C <sub>4</sub> H <sub>6</sub> O <sub>4</sub>	118
benzene-1,2-dicarboxylic acid: C <sub>8</sub> H <sub>6</sub> O <sub>4</sub>	166
<b>Aromatic organic compounds: Phenol derivatives</b>	<b>Molecular weight (g/mol)</b>
m-ethyl-phenol: CH <sub>3</sub> C <sub>6</sub> H <sub>4</sub> (OH)	108
Phenol, 3,5-dimethoxy: C <sub>8</sub> H <sub>10</sub> O <sub>3</sub>	154
p-Phenylphenyl acetate: C <sub>14</sub> H <sub>11</sub> O <sub>2</sub>	211
2,6-diphenylphenol: C <sub>18</sub> H <sub>14</sub> O	246
<b><u>Esters</u></b>	<b>Molecular weight (g/mol)</b>
<u>Butanoic acid, 3-methyl-, 2-phenylethyl ester</u> C <sub>13</sub> H <sub>16</sub> O <sub>2</sub>	206
<b><u>Cyclic compounds</u></b>	<b>Molecular weight (g/mol)</b>
1,3-2H-Isobenzofuranone: C <sub>11</sub> H <sub>12</sub> O <sub>5</sub>	202

<b><u>N-Heterocyclic compounds : Amine/Amide derivatives</u></b>	<b>Molecular weight (g/mol)</b>
2-Hydroxypyridine-3-carboxylic acid: C <sub>6</sub> H <sub>5</sub> NO <sub>3</sub>	139
1-Methyl-2-piperidinone: C <sub>6</sub> H <sub>11</sub> NO	113
3,4-dihydropyrimidin-2(1h)-one: C <sub>4</sub> H <sub>6</sub> N <sub>2</sub> O	98
Pyridine, 2-methoxy-5-nitro: C <sub>6</sub> H <sub>6</sub> N <sub>2</sub> O <sub>3</sub>	154
N,N'-Dimethylpiperazine: C <sub>6</sub> H <sub>14</sub> N <sub>2</sub>	114
Acetamide, N,N-bis(1-methylethyl): C <sub>8</sub> H <sub>17</sub> NO	143
1-Methyl-9 Pyrid [3,4-b]indole: C <sub>12</sub> H <sub>10</sub> N	182
9-Octadecenamide: C <sub>18</sub> H <sub>35</sub> NO	281
Tetradecanamide: C <sub>14</sub> H <sub>29</sub> NO	227
N-Methyl hexadecanamide : C <sub>17</sub> H <sub>35</sub> NO	269
Octanamide, N,N-dimethyl: C <sub>13</sub> H <sub>28</sub> N <sub>2</sub> O	171

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N,N-Dimethylacetamide : C <sub>4</sub> H <sub>9</sub> NO	87
3-Benzyl-6-isopropyl-2,5-piperazinedione: C <sub>14</sub> H <sub>18</sub> N <sub>2</sub> O <sub>2</sub>	246
N,N-Diethyldodecanamide: C <sub>16</sub> H <sub>33</sub> NO	255
Isocyanuric acid : C <sub>3</sub> H <sub>3</sub> N <sub>3</sub> O <sub>3</sub>	129
Acetamide, N-(2-phenylethyl): C <sub>16</sub> H <sub>17</sub> NO	239
3-Benzyl-piperazine-2,5-dione: C <sub>11</sub> H <sub>12</sub> N <sub>2</sub> O <sub>2</sub>	204

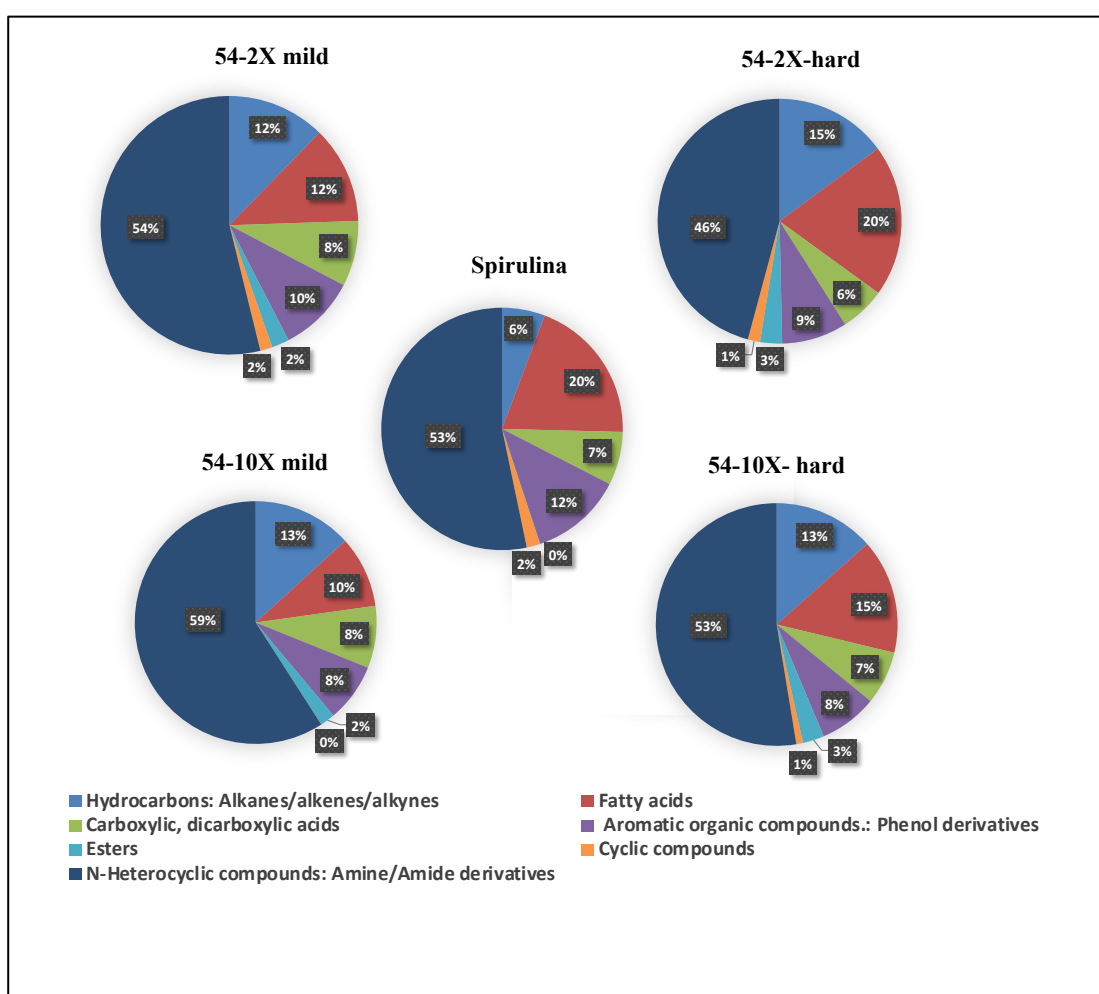


Figure 55: Distribution of the different chemical groups in the biocrude oil issued after HTL conversion of the biomass obtained before from mild and hard treatment.

### 3.2. Thermorheological behavior

To assess the potential of HTL oil fractions as a substitute of petroleum-based bitumen, their rheological properties were investigated.

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Figures 56 and 57 present Black diagrams plotted from rheological data, which help identify whether the oils exhibit bitumen-like behavior. In these plots, the measured phase angles ( $\Phi$ , in degrees) are plotted against the complex modulus ( $G^*$ ).

A phase angle close to  $90^\circ$  indicates nearly liquid behavior, while an angle close to  $0^\circ$  reflects a nearly solid state. Values between these extremes represent viscoelastic behavior. For conventional bitumen, the plot forms a characteristic continuous curve, as shown in Figure 56a. At the lowest tested temperatures, the material behaves more like a solid, exhibiting a high modulus and a low phase angle. As the temperature rises, the modulus decreases, and the phase angle progressively increases, eventually approaching liquid-like behavior. This trend is crucial for distinguishing between materials that mimic bitumen and those that do not.

In Fig. 56b and 56c, the data for the biocrude oils derived from 2X mild and 10X mild biomasses clearly do not exhibit a thermo-rheological behavior comparable to conventional bitumen. Their rheology is predominantly characterized by viscous behavior, with phase angles ( $\Phi$ ) around  $80$ - $90^\circ$ . However, at the lowest temperatures tested, the oil from 10X mild biomass shows a more solid-like behavior with no continuation between both states. This rheological behavior can be explained by the dominance of compounds with low molecular weight, approximately  $290$  g/mol for 2X mild and  $314$  g/mol for 10X mild. These fractions make up the majority of the biocrude oils, while only a small portion of higher molecular weight compounds, above  $1000$  g/mol, are present (as shown in (Fig. 54b and 54c). This molecular distribution, with a lack of significant high molecular weight components, contributes to the predominantly viscous behavior observed in the rheological analysis.

In contrast, it was notable from the rheology of biocrude oil samples derived from QUCCCM 54 2X hard, QUCCCM 54 10X hard and *Spirulina* biomass, that they exhibited a viscoelastic behavior comparable to that of a conventional bitumen and to the previously reported for *Spirulina* processed with a 300 mL HTL reactor [231]. In point of fact, the rheological data plotted in the black diagram (Fig. 57b and 57c) showed a continuous curve from  $80^\circ\text{C}$  to  $0^\circ\text{C}$  with newtonian behavior at high temperatures where the material is viscous and presented much smaller modulus ( $1\text{E}^{+2}$  Pa), and at lower temperatures, the complex viscosity tends to decrease, and the dynamic modulus increased ( $1\text{E}^{+8}$  Pa) showing a transition towards more elastic solid material. Moreover, the continuity of the curve reflects the thermostability of the

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material molecular structures [205]. This rheological behavior may be ascribed to the changes of composition of the biocrude oil when HPH treatment is performed. Indeed, the SEC analysis revealed a consistent trend across all these biocrude oils in terms of molecular populations: a dominant population centered around 300 g/mol, accounting for 50-60% of the total area, alongside a second significant population above 1000 g/mol, representing approximately 20-30% of the total area mostly generated by polymerization reactions in the biocrude. The increase in fatty acids and decrease of nitrogenous and oxygenated compounds highlighted in the GC MS data also supports this assumption.



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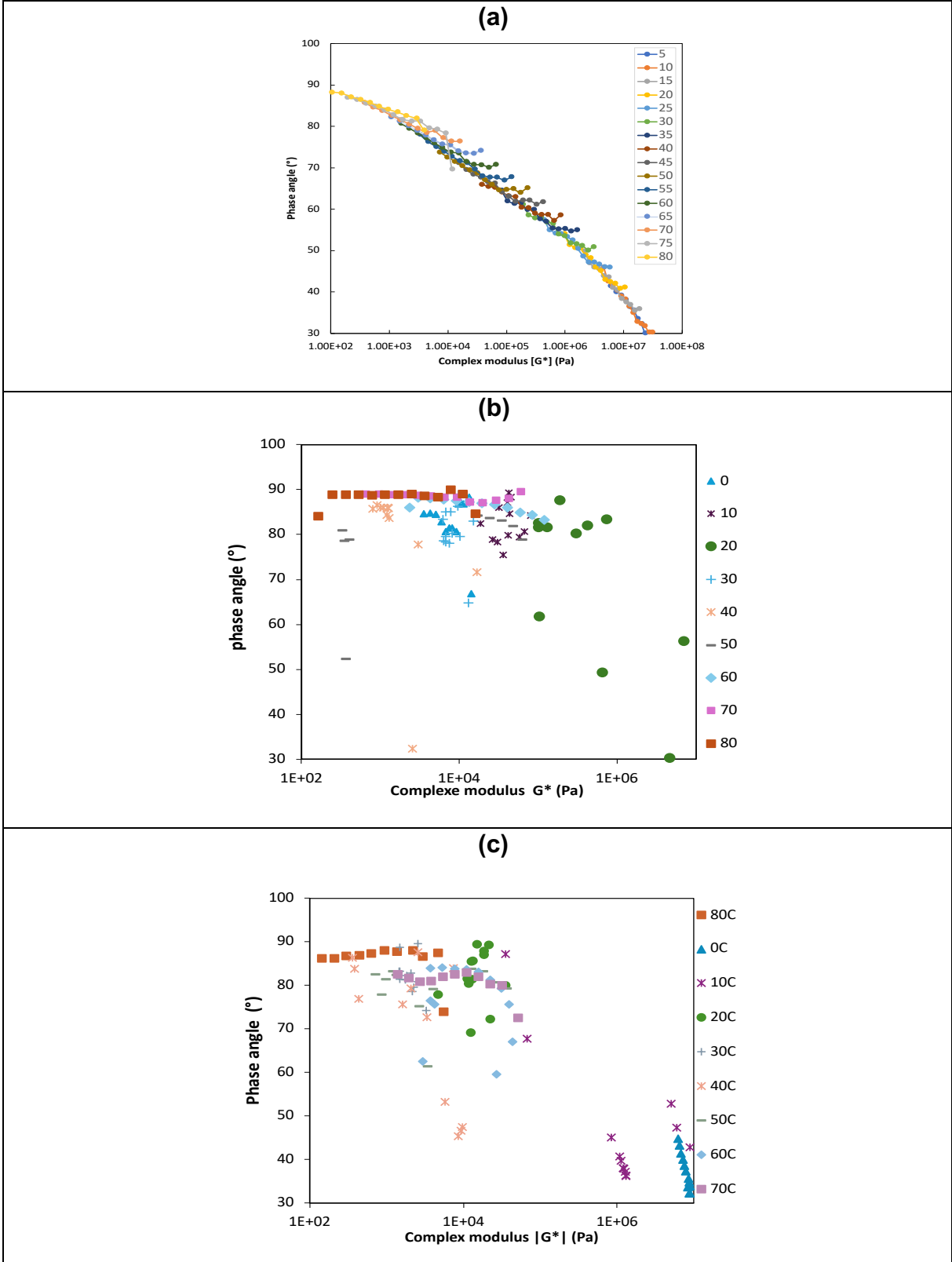


Figure 56 : Black diagrams for conventional bitumen (a), and oils obtained from biomass residues 2X mild (b) and 10X mild (c).

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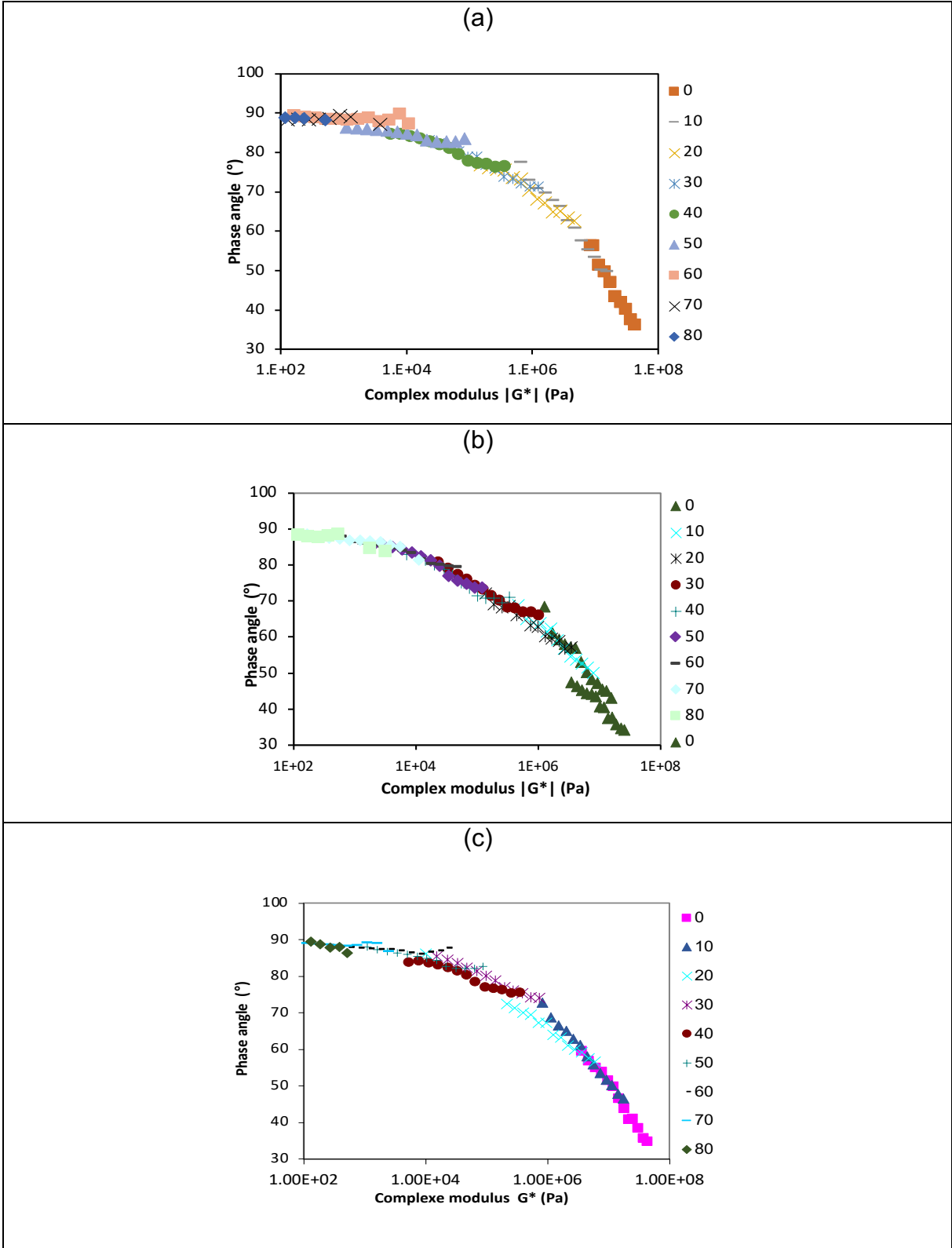


Figure 57: Black diagrams for oils from 2X hard (a), 10X hard (b) and Spirulina biomass (c.)

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Fig. 58 shows the norm of the complex modulus (at 1 Hz) versus temperature, for the biocrude oil fractions of QUCCCM 54 2X hard, QUCCCM 54 10X hard and *Spirulina* sp, compared to standard bitumen. Both biocrude oil from cyanobacterial residues and conventional bitumen exhibited temperature dependent stiffness changes. However, even though the overall viscoelastic signature was similar to conventional bitumen, the stiffness measured at 1 Hz of the HTL fractions of both QUCCCM 54 and *Spirulina* sp., residues was lower than the petroleum bitumen except for the high temperature range (50-80 °C) where the stiffness of the hydrophobic fraction obtained from QUCCCM 54-10X hard was close to the conventional bitumen. The extent of this sensitivity may vary based on the specific characteristics of each type. Variations in processing conditions between bio bitumen and the conventional one can also influence the resulting material properties leading to differences in stiffness. Difference in the chemical composition of algal biocrude also can explain the difference observed in the stiffness. Indeed, lower molecular weight compounds can contribute to reduced stiffness. Additionally, presence of oxygen-containing compounds in the biocrude oil derived from algae biomass imparts the flexibility and reduces stiffness compared to hydrocarbons in conventional bitumen. The molecular weight and structure of the hydrocarbons in bio bitumen may also differ from those in conventional bitumen. Low stiffness generally is regarded in situations where the material needs to accommodate movement as it offers better flexibility, making it suitable for areas with temperature fluctuations or where deformation resistance is less critical. However, it would not be ideal for heavy-duty applications that require high stiffness and load-bearing capacity.

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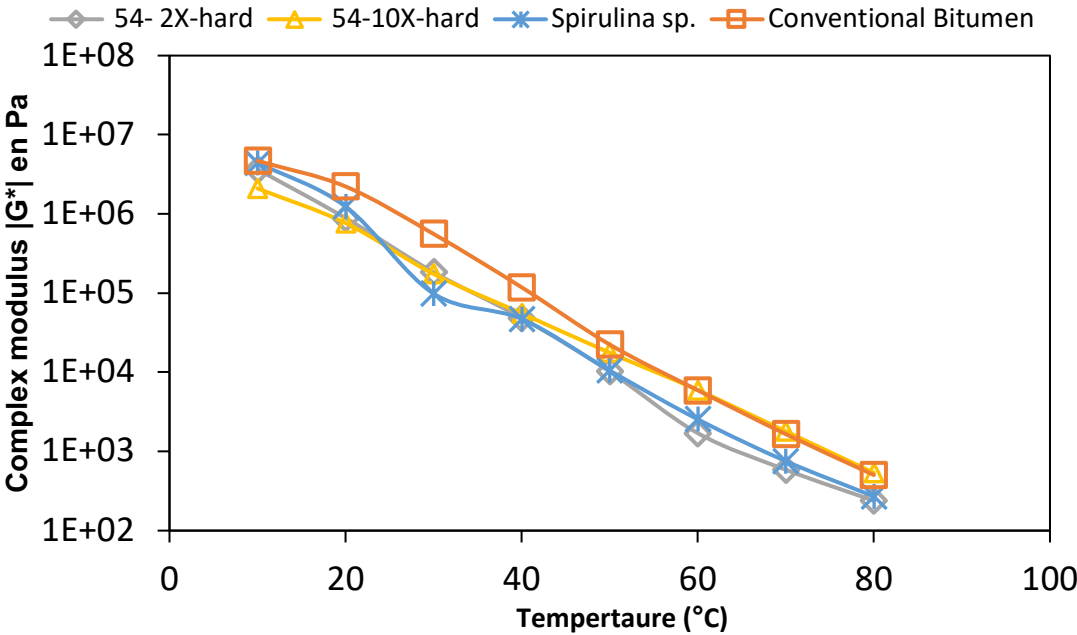


Figure 58 : Norm of the complex modulus as a function of temperature, measured at 1 Hz for the biocrude oil fractions obtained from HTL conversion of the different biomass feedstocks, in comparison with a conventional bitumen.

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### Conclusion

The findings of this study highlight the potential of applying the biorefinery concept for producing bio-bitumen from biomass residues of marine strains through hydrothermal highlighting the possible extrapolation of findings from freshwater strains to selected Qatar marine cyanobacteria species.

Preliminary hydrothermal conversion studies using industrial *Spirulina* residues indicate that the 30 mL Swagelok reactor produces a hydrophobic phase yield of 50 %, which is comparable to the yield obtained from larger, stirred, and better-controlled reactors previously applied for the conversion. Thus, it was used for a comparative HTL analysis on the limited biomass residues from the four cyanobacteria strains cultivated indoors and processed using a "mild" pigment extraction method, as described in Chapter 3. Nevertheless, after separation of biocrude oil and solid particles, the data of the resulting biocrude oil phase yields from the local strains are relatively low, ranging from 9-28%. This decrease in yield is likely associated with the higher ash content present in the biomass before conversion.

Despite the low conversion yields, the rheological study of the extracted oils provides proof of concept for the feasibility of bio-bitumen production from certain local isolates. Indeed, the study shows that the biocrude oil from both *Chroococcidiopsis* sp. QUCCCM 34 and *Cyanobacterium aponium*. QUCCCM 129 exhibit bitumen-like rheological properties, indicating their potential for bio-bitumen production. Conversely, *Pleurocapsa* sp. QUCCCM 54, which displayed the highest growth/PBPs content, results in the lowest biocrude oil phase yield with no bitumen-like behavior. This contrast underscores the variability among strains and highlights the need for further optimization and analysis of different strains to maximize bio-bitumen yield and quality.

The second part of the study was targeting the *Pleurocapsa* biomass obtained for outdoor cultivations and focused on the ash content before hydrothermal conversion. It was observed that only the simulated "hard" pigment extraction process leads to significant reduction in the ash content. This decrease presents a strong influence on

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hydrothermal conversion process efficiency, leading to a higher biocrude oil phase yield and a modification of its composition in terms of molecular mass distribution. In particular, a significant fraction of molecules with masses above 1000g/mol appears, similar to the composition of biocrude oil derived from *Spirulina*. Concurrently, the rheological behaviour of these biocrudes exhibited bitumen-like characteristics, suggesting that this property can only be obtained if higher molecular mass molecules are formed during the hydrothermal conversion.

Besides, it was observed that the culture conditions in particular the urea concentration didn't influence strongly the yield of the hydrothermal products. However, the bio bitumen obtained slightly differ in terms of stiffness. Therefore, the cultivation conditions may affect the potential applications, and the type of petroleum bitumen that may be replaced or amended with the bio-bitumen produced.

Finally, this study emphasizes the possible integration of biorefinery concept to produce bio bitumen from biomass treated with HPH, promoting a more sustainable approach to biomass valorisation. It also highlights the challenge posed by the high ash content found in the local strains. However, this issue can be effectively addressed by optimizing the downstream processing steps prior to hydrothermal conversion.

# **General Conclusion and Perspectives**

## Conclusion and Perspectives

### 1. General conclusion

This thesis aimed to contribute to Qatar's commitment to achieving environmental sustainability and contributing to global efforts to mitigate climate change and minimize CO<sub>2</sub> emissions. Focusing on microalgae due to their unique ability to capture atmospheric carbon dioxide and convert it into valuable biomass through photosynthesis, this study, like other recent works, highlights the potential of microalgae in carbon sequestration. However, it stands out as the first research to explore the ability of local extremophilic strains from Qatar to produce phycobiliproteins under outdoor conditions, while evaluating the potential valorization of the coproducts as bio-bitumen, offering an innovative approach to resource utilization and sustainability. Given the scarcity of freshwater resources in the region, four marine local strains were chosen for this study and were assessed for their ability to grow under varying temperature and salinity ranges reflective of Qatar's natural environment.

In the first part of our experimental study, presented in chapter 3, the strains were initially identified based on their 16s rDNA sequence as *Chroococcidiopsis* (QUCCM 34), *Pleurocapsa* (QUCCM 54), *Euhalothece* (QUCCM 77) and *Cyanobacterium* (QUCCM 129). The primary data highlighted their thermo and halo tolerance along with their suitability as viable candidates for PBPs production, which are highly valuable products known for their diverse biotechnological applications. The study also allowed to determine the conditions leading to the highest growth and pigment expression. Bioprospecting of the crude PBPs extracts reported remarkable antioxidant properties which qualifies them as potential candidate for several uses such as natural colorant, in cosmetics, pharmaceutical and health food industries. Among the strains studied, *Pleurocapsa* sp. displayed the highest biomass productivity and CO<sub>2</sub> capture rate with ability to produce its highest phycobiliprotein in particular phycocyanin at early growth stage allowing faster utilization of resources, reducing the risk of contamination, and enhancing the economic profitability by minimizing the time and resources required for both biomass and pigment production. In addition, the strain displayed ability to self-settle due to its large size and colony forming characteristics. This trait significantly benefits large-scale biomass harvesting, as it enables easy separation from the culture



## Conclusion and Perspectives

through sedimentation—a low-energy, cost-effective process that further enhances the strain's potential for industrial applications.

One of the challenges for biomass production is not only identifying strains that can produce marketable products but also ensuring their growth under relevant outdoor conditions. In light of this, the second part of our experimental work, presented in chapter 4, focused on the outdoor cultivation of *Pleurocapsa* sp to further assess its potential for commercial biomass production under real Qatar's desertic environment. As strategy to reduce the cost of high nitrogen requirement in mass culturing, urea, which was supplied by a local fertilizer company and proved efficient in terms of biomass and pigment productivity at laboratory scale, was adopted as alternative nitrogen source in large scale production.

Outdoor cultivations were performed using 200L raceway ponds, with a specific focus on adjusting urea concentrations as approach to minimize nitrogen usage while enhancing biocrude yield and potentially the rheological properties of the resulting biomass. The initial outdoor cultivation using a 10% inoculum proved unsuccessful. Consequently, the experiment was repeated with a higher initial biomass density of 20% and included partial shading of the ponds after inoculation. By implementing these measures, the strain displayed its aptitude to grow outdoor and withstand the harsh uncontrolled environmental conditions. However, the performance of the outdoor culture differed significantly from that observed in indoor experiments, with lower biomass productivity and reduced PBPs accumulation. This discrepancy suggests that the strain encountered difficulties adapting to the outdoor environment. Factors such as elevated light intensities, UV radiation, and low inoculum volume, which were not simulated under laboratory conditions, are hypothesized to contribute to the observed strain behavior outdoors, highlighting the importance of further optimization and adaptation strategies for successful outdoor cultivation.

The last part of this work, presented in chapter 5, focused on applying the biorefinery concept to optimize biomass utilization post-phycobiliprotein extraction. This was achieved by testing a lab scale hydrothermal conversion process to transform the biomass residues into bio-bitumen, building on previous research work conducted with industrially cultivated strains such as *Spirulina*, which served as a reference during our

## Conclusion and Perspectives

study. The results confirmed the feasibility of bio-bitumen production for some indigenous strains. However, a significant challenge emerged due to the high ash content in their biomass, which posed a disadvantage in the conversion process. Indeed, for the selected *Pleurocapsa* strain, this has resulted in a low biocrude oil yield and the absence of bitumen-like rheological behavior. Nonetheless, it was possible to overcome this issue by modifying the pigment extraction treatment. While a “mild” extraction process leaves the ash content almost unchanged, a “hard” extraction process, simulated through high-pressure homogenisation treatment, significantly reduced the ashes. Subsequently, the yield in biocrude oil obtained by hydrothermal conversion increases, and the bitumen like rheology is observed. Molecular mass distribution analysis of the biocrude oil samples revealed a predominant population around 300 g/mol, typical of smaller molecular structures. However, in samples displaying bitumen-like rheology, a significant fraction of molecules with masses above 1000 g/mol was observed—similar to the bio-bitumen obtained from *Spirulina*. This indicates that the presence of larger, more complex molecules is crucial for achieving the rheological properties characteristic of bitumen.

All in all, this thesis marks a pivotal step forward in harnessing the potential of native extremophilic cyanobacteria for phycocyanin production in desert environments. The successful outdoor cultivation of *Pleurocapsa* sp, QUCCCM 54 underscores its adaptability to harsh environmental conditions, presenting a viable pathway for large-scale biomass production without excessive resource input. This is particularly relevant in regions with arid climates like Qatar, where conventional agricultural practices encounter challenges due to water scarcity and high temperatures. Furthermore, the research highlights the importance of valorizing residual biomass generated from pigment extraction processes. By converting these residues into valuable bioproducts such as bitumen, the study promotes a circular economy approach, reducing waste generation and maximizing resource utilization, thus, enhancing the economic and environmental sustainability of cyanobacterial cultivation.

Additionally, the successful application of hydrothermal liquefaction to marine biomass indicates the potential to diversify the range of feedstocks available for bio-bitumen production. This expansion could be particularly advantageous for Qatar's commitment

## Conclusion and Perspectives

to renewable energy initiatives and the diversification of its economy, ultimately contributing to sustainable production practices for future generations.

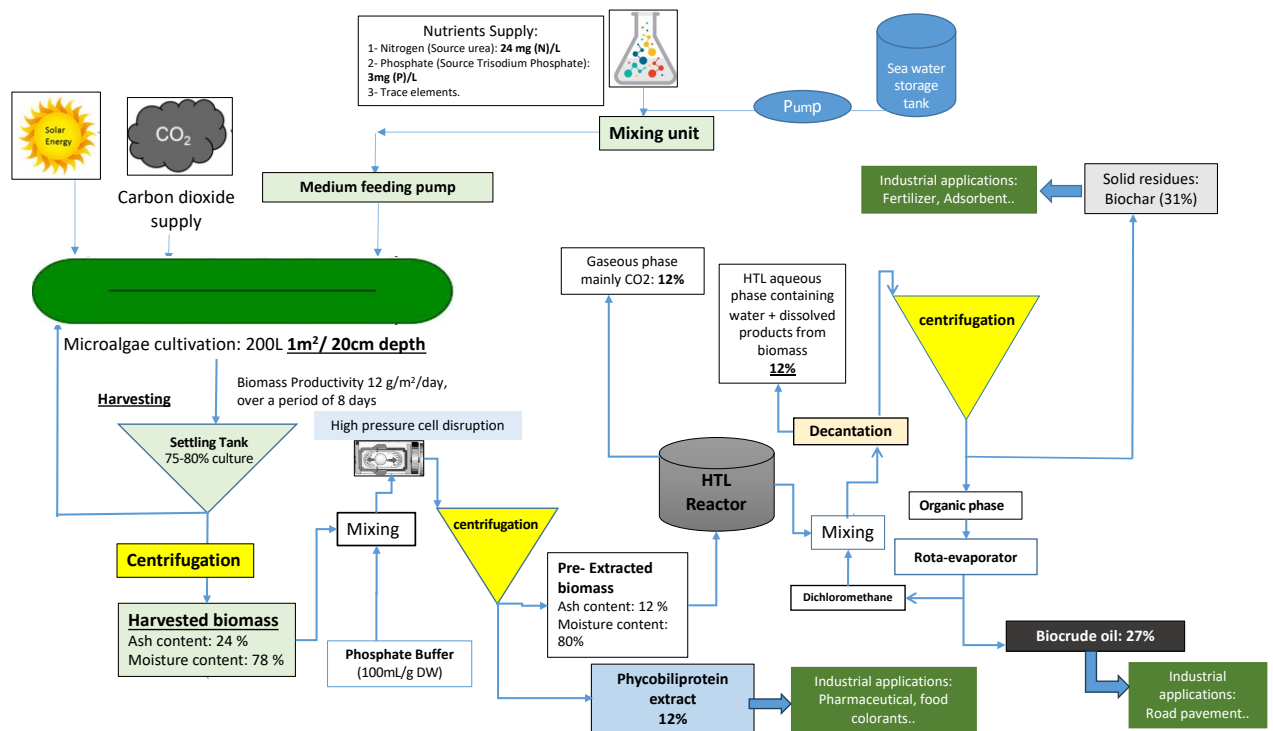


Figure 59: Schematic presentation of the full process from biomass cultivation to phycobiliprotein extraction, and finally conversion of the leftover biomass through HTL reaction.

## 2. Opened questions for future studies

Despite the promising results of the current study, challenges remain, and several areas can be improved through investigation of additional scientific aspects related to strain cultivation, biomass pre-treatment, PBPs production, subsequent biomass conversion and HTL process.

The methodology developed in this work focused on investigating temperature and salinity as cultivation factors relevant to Qatar's climate, and that their indoor study in controlled photobioreactors would allow a smooth transition to outdoor cultivation. However, the significantly lower productivity observed in outdoor cultivation suggest that light may also be a dominant factor. Study of this parameter at the PBR scale using artificial light able to reproduce the daily diurnal fluctuations across different seasons, and their effects on the growth performance, metabolite profiles, and PBPs

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synthesis of the selected *Pleurocapsa* sp. would be interesting and can aid in rational optimization of microalgal cultures under outdoor conditions.

Besides, the observed high ash content was quite unexpected. It would be interesting to further investigate the metabolism of the strains to understand where it comes from. Notably, the EDX characterization revealed substantial amounts of calcium present in the biomass of *Pleurocapsa*. This finding suggests that the strain may possess the capacity to produce calcium carbonate, a process that serves as an effective mechanism for CO<sub>2</sub> capture.

In terms of downstream processing, it would be necessary to obtain higher biomass quantities to fully assess:

- The effect of the pigment extraction process on the ash content and remaining material. The simulated “hard” extraction process should be replaced by the real pigment extraction processes to optimize the biorefinery scheme.
- The potential of the indigenous strains in terms of biocrude oil yield. By comparing the tests conducted with previous results of the reference *Spirulina* biomass obtained in larger, more controlled reactors, it was concluded that the conversion process may not be optimal with the small reactor. Optimization of the process can be proposed through changes in the reaction time to achieve optimal thermal conditions, exploring the use of catalysts to increase reaction efficiency, and optimizing the loading ratio of biomass to solvent.
- It would be equally interesting to look further into the storage stability of the bio bitumen produced. Understanding its stability over time is crucial for its practical application in real-world scenarios.

Last but not least, addressing the valorization of both the aqueous phase and the solid residues is critical for enhancing the overall sustainability of the biorefinery process. The aqueous phase, rich in essential nutrients such as nitrogen and phosphorus, presents opportunities for further nutrient recycling, particularly for algal cultivation or other biomass resources. Alternatively, the remaining carbon source in the aqueous phase can be used to produce synthetic natural gas by gasification or methane by anaerobic digestion. Moreover, given the high amount solid particles produced, it may be more interesting from an economical point of view to investigate the production of

## **Conclusion and Perspectives**

biochar that may serve as a soil amendment for agricultural applications or as an adsorbent in wastewater treatment.

Finally, the economic and environmental merits are key factors in determining the viability of the proposed process. Comprehensive modeling of the entire cultivation and downstream processing framework is essential to understand the associated costs and environmental impacts; however, this was not feasible in the present study due to the challenges of evaluating certain parameters at the laboratory scale.

By addressing these key areas, it would be possible to develop a sustainable scalable PBPs production and HTL process, ensuring consistent quality and yield in industrial applications and/or highlight areas for improvement of more durable and reliable process.

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## Appendix

Table 18: % C-PC and %APC in the crude extract of the 4 screened cyanobacteria strains during the different cultivation days.

		% C-PC						
		Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12
QUCCCM	34-20C-40ppt	2.013	1.579	2.130	2.766	5.453	0.674	0.837
QUCCCM	34-30C-40ppt	2.013	2.839	3.100	3.204	4.036	2.114	2.540
QUCCCM	34-40C-40ppt	2.013	5.045	5.04				
QUCCCM	34-20C-60ppt	2.078	3.200	5.560	6.432	7.419	1.620	4.347
QUCCCM	34-30C-60ppt	2.078	3.100	6.370	6.257	6.189	2.765	3.980
QUCCCM	34-40C-60ppt	2.078	3.900	9.60	7.836	7.232	6.950	6.900
		Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12
QUCCCM	77-30C-40ppt	3.214	6.320	8.622	9.776	10.840	11.259	
QUCCCM	77-40C-40ppt	3.214	6.980	7.379	5.900	4.620	3.145	
QUCCCM	77-30C-60ppt	3.872	7.200	9.196	9.670	10.630	9.441	
QUCCCM	77-40C-60ppt	3.872	4.630	9.915	9.963	7.650	5.919	
		Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12
QUCCCM	54-20C-40ppt	1.648	1.960	2.143	1.406	1.520	1.745	2.701
QUCCCM	54-30C-40ppt	1.648	2.759	5.817	6.719	6.984	6.499	4.961
QUCCCM	54-40C-40ppt	1.648	5.149	10.801	8.189	7.657	7.639	7.356
QUCCCM	54-30C-60ppt	1.039	4.808	5.468	6.015	5.673	7.033	4.131
QUCCCM	54-40C-60ppt	1.039	6.312	7.823	8.441	5.804	6.485	5.247
		Day 0	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12
QUCCCM	129-20C-40ppt	0.610	0.538	2.527	2.477	4.371	6.026	
QUCCCM	129-30C-40ppt	0.610	2.448	4.941	6.754	8.002	7.849	
QUCCCM	129-40C-40ppt	0.610	6.047	6.957	7.537	8.760	6.643	

## Appendix

<b>QUCCCM</b>	<b>129-30C-60ppt</b>	2.025	0.701	1.019	5.872	6.883	5.900	
<b>QUCCCM</b>	<b>129-40C-60ppt</b>	2.025	1.176	3.775	9.661	6.979	3.528	

### % APC

		<b>Day 0</b>	<b>Day 2</b>	<b>Day 4</b>	<b>Day 6</b>	<b>Day 8</b>	<b>Day 10</b>	<b>Day 12</b>
<b>QUCCCM</b>	<b>34-20C-40ppt</b>	1.419	0.011	0.006	0.020	0.035	0.005	0.005
<b>QUCCCM</b>	<b>34-30C-40ppt</b>	1.419	0.020	0.018	0.016	0.016	0.010	0.010
<b>QUCCCM</b>	<b>34-40C-40ppt</b>	1.419	0.058	0.044				
<b>QUCCCM</b>	<b>34-20C-60ppt</b>	2.047	0.025	0.027	0.037	0.049	0.027	0.052
<b>QUCCCM</b>	<b>34-30C-60ppt</b>	2.047	0.039	0.013	0.036	0.133	0.021	0.017
<b>QUCCCM</b>	<b>34-40C-60ppt</b>	2.047	0.070	0.006	0.063	0.060	0.042	0.038
		<b>Day 0</b>	<b>Day 2</b>	<b>Day 4</b>	<b>Day 6</b>	<b>Day 8</b>	<b>Day 10</b>	<b>Day 12</b>
<b>QUCCCM</b>	<b>77-30C-40ppt</b>	2.228	2.432	2.450	2.959	2.968	3.476	
<b>QUCCCM</b>	<b>77-40C-40ppt</b>	2.228	4.125	3.283	2.603	2.485	2.302	
<b>QUCCCM</b>	<b>77-30C-60ppt</b>	0.979	1.950	2.810	3.394	2.945	2.515	
<b>QUCCCM</b>	<b>77-40C-60ppt</b>	0.979	1.895	2.843	2.607	2.012	1.590	
		<b>Day 0</b>	<b>Day 2</b>	<b>Day 4</b>	<b>Day 6</b>	<b>Day 8</b>	<b>Day 10</b>	<b>Day 12</b>
<b>QUCCCM</b>	<b>54-20C-40ppt</b>	1.397	2.170	2.865	1.303	2.054	1.346	1.199
<b>QUCCCM</b>	<b>54-30C-40ppt</b>	1.397	2.545	4.284	4.024	3.899	3.963	4.525
<b>QUCCCM</b>	<b>54-40C-40ppt</b>	1.397	4.094	5.606	5.018	5.242	4.990	4.372
<b>QUCCCM</b>	<b>54-30C-60ppt</b>	0.680	1.892	2.527	2.672	2.468	3.162	2.008
<b>QUCCCM</b>	<b>54-40C-60ppt</b>	0.680	2.583	3.358	3.486	3.174	2.556	2.654
		<b>Day 0</b>	<b>Day 2</b>	<b>Day 4</b>	<b>Day 6</b>	<b>Day 8</b>	<b>Day 10</b>	<b>Day 12</b>
<b>QUCCCM</b>	<b>129-20C-40ppt</b>	0.332	0.325	0.855	1.560	1.310	1.235	
<b>QUCCCM</b>	<b>129-30C-40ppt</b>	0.332	1.026	2.174	2.548	3.233	2.190	

## Appendix

<b>QUCCCM</b>	<b>129-40C-40ppt</b>	0.332	2.767	3.157	3.422	4.076	2.333	
<b>QUCCCM</b>	<b>129-30C-60ppt</b>	1.059	0.425	0.417	8.457	9.088	2.358	
<b>QUCCCM</b>	<b>129-40C-60ppt</b>	1.059	0.830	1.424	4.655	7.439	3.315	

Appendix

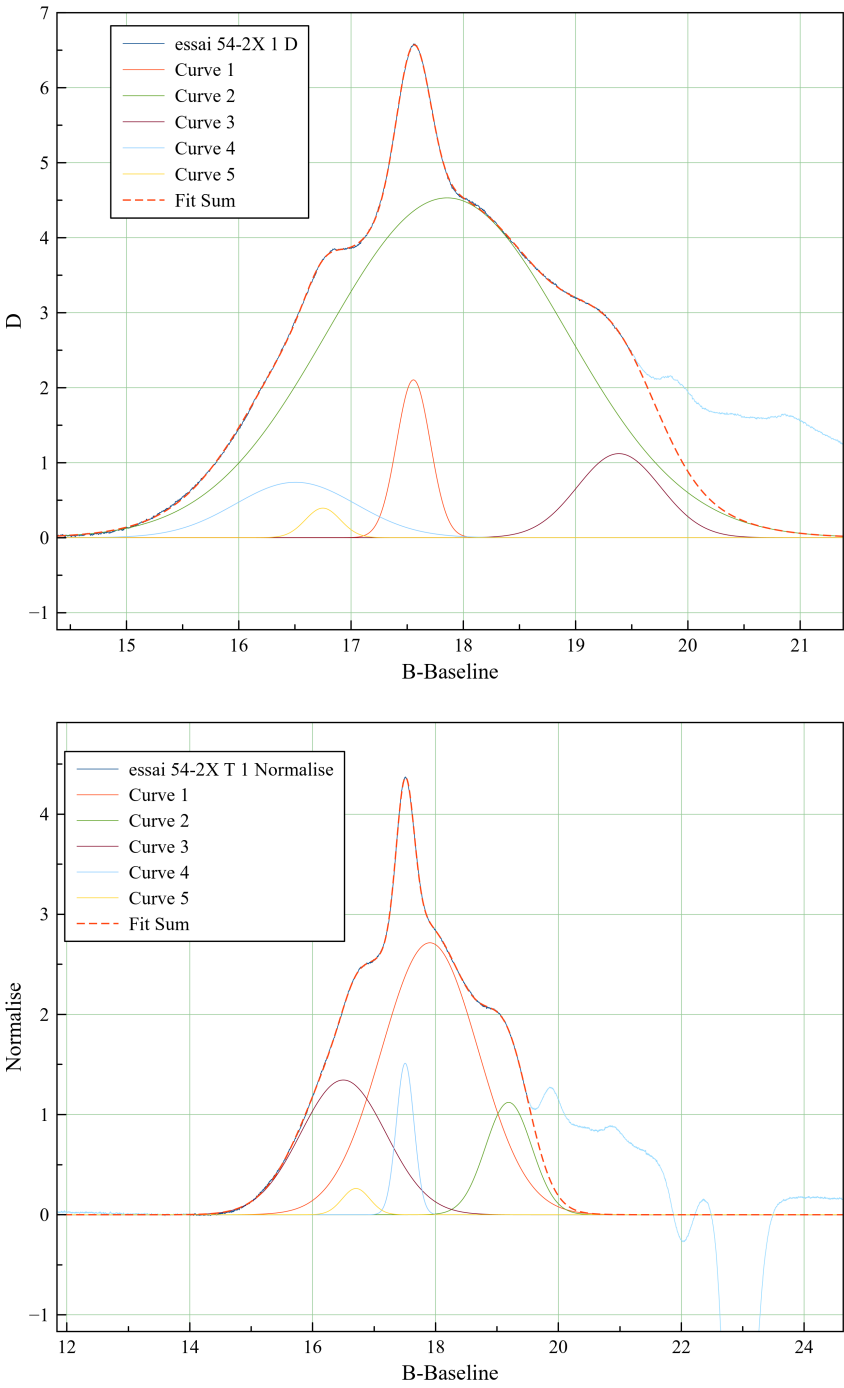


Figure 60: Fitting example for the biocrude oil from Pleurocapsa at biomass cultivated at 2 XF2, without treatment (54-2X), and with treatment (54-2X T).



Appendix

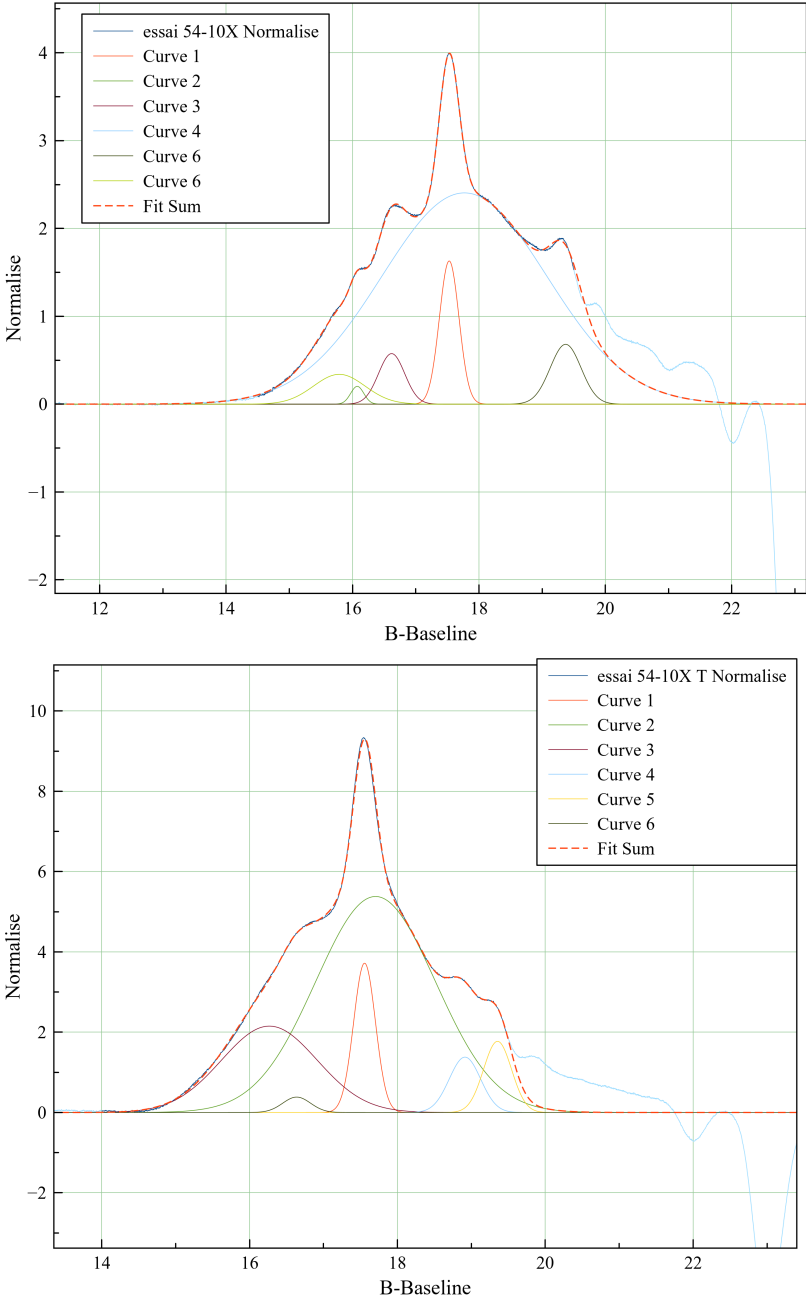


Figure 61: Fitting example for the biocrude oil from Pleurocapsa at biomass cultivated at 10 XF2, without treatment (54-10X) and with treatment (54-10X T).

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**Titre :** Culture et bioraffinerie d'isolats de cyanobactéries du Qatar pour la production de pigments et de bio-bitume en environnement désertique.

**Mots clés :** Cyanobactérie, Phycobiliprotéines, Température, Salinité, bioraffinerie, Bio-bitume.

**Résumé :** Les cyanobactéries marines extrêmophiles sont des candidates potentielles pour être cultivées en conditions solaires dans des environnements désertiques chauds. Elles offrent une solution viable au manque de terres arables et d'eau douce pour la production de pigments naturels à forte valeur ajoutée, comme la phycocyanine, dans la péninsule arabique. Cette thèse évalue le potentiel de quatre isolats de cyanobactéries du Qatar. Leur croissance et leur productivité en phycobiliprotéines sont d'abord étudiées à différentes températures et salinités en laboratoire en utilisant des photobioréacteurs, conduisant à la sélection d'une souche thermotolérante et halotolérante capable de produire des extraits riches en phycocyanine aux propriétés antioxydantes remarquables.

Après cette étape de criblage, la culture solaire de la cyanobactérie sélectionnée est étudiée en extérieur au Qatar en utilisant l'urée comme source d'azote à faible coût, confirmant son potentiel pour une culture à grande échelle dans le climat désertique de la péninsule arabique. Finalement, la faisabilité de la co-production de bio-bitume par conversion hydrothermale des résidus de biomasse après récupération des pigments est évaluée. Les résultats mettent en évidence l'importance du procédé d'extraction des pigments afin de réduire le taux de cendres avant le traitement hydrothermal, ce qui permet d'augmenter le rendement en phase huileuse possédant des propriétés rhéologiques similaires à celles d'un bitume conventionnel.

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**Title:** Cultivation and biorefinery of cyanobacteria isolates from Qatar for the production of pigments and bio-bitumen in desertic environment

**Keywords:** Cyanobacteria, Phycobiliproteins, Temperature, Salinity, Biorefinery, Bio-bitumen

**Abstract:** Extremophilic marine cyanobacteria are potential candidates for their solar cultivation in hot desertic environment, providing a sustainable solution to the lack of arable land and fresh water, for the production of valuable natural pigments such as phycocyanin in the Arabic Peninsula.

In this context, this thesis evaluates the potential of four cyanobacteria isolates from Qatar. Their growth and phycobiliprotein productivities are first studied indoor using photobioreactors at different temperatures and salinities, allowing the selection of a thermotolerant and halotolerant strain, able to produce phycocyanin rich extracts with remarkable antioxidative properties.

After this screening step, the outdoor solar cultivation of the selected cyanobacteria is studied in Qatar using urea as a low-cost nitrogen source, confirming its potential for large scale cultivation in the desert climate prevalent in the Arabic peninsula.

Finally, the feasibility of coproducing bio-bitumen by hydrothermal conversion of biomass residues after pigment recovery is assessed. The results point out the crucial role of the pigment extraction process in order to reduce the ash content, prior to the hydrothermal treatment, resulting in an increase of the yield in oil phase with rheological behavior similar to that of conventional bitumen.

