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Tania Yehya

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HAL Id: tel-01424123

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UNIVERSITE BLAISE PASCAL
N° D. U. 2660

UNIVERSITE D'AUVERGNE
Année: 2015

ECOLE DOCTORALE SCIENCES POUR L'INGENIEUR

N° d'ordre : EDSPIC: 736

Thèse

Présentée à l'Université Blaise Pascal

Par

Tania YEHYA

Pour l'obtention du grade de

DOCTEUR D'UNIVERSITE

(SPECIALITE: GENIE DES PROCEDES)

Etude de procédés électrochimiques et biologiques pour le traitement des eaux : Application à l'élimination des nitrates et de la carbamazépine

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Order no: EDSPIC: 736

Thesis

Submitted to Université Blaise Pascal

Defended by

Tania YEHYA

To obtain the degree of

DOCTOR OF PHILOSOPHY

(SPECIALITY: PROCESS ENGINEERING)

**Study of electrochemical and biological processes for
the removal of water pollutants: Application to
nitrates and carbamazepine**

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ACKNOWLEDGMENTS

This dissertation would not have been possible without the help of **God** who provided me with courage, strength, determination, patience, and health to finish this work.

Firstly, I would like to acknowledge CIOES - Lebanon for giving me the opportunity to do my Ph.D. in France.

I warmly thank Prof. Gilles DUSSAP, the Head of the GePEB research group for welcoming me in the laboratory.

I would like to express my sincere gratitude to my thesis supervisor, Prof. Christophe VIAL, for the continuous support of my Ph.D. study and related research, for his kindness, and immense knowledge. Thanks to his gentleness, patience, and his high-quality scientific guidance which helped me in all the time of research and during the writing of this thesis.

My sincere thanks also goes to Dr. Fabrice AUDONNET, and Dr. Lidia FAVIER, my Co-supervisors, who were always there whenever I needed help and support.

I would also like to thank my thesis committee: Mr. Samir TAHA, Professor at the Lebanese University, Director of the LBA3B laboratory and Head of M2R Applied Biotechnology in the Lebanese University, and Mrs. Claire ALBASI, CNRS Research Director at the LGC in Toulouse (France) for giving me time to evaluate my work and for accepting to be its reviewers. I equally thank Mr. François LAPIQUE, CNRS Research Director at LRGP in Nancy (France), and Mr. Christian LARROCHE, Professor, UBP, Institut Pascal, Clermont-Fd, France for being the examiners of this work and participating to the thesis jury.

I thank my colleagues for the stimulating discussions, for the sleepless nights we were working together before deadlines, and for all the fun we have had in the last three years.

I would like to thank my family: my parents, mom and dad, my brothers, my mother-in-law, and my sisters-in-law for supporting me spiritually throughout writing this thesis. Without their support and motivation, this thesis would never be possible.

I dedicate this thesis to the innocent soul and memory of my brother who inspired me through the tough times of this work whose role in my life was, and remains, immense.

This last word of acknowledgment I have saved for my dear husband, Nidal FAYAD, who has been with me the mentor, and the real friend all these years and has made them the best years of my life, and for my daughter-to-be, my everything, Anna-Bella, for being the most real incentive to work hard.

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ABSTRACT

This work concerns the quantitative removal, in a respectful manner for the environment, of water pollutants, with a special focus on a pharmaceutical biorefractory micropollutant, carbamazepine, and on an inorganic pollutant, nitrate anions. The work is centered on the analysis of non-conventional treatments. The first one is an electrochemical method, electrocoagulation (EC), which exhibits the advantages to be non-specific and to combine various depollution mechanisms (adsorption, electro-oxidation...). The second is an innovative and low-cost biological treatment using green algae, *Ankistrodesmus braunii*.

First, EC treatment using aluminum electrodes was used for denitrification and then for Carbamazepine (CBZ) removal from water. In the case of nitrate removal, it was found that nitrate was reduced into nitrite and finally into ammonium which then was found to be adsorbed onto the flocs produced during EC; this mechanism has never been previously analyzed. Nitrate was eliminated up to 95% when starting with a 50-200 mg/L concentration range, after two hours of EC treatment. For the first time studied, EC was found also efficient for CBZ removal: after two hours of treatment, up to 62% of CBZ was eliminated when starting with a 12.5 mg/L concentration. It was also found that CBZ was oxidized into several metabolites among which five were identified and one of them (10-OHCBZ) is adsorbed on the flocs and corresponded to about 20% of the total initial carbon amount.

Phycoremediation, *i.e.* water treatment using algae and microalgae, is a recent process. In this work, a biological treatment using a green algae, *Ankistrodesmus braunii*, was found to be more efficient than EC for the treatment of CBZ over a 60 days study. The respective effects of the culture medium, the initial inoculum concentration of *A. braunii* and the initial CBZ concentration on CBZ removal were studied. Lastly, the mechanism of CBZ elimination by *A. braunii* was investigated. The highest percentage of CBZ elimination achieved was 87.6%. The bold's basal medium was shown to favor CBZ removal in comparison to a proteose peptone medium. CBZ exhibited a toxic effect on the growth of algae, but the removal yield remained always higher than 70% and the elimination was faster at high initial concentrations of CBZ. The removal mechanism was mainly the bioaccumulation of CBZ inside the *A. braunii* cells, but the biotransformation of about 20% of the initial CBZ into the metabolite 10-OHCBZ inside the cells was also observed.

As biosorption/bioaccumulation was the prevailing removal mechanism using *A. braunii*, CBZ removal using *Saccharomyces cerevisiae* immobilized on alginate beads was studied for comparison purpose, as well as another biorefractory micropollutant, diclofenac. A textile dye, Orange II (OII), was used as a reference, as it has been extensively studied in the literature as a typical azo dye. The respective effects of the presence of *S. cerevisiae*, pH, mass of alginate beads, diameter of the beads and initial concentrations of each pollutant were investigated. The experiments were driven in batch conditions as a function of treatment time. The highest removal yields were obtained when starting with the highest pollutant concentrations, especially with CBZ and diclofenac. These reached 80% for OII, while it was lower for the other pollutants, around 55%, and limited to very low pH, which confirms the high efficiency of microalgae *A. braunii* previously studied.

In conclusion, this work proved the efficiency of EC, with aluminum electrodes, for almost fully treating nitrates, even though it is more expensive than the conventional biological treatment. The advantage is that EC is also able to remove other kinds of pollutants, including CBZ. It has also proved the efficiency of biological treatment using *Ankistrodesmus braunii*

for the treatment of CBZ that acts mainly through by biosorption/bioaccumulation. Phycoremediation was shown to be far more efficient to remove pharmaceutical micropollutants than *S. cerevisiae* immobilized on alginate beads, even though this is effective to azo dyes. This opens the way to a coupling with EC and phycoremediation, as EC is able to harvest microalgae.

INTRODUCTION

Freshwater resources are limited and comprise only 2.66% of the total global water resources. They include mainly groundwater, surface water (lakes and rivers), polar ice and glaciers. Only a smaller fraction, about 0.6% of the water resources, can be effectively used as drinking water. Due to the combined effects of global warming, population growth and industrial development, water scarcity becomes a key problem in many countries of the world. For this reason, wastewater which has been altered by human activity, whether domestic, industrial or agricultural, must necessarily be treated, with the aim to preserve the resource, while promoting cost and energy savings. Non-treated wastewater may, indeed, makes the resource unsafe for people and animals, and disrupt the aquatic ecosystem. For example, domestic wastewater may contain many, such as detergents, drugs, estrogen, dyes and endocrine disruptors, that is to say all synthetic substances that cannot be destroyed naturally. Their presence is directly related to their daily use by human. Some of them may be toxic to the environment or may, at least, modify the ecosystems. A key problem is that these cover thousands of compounds that cannot be followed individually. For a long time, these were disregarded because they could not be detected, but their accumulation and the advances in analytical tools has highlighted their presence in the water resource since the 1980s and is in continuous increase. That's why, in the last ten years, research on the behavior and the impact of these molecules on the environment and human health have been intensified, together with that on the development of effective water treatment technologies.

This work deals with two of these pollutants: nitrates as an inorganic compound that is widely used in agricultural activities, and CBZ as a pharmaceutical micropollutant that can be found in hospital and municipal wastewater and is considered as biorefractory. Dyes will be used as a reference to represent industrial biorefractory pollutants, as their removal has already been extensively analyzed. Various treatment methods have been studied to remove all these pollutants, mainly by adapting the standard treatments used in sewage treatment plants (STPs) that are conventionally classified into pre-treatment, primary, secondary and tertiary:

- Primary treatment is the physical separation of suspended solids from the wastewater flow by settling, but is not effective on removing pharmaceutical compounds and nitrates.
- Secondary treatment is most commonly led by biological means, mainly by the activated sludge process that is primarily devoted to treat organic pollutants under aerobic conditions. A physicochemical step can be added to promote flocculation and coagulation of sludge. Nitrates can be treated when activated sludge is driven under anoxic conditions. Conversely, biorefractory compounds, such as azo dyes, are difficult to treat and the same stands for some pharmaceuticals, such as carbamazepine. Many studies have been aimed at improving the efficiency of this process by biostimulation or bioaugmentation of activated sludge, but removal efficiencies remain low for some biorecalcitrant compounds such as CBZ. Recent alternatives suggest the use of fungi and algae (phycoremediation), but these are not currently applied at the industrial scale.
- Tertiary treatment section includes techniques for specific removal of undesirable compounds, particularly heavy metals, but also the refractory contaminants that the secondary treatment is not able to clean. In order for this problem to be solved, the most recent work has focused on advanced oxidation processes, such as ozonation which are usually reserved for the treatment of drinking water. Alternatives include membrane treatments and photochemical or electro-oxidation processes. However, these techniques

when found effective, are not used for wastewater treatment because they remain too expensive.

The objective of this Ph.D. thesis is, therefore, to develop low-cost treatments for the removal of these compounds. A representative reactive azo dye of the most common industrial dyes (orange II, noted OII) and pharmaceutical molecules, such as diclofenac (DCF) and carbamazepine (CBZ) have been studied, together with nitrates. The work focuses, first, on an electrochemical treatment, electrocoagulation (EC), and on the opportunity to couple EC to biological treatments, including biosorption/bioaccumulation, such as the elimination of pollutants using a green algae, *Ankistrodesmus braunii*, or adsorption/degradation using *Saccharomyces cerevisiae* immobilized on alginate beads.

As a result, this Ph.D. thesis comprises six chapters. Chapter I is a submitted literature review about the first pollutant treated in this study, nitrates. This summarizes the origin and effect on the environment of the presence of nitrates in water; it explains, then, the metabolism of nitrates in the human body, along with health effects when this pollutant reaches the human body in high concentrations. Finally, this review focuses on the recent advances of water technology for removing nitrates from water.

Chapter II is a published article that describes the treatment of nitrates with EC. This article details the principles of experimental techniques used in this work along with the experimental setup available for nitrate removal. The elimination mechanisms involved in nitrate removal are explicated along with nitrate speciation and separation between the liquid and solid phases. Finally, a conclusion of the efficiency of EC for nitrate elimination is drawn.

Chapter III is a published article that describes the denitrification process by EC and how each parameter studied influences the efficiency of the process. Moreover, a simple denitrification model was developed that could describe both the reduction of nitrates into ammonium and the amount of adsorbed ammonium on the solid phase. A clear cost analysis of the whole process used was discussed at the end of this article.

Chapter IV is a submitted literature review about the second pollutant treated in this study, CBZ. Here, CBZ is introduced as a refractory organic compound in water. Its occurrence in water and human body fluids is also detailed. Then, CBZ metabolism and its different health issues and toxicity are explained. Finally, the analytical techniques and the different possible treatment methods are explained in details and the recent advances in this field are used to compare their performance.

Chapter V is a published article that tells how CBZ behaves electrochemically during the EC process. It also details how CBZ is affected by the different parameters studied. Moreover, this article shows the formation of a new metabolite as an effect of chemical transformation of CBZ. This metabolite was found to be detected in the solid phase of EC.

Chapter VI is a submitted article about the biological treatment of CBZ by the green algae, *Ankistrodesmus braunii*. In this paper, the effect of two different culture media on the growth of algae and on CBZ elimination are investigated. The toxic effect of CBZ on the algal growth has also been studied. Finally, the possible mechanisms of CBZ elimination, bioaccumulation and metabolization, are discussed and analysed.

Finally, the last chapter, Chapter VII, comprises unpublished results that can be useful for further work and sometimes open the way to new perspectives. It deals with the elimination

of Orange II, Diclofenac, and Carbamazepine by *Saccharomyces cerevisiae* immobilized on alginate in wastewater. In this abstract, the effectiveness of *S. cerevisiae* for the removal of OII dye, diclofenac and CBZ is compared. It also describes the different influences of the parameters studied on the elimination process of each pollutant.

CHAPTER I: NITRATES IN WATER AND THEIR METHODS OF TREATMENT

This review article is submitted online to Journal of Environmental Management. Consequently, this chapter follows the guidelines of this journal.

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Water resources are limited and those that are considered as fresh water comprise only 2.66% of the total global water resources such as groundwater, lakes and rivers, polar ice and glaciers. A smaller fraction of 0.6% of water can be used as drinking water. Moreover the water destined for human consumption of water resources is highly elevated. Water scarcity affects 2.8 billion people each year. Even in developed countries, the situation is getting worse. If France is not threatened by water scarcity at the moment, the situation of Great Britain is more difficult, as 80% of the surface waters and 20% of underground water is said to be used by humans. For these reasons, water resources must be necessarily treated properly and wastewater treatment must be done efficiently. One of the hazardous pollutants is nitrates. Nitrates, mainly used in fertilizers, correspond to a major pollution source in the regions of intensive agricultural activities. In this literature review, nitrate is introduced as a water contaminant and environmental concerns arising from its presence are discussed. Moreover, the different processes and methods applied for its removal, in addition to their advantages and disadvantages and a detailed comparison between these processes, are explained in detail.

1. INTRODUCTION

Nitrogen is a widespread essential element for life. It comprises 78% of the gases of the atmosphere. It is also present in water under different ionic forms, such as nitrates (NO_3^-), nitrites (NO_2^-), ammoniacal nitrogen (NH_3 and NH_4^+), and organic nitrogen. It is one of the main components of amino acids, and nucleic acids, the building blocks of proteins and DNA.

Despite this nitrogen abundance, nitrate, a colorless, odorless, and tasteless anion, has a fatal effect on different creatures when found at high concentrations, on the human health and on the environment as well. It could be exogenously consumed as well as endogenously produced in the human body. The increased nitrates concentration leads to increased reduction of nitrates by buccal bacteria into nitrites which in turn cause stomach cancer, and methemoglobinemia (blue baby syndrome) in infants. Moreover, nitrates, when found in water bodies, cause water eutrophication which strongly impacts the aquatic life. Consequently, the World Health Organization published health reports describing the health risks of nitrates in 1985 and 1993. The set admissible daily intake, ADI, is 3.65 mg/kg of body weight. Accordingly, the European Community authorized the maximal limit of nitrates at 50 mg/L in drinking water with a recommended level of 25 mg/L (1991, the EU Directive N°891/676/CEE (12/12/91) named “Nitrates Directive”). Moreover, the maximum accepted concentration of nitrate (MAC) set by the United States Environmental Protection Agency and Canada at 44 mg/L (Ghafari et al., 2008), and that of nitrite at 3.2 mg/L in drinking water. The same concern in Europe lead to a guideline value of 12 mg/L of nitrate in drinking water and 11.3 mg/L in effluent discharges (European Council Directive, 1998). Moreover, it

was approved that for values higher than 100 mg/L of nitrate, the water then should neither be drunk, nor used for alimentary purposes.

Nitrates diffuse easily via the surface waters to the underground water when their concentration exceeds the need of vegetation (Gingras et al., 2002). The oxidation of nitrogen gas leads to nitrates and nitrites. But due to the stable nature of nitrates, a high number of other nitrogenous species forms tend to reform into nitrates (Wehbe, 2008). Nitrate levels have been gently increased due to increased anthropogenic activities such as the usage of nitrogenous fertilizers. Although most researchers relate the groundwater contamination to non-agricultural sources (Ghafari et al., 2008), this however, could be also related to changes in land-use patterns from pasture to arable, and increased recycling of domestic wastewater in low-land rivers (Kapoor and Viraraghavan, 1997). In particular, 55% of nitrate pollution is caused by agricultural activities by the usage of fertilizers, 35% by the local wastewater, and 10% are due to the industrial activities, such as those which use the nitrites as antimicrobial agents in meat salting (Wehbe, 2008). Industrial effluents in general comprise very high nitrates concentrations that sometimes can exceed 200 mg/L (Peyton et al., 2001). Other higher range of nitrates concentrations of 1000 mg/L can be found in the effluents of some industries producing explosives, fertilizers and pectin. Even higher nitrates concentrations of 50000 mg/L are reported in the wastewater of industries producing nuclear weapon (Ghafari et al., 2008).

2. NITRATES REMOVAL FROM WATER

A 1985 American Water Works Association (AWWA) survey showed that 23% of primary drinking water standard violations were due to excessive nitrates concentrations. Nitrates are present in most surface water and groundwater supplies at levels below 4 mg/L, with levels exceeding 20 mg/L in about 3% of surface waters and 6% of groundwater (WHO, 2011). Consequently, chemical, physicochemical and biological nitrates removal techniques have been applied to avoid the potential risks of nitrate to public health, when found at elevated concentrations in wastewater or potabilizing water. The most common treatment methods for nitrate removal from water include chemical denitrification using zero-valent iron (Ahn et al., 2008) zero valent magnesium (Kumar et al., 2006), ion exchange and adsorption (Samatya et al., 2006), reverse osmosis (Schoeman and Steyn, 2003), electrodialysis (Hell et al., 1998; El Midaoui, 2002), catalytic denitrification (Pintar et al., 2001), and biological denitrification (Soares et al., 2000). On the one hand, physicochemical techniques, in particular ion exchange, adsorption, electrodialysis, and reverse osmosis, etc. lead to pollution transfer rather than its elimination or degradation. On the other hand, biological processes are performed using heterotrophic or autotrophic denitrification processes that reduce nitrates into gaseous nitrogen, but these are slow (Paugam et al., 2001), nitrite producing (which is a byproduct that has bactericidal properties) (Foglar et al., 2005) and are only efficient for treating water with nitrate concentrations below 1000 mg/L in order to avoid the denitrifying bacterial growth inhibition (Puckett et al., 1995). Moreover, biological denitrification requires a continuous follow-up of the pH and temperature for the bacterial growth (Mateju et al., 1992). Finally, chemical processes also produce a large amount of sludge and sometimes secondary pollutants.

Nitrates, as a conclusion, are reported to be hard to treat in water. For example, most of the conventional processes including coagulation, filtration and disinfection employed for water potabilisation are not proved to be efficient enough for the elimination of nitrates. The same

stands for other water treatment processes, such as precipitation. The key difference between physicochemical and biological treatments are the capital and operating costs which can induce major advantages of one method over the others. For example, the capital costs for ion exchange plants are about two and a half to three times lower than for heterotrophic denitrification plants. (WHO, 2011). Moreover, the operational costs of ion exchange, adsorption and biological treatments are considered as medium costs when compared to the higher operational costs of reverse osmosis and chemical treatment methods (Bhatnagar and Sillanpaa, 2011).

In this chapter, physicochemical and chemical treatments will be reviewed first. Then, biological treatments will be discussed, before reviewing hybrid biological-physicochemical treatments for nitrates removal. Finally, the pros and cons of each method will be summarized with a detailed comparison between all these denitrification processes.

3. PHYSICOCHEMICAL TREATMENTS OF NITRATES

3.1. ION EXCHANGE

Ion exchange (IX) is an established water treatment process. It most widely used at the industrial scale (Ratel, 1992). It was applied first for drinking water treatment in 1974 in the United States, and secondly in Great Britain where two treatment stations were established in 1976 and 1978. In France, the agreement of anionic resins delayed its use until 1985. Ion exchange is a conventional process that has been used for nitrates removal. With the potential for multiple contaminants removal, IX can also be used to address other water quality concerns including arsenic, perchlorate, selenium, chromium (total and Cr(VI)), and uranium (AWWA, 1990, Boodoo, 2004). Figure 1 shows the principles and resins functions in the IX process in water treatment.

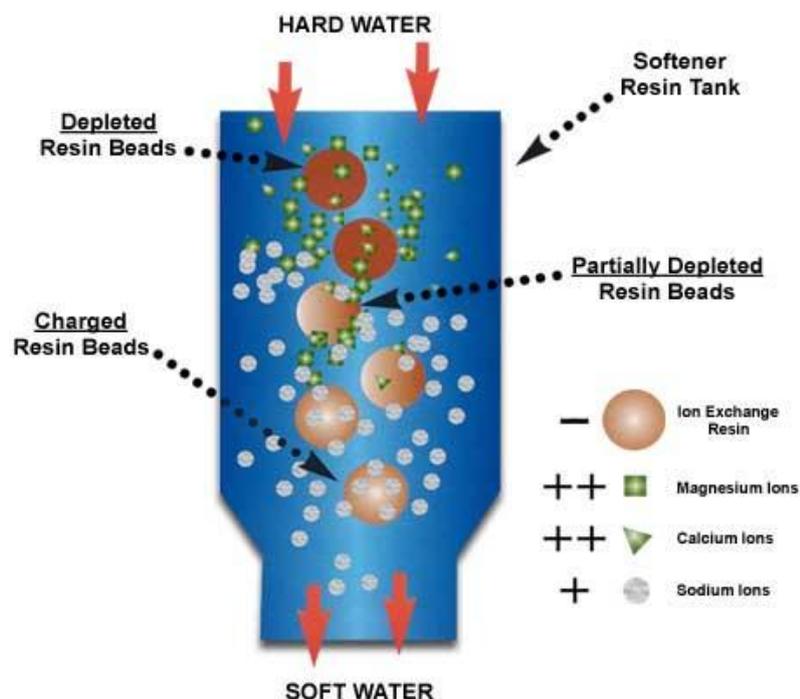


Figure 1: Ion exchange process principles in softening water process: highly positive Mg and Ca ions are exchanged for less-positively charged Na ions. Image credit: ML Ball

In conventional IX treatment, pre-treated water passes through strong base anion (SBA) exchange resin on which the nitrate ions are fixed and chloride ions are liberated in an equivalent amount (Aouina, 2010). Eq. 1 and Eq. 2 summarize the reactions that happen at this stage:



To prevent nitrate breakthrough, regeneration is necessary when the resin is exhausted of chloride ions. The media is backwashed with a high salt solution (0.5 – 3 M), (Clifford, 2007), which results in a brine waste stream highly enriched in nitrates and other ions as a secondary pollution.

However, competing anionic species found in the treated water can cause a disorder in the nitrate removal process. The increasing selectivity order of ion selectivity for resins is bicarbonate, chloride, nitrate, and then sulfate (Clifford et al., 2010). This necessitates the early resins regeneration to avoid sulfate displacement of nitrate leading to nitrate dumping, nitrate peaking, or chromatographic peaking. This issue can be typically solved by measures which are reported in Table 1. Some denitrification studies using IX are summarized in Table 2.

Solution	Characteristics	References
Use of ethyl rather than methyl group around the ammonium nitrogen in the resin structure.	Renders the selectivity of the sulfate lower than that of nitrate by 10 times	Kapoor and Viraraghavan, 1997
Carbon dioxide regenerated ion exchange process (CARIX)	<ul style="list-style-type: none"> - Combines anion and cation exchange for hardness reduction - Bicarbonate is the anion exchanger and CO₂ is liberated - Removes nitrates up to 63% with the CO₂ amounted to 0.35 kg/m³ of treated water - Poor efficiency of regeneration by CO₂ - Resins do not need to be regenerated 	Holl, 1995 Guter, 1995

Study	Conditions	Adsorption isotherm and kinetics	Results	Reference
Removal of nitrates by selective strong base anion exchange resin, Pyrolite A 520	<ul style="list-style-type: none"> - It was carried out with column method - Influent nitrate concentration = 22.6 mg/L as N (100 mg NO₃⁻/L) 	<ul style="list-style-type: none"> - Langmuir and Dubinin-Radushkevich (DR) adsorption isotherm - No data on kinetics 	<ul style="list-style-type: none"> - With no sulfate and chloride: resin capacity 126.4 mg NO₃⁻/g - Nitrates were effectively removed from groundwater - Nitrates were eluted quantitatively with 0.6 M NaCl. 	Samatya et al., 2006
Removal of nitrates by selective strong base anion exchange resin, Pyrolite A 520 in batch and a fixed bed column	<ul style="list-style-type: none"> - Resin doses: 1.5-3 g/L - Nitrate influent concentration : 20 mg/L 	-Langmuir adsorption isotherm	- Maximum adsorption capacity : 32.3 mg/ N g	Nur et al., 2015

Removal of NO ₃ ⁻ by ion exchange resin Amberlite IRA 400 from aqueous solution was investigated under different initial concentrations	- Influent nitrate concentration: 1 – 8 mg NO ₃ ⁻ /L	- Freundlich adsorption isotherm - Reversible first-order with intra-particle diffusion	- The maximum sorption capacity was 769.2 mg/g at 25 °C - Removal efficiency 96%.	Chabani et al., 2006
An anion exchange resin (NDP-2), D201 and Pyrolite A 300	- Resin: 0.1 g - Initial concentrations of nitrate: 50, 100, 200, 400 and 600 mg/L	- Langmuir adsorption isotherm model - Pseudo-first order and pseudo-second order kinetic models.	- NDP-2 were the best sorption resins even in the presence of competing ions, such as SO ₄ ²⁻ , Cl ⁻ and HCO ₃ ⁻ , in aqueous solution.	Song et al., 2012

Modifications of conventional IX have led to the emergence of more efficient processes, including multiple vessel configurations, counter-current configurations, the use of specialized resins (nitrate selective resins, (Jensen et al., 2014)) improved hydraulics, and weak base anion exchange (WBA IX) (Jensen and Darby, 2012). Some of these process modifications are summarized in Table 3.

Modifications to Conventional IX	Developed by	Innovations	Reference
Magnetic ion exchange (MIEX)	Orica Watercare	- Offers low brine using a unique SBA Type I resin - The resin is fluidized in a contactor with spent resin removed from the contactor for regeneration outside of the process water stream and then returned to the contactor (in conventional IX, the resin is stationary) - Proposes a fluidized bed process tolerant of suspended solids and low levels of oxidants	Orica Watercare, 2008
Improved Hydraulics and Nitrate Selective Resins	The Layne Christensen Company and Rohm and Haas offer Advanced Amberpack® system	- Utilizes nitrate selective resins to increase the treated water volume and decrease the waste brine - Shows improved removal efficiency due to nitrate selective resins, especially in waters where the sulfate to nitrate ratio is greater than one	Rohm and Haas Company 2007.
Multiple Vessel Carousel Configuration	Calgon Carbon's Continuous Ion Exchange Separation System (ISEP® System)	- Utilizes a carousel configuration which has the potential to avoid downtime for regeneration - Needs a minimal amount of resin - Exhibits maximum regeneration efficiency	Calgon Carbon Corporation. (2003) ISEP® for Nitrate Removal
	Envirogen Technologies, Inc.	- Uses multiple beds operated in a staggered design which maximizes resin capacity and minimizes waste and chemical use - Implements a low brine IX system for nitrate and uranium removal	Envirogen, 2010

<p>Weak Base Anion Exchange (WBA IX)</p>	<p>Applied Research Associates, Inc. (ARA) and The Purolite Company (ARA & Purolite N.D.)</p>	<ul style="list-style-type: none"> - Is effective for nitrate removal from potable water, but highly pH dependent - Removes strong acids WBA IX resin; acid addition protonates the WBA resin, then the positively charged resin sites remove anions, like nitrates - Uses weak bases to neutralize the WBA resin rather than the high salt solution for SBA resins - Provides waste stream with lower salt content that can potentially be recycled as a fertilizer (NH_4NO_3 and $\text{Ca}(\text{NO}_3)_2$) - Exhibits corrosion risk and sensitivity to pH that must be adjusted (influent pH must be between 3 and 6). - Exhibits more sensitivity to temperature (operation below 95°C) - Provides a nitrate-containing effluent representing typically less than 0.2% of the treated water. 	<p>Clifford, 2007 Dow, 2010 Nur et al., 2015</p>
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3.2. REVERSE OSMOSIS

Reverse osmosis (RO) is a water purifying process in which ions are removed by forcing the water across a semi-permeable membrane and leaving water nitrates and other ionic species behind (Figure 2). RO can treat multiple contaminants simultaneously including ionic (e.g. nitrate, arsenic, sodium, chloride and fluoride), particulate (e.g., asbestos and Protozoan cysts), and organic constituents (e.g. some pesticides) (Dvorak and Skipton, 2008). Reverse osmosis requires high energy input to develop pressures needed to operate, thus it is expensive to operate. The collected concentrate is highly concentrated in nitrate and other rejected constituents (salts) and requires appropriate disposal. For example, rejection rates for sodium chloride and sodium nitrate can be as high as 98% and 93%, respectively (Elyanow and Persechino, 2005).

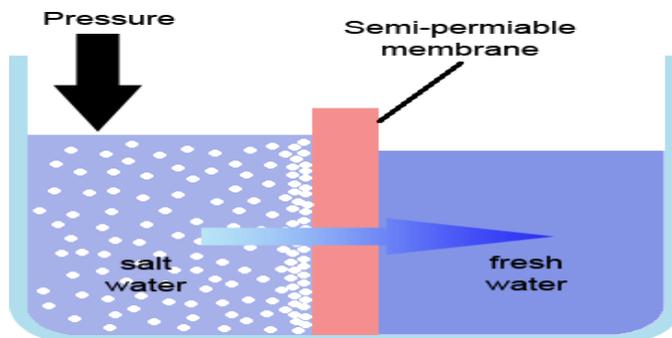


Figure 2: The reverse osmosis principle in water treatment. www.luminoruv.com

Membranes commonly used are made of cellulose acetate, but others made of polyamides and composite membranes are also available. The drawback of membrane fouling (scaling, colloidal fouling, biological fouling and organic fouling) and deterioration with time necessitated some improvements to the standard RO process. The lifetime of the RO membranes and prefilters and the frequency of membrane cleaning directly depend on water quality and the efficiency of pretreatment measures. For example, when the salt concentration in the feed water exceeds the saturation point at the membrane surface, precipitation of solids such as precipitates of silica, calcium, barium, and strontium salts pose a significant threat on the membrane which can diminish the removal efficiency (Elyanow and Persechino, 2005). Silica can be a particularly problematic constituent for RO membranes that are difficult to remove. Modifications to conventional RO have emerged to manage high silica source waters. Some modifications to RO process are detailed in Table 4.

Modified Process Name	Innovations	Reference
High Efficiency Reverse Osmosis (HERO™) patented by Debasish Mukhopadhyay	<ul style="list-style-type: none"> - Multi-step process with increased water recovery (> 90%) and minimized cleaning requirements - Limited scaling by incorporating hardness reduction, CO₂ stripping, and pH adjustment, - Production of ultra-pure water for use in electronics applications 	Engle, 2007 GE, 2010 Water Corporation, 2007
Ultra-low Pressure Reverse Osmosis (ULPRO)	<ul style="list-style-type: none"> - ULPRO membranes for use with lower operating pressures (3.44 to 8.61 bar, compared to 13.78 bar in conventional RO) and improved flow rates - Pretreatment to prevent membrane scaling and fouling similar to those necessary for conventional RO membranes - Poor membrane recovery after cleaning 	Drewes et al., 2008

Some research studies have shown the efficiency of nitrate removal using RO. For example, the high efficiency reverse osmosis (HERO) study in Australia, removes both nitrates and silica from brackish water and resulted in a water recovery of 95% with more than 85% waste reduction and as low as 10% of the waste produced in the conventional RO (Water Corporation, 2009).

3.3. ELECTRODIALYSIS

The use of electrodialysis (ED) or electrodialysis reversal (EDR) in potable water treatment has increased in recent years, offering the potential for improved water recovery, and the minimization of chemical and energy requirements (Sahli et al., 2008; Banasiak and Schafer, 2009). In this process, ions are transferred from a less concentrated to a more concentrated solution due to the passage of a direct current through a series or stack of anion and cation exchange membranes (Figure 3). Nitrate ions (and other anions) move through the anion exchange membrane toward the anode. In its way to the anode, nitrate is rejected by the anion-impermeable cation exchange membrane and trapped in the recycled waste stream. Recent modifications (Table 5) were applied to electrodialysis to improve the selectivity of the membranes to certain treated contaminant. Moreover, some research studies with their denitrification results are shown in Table 6.

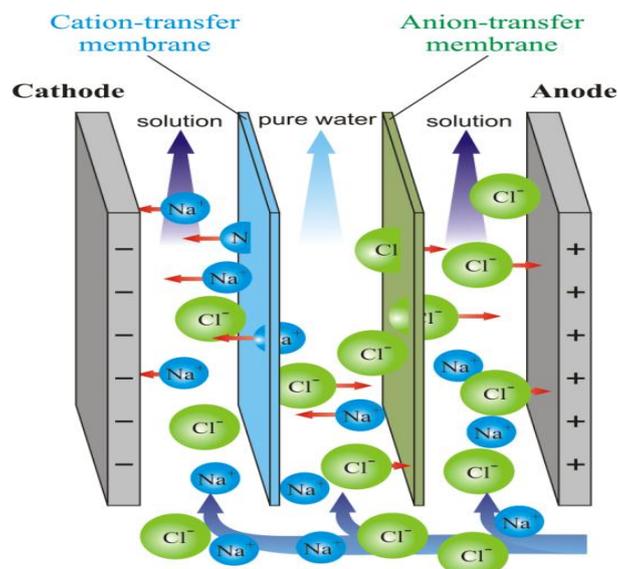


Figure 3: An explanatory schema of Electrodialysis process. <http://glossary.periodni.com/glossary.php?en=electrodialysis>

New process	New innovations	References
Selective electro dialysis (SED) Developed by Shikun & Binui (formerly Nitron, Ltd.)	<ul style="list-style-type: none"> - High water recovery (up to 95%), and minimized waste volume - up to 70% removal of nitrates and sulfate ions rejection by nitrate selective membranes - Low pressure operation (2.06 – 4.13 bar) - No pH adjustment or remineralization in post-treatment 	Nitron, 2009 Nitron, 2010
NitRem	<ul style="list-style-type: none"> - Nitrates removal without the addition of chemicals - No need for extensive pre-treatment 	Miquel and Oldani, 1991

Study	Conditions	Results	References
Full-scale installation in Austria with 1 m ³ /h capacity	<ul style="list-style-type: none"> - Influent nitrate concentration 18–23 mg N/L (80–100 mg/L NO₃⁻) - Monovalent selective anion exchange membranes with capacity from 48 to 144 m³/h 	<ul style="list-style-type: none"> - Hardness reduction 23% - Nitrate removal 66% - Desalination 25% 	Hell et al., 1998
Variations of voltage, flow rate and temperature for minimizing precipitation, scaling and chemical use with selective membranes in Morocco	<ul style="list-style-type: none"> - Influent concentration: 20 mg N/L (90 mg/L NO₃⁻) - Total dissolved solids (TDS) concentration 800 mg/L 	Ion removal increased with higher temperature	Midaoui et al., 2002
Removal of nitrate from potable water in Delaware (USA)	Influent nitrate concentration: 13.5 mg N/L (60 mg/L as NO ₃ ⁻)	<ul style="list-style-type: none"> - Demineralization 88% - Water recovery 90% - pH decrease 6.2 to 5.4 - Nitrates in treated water 1 mg N/L (4.4 mg/L NO₃⁻). 	Prato and Parent, 1993
Investigation of the impact of different voltage (from 40 to 50 V) across several ionic species on nitrate removal	Alternating anion and cation exchange membranes	94% nitrate removal with a reduction in the removal rate at 50 V due to back-diffusion and fouling	Nataraj et al., 2006

3.4. NITRATE ADSORPTION ON EMERGING ADSORBENTS

Adsorption technology has been found successful in removing different types of inorganic anions, e.g., fluoride (Viswanathan et al., 2010), nitrate (Guan et al., 2010), bromate (Bhatnagar et al., 2009), perchlorate (Mahmudov et al., 2010), from water by using various materials as adsorbents. All the common materials used as adsorbents for various water treatments described in the literature have been tested for nitrate removal. These include activated carbons of various types (granular, powdered... and from different origins: baboo, coconut, organic solid waste...), natural clays and synthetized layered double hydroxides (LDH), zeolites (natural, synthetized or from waste...), biopolymers (chitosan...), organic or industrial waste or agro-waste. Key results are summarized in Table 7.

Table 7: Denitrification studies using adsorbents.

Materials	Aim of study	Conditions	Results and notes	References
I. Carbon-based adsorbents				
a. Carbon cloth	Study the effects of functional groups on the adsorption of NO_3^- and NO_2^-	a. Carbon cloths were etched in 4 M H_2SO_4 after deionization cleaning procedure and used for adsorption at pH 7	- Increased electrostatic adsorption of anions due to surface protonation was reported - Adsorption capacity was: - 2.03 mmol/g NO_3^- - 1.01 mmol/g NO_2^-	Afkhami et al., 2007
		b. Carbon cloth were treated with distilled water	- Adsorption capacity was: - 0.38 mmol/g for NO_3^- - 0.05 mmol/g NO_2^- - No effect of competing ions was found on the adsorption	
b. Powdered activated carbon (PAC) and carbon nanotubes (CNT)	Remove NO_3^- from aqueous solutions	- pH 5	- Adsorption capacity was: - 10 mmol/g for PAC - 25 mmol/g for CNT (decreased at pH above 5) - The equilibrium time for maximum uptake was 60 mins.	Khani et al., 2008
c. Granular activated carbon (GAC)	Study the influence of process variables (chemical ratio and activation temperature) on NO_3^- removal	GAC produced from coconut shells by steam activation were chemically activated with ZnCl_2	- Optimal removal was for chemical weight ratio: 200% at 500°C, resulting in the development of some new pores, and hence, increased adsorption of NO_3^- - 400°C: low adsorption, - 600°C: decreased adsorption	Bhatnagar et al., 2008
	Compare between the treated and untreated GAC in the removal of NO_3^-		Adsorption capacity was: - treated GAC: 10.2 mg/g - untreated GAC: 1.7 mg/g	
d. Activated carbon (AC) and charcoal (CB)	Study the adsorption behaviour of NO_3^- from aqueous solutions	Activated carbon and charcoal were prepared from coconut shells and bamboo, respectively	- Show maximum removal of NO_3^- reported at equilibrium pH 2- 4 - Adsorption capacity was: - 2.66×10^{-1} mmol/g for AC - 1.04×10^{-1} mmol/g for CB	Ohe et al., 2003
e. Bamboo powder charcoal	Remove NO_3^- from water	The bamboo powder heated in an electric	- Uptake values at 10°C was: - 1.25 mg/g for bamboo	Mizuta et al., 2004

		furnace at 900 °C for 1h.	powder charcoal - 1.09 mg/g for commercial activated carbon (CAC)	
f. Activated carbon F400	Study the competitive behaviour of nitrates with inorganic anions (bromate, chlorate, chloride, iodate, perchlorate, sulphate, and dihydrogen-phosphate)	- pH 4 - NO ₃ ⁻ content range: 0.1-1.0 mM	- Adsorption density for nitrate was 0.29 mmol/g - Competitive adsorption was observed with perchlorate	Mahmudov et al., 2011
g. Activated carbon (AC) and a composite of activated carbon and Fe ₂ O ₃ nanoparticles (Fe-AC)	Study nitrates adsorption on both adsorbents	- AC : 0.53 g/50 mL, - pH = 3 - C ₀ = 147.31 mgL ⁻¹ - Fe-AC: 0.53 g/50 mL - pH = 5.1 - C ₀ = 69.16 mg L ⁻¹	- Maximum nitrate removal percentages : -AC: 68.45% -Fe-AC: 95.56% - Langmuir adsorption isotherm	Mehrabi et al., 2015
h. Activated carbon (ACR) residue, carbon residue (CR) and activated carbon (AC)	Study the effect of activation process on nitrate adsorption on adsorbents	- Optimal pH : - ACR: 6 - CR: 6 - AC: 4 - Optimal nitrate concentration: 25 mg L ⁻¹ for all adsorbents - Adsorbent : 5 g L ⁻¹	- Removal efficiencies: - AC: > 75 % - ACR: > 10% - CR: > 4% - Langmuir adsorption isotherm	Kilpimaa et al., 2014
II. Clay adsorbents				
a. Calcium montmorillonite	Study NO ₃ ⁻ removal by adsorption on acid modified clays	Calcium montmorillonite activated by HCl	- Removal capacity was 22.28% - No direct relation was found between the surface area and the nitrate removal capacity	Bhattacharyya et al., 2008 Mena-Duran et al., 2007
b. Queensland bentonite (QDb), kaolinite, halloysite	Characterize surfactants clay minerals for nitrate adsorption	Raw and treated clays with non-functional surfactant hexadecyltrimethylammonium bromide (HDTMA) in 2 or 4 cation exchange capacity (2CEC and 4CEC, respectively) were used for NO ₃ ⁻ removal	- Untreated QLDb and kaolinite showed no adsorption of NO ₃ ⁻ - Halloysite adsorbs 0.54 mg/g. - HDTMA modified QLDb showed the best removal result: - H-B-2CEC: 12.83mg - H-B-4CEC: 14.76 mg/g - But HDTMA modified kaolinite (H-K) and halloysite (H-H) were found to remove less NO ₃ ⁻ compared to Bentonite (H-B) - 2CEC were less efficient than 4CEC: - H-K-2CEC = 1.54 mg/g - H-H-2CEC = 1.78 mg/g - H-H-4CEC = 1.93mg/g - An increase of concentration of HDTMA 4CEC increased the removal capacity (4.87 mg/g on H-K-4CEC).	Xi et al., 2010
II. Layered double hydroxides (LDH)				
a. Zn-Al-Cl LDH	Characterize the physicochemi-	Zn-Al-Cl LDH was synthesized by co-precipitation method:	- Removal of NO ₃ ⁻ was 85.5% - Removal decreased with the: - increase of pH (optimal pH 6)	Islam et al., 2010

	cal properties of Zn/Al chloride LDH and evaluation of nitrate removal efficiency	<ul style="list-style-type: none"> - Neutral conditions - 0.3 g of LDH in 100 mL NO_3^- solution - Initial concentration 10 mg/L 	<ul style="list-style-type: none"> - competitive anions present in the order of carbonate > phosphate > chloride > sulphate. 	
b. Ni-Fe LDH	Study the selective adsorptive properties of Ni-Fe LDH (Ni-Fe), for NO_3^- removal from seawater	<p>LDH (Ni-Fe) with Cl^- in the interlayers was synthesized by coprecipitation at constant pH</p> <p>Dissolution of Ni and Fe from LDH (Ni-Fe) was less than 0.6% for Ni and less than 0.1% for Fe at pH 8, indicating that LDH (Ni-Fe) was sufficiently stable in seawater</p>	<p>Results showed a higher equilibrium constant for NO_3^- removal than for other anions (HPO_4^{2-} and SO_4^{2-})</p>	Tezuka et al., 2005
	Study the potential of LDH (Ni-Fe) for NO_3^- removal by batch method from seawater	NO_3^- concentration was 40 mol/L	<ul style="list-style-type: none"> - Equilibrium time: 4 h - maximum uptake: 83%, <i>i.e.</i> 0.33 mmol/g when 0.10 g LDH (Ni-Fe) was added to 1 L at pH 8 	
c. Other LDH	<p>Study:</p> <ul style="list-style-type: none"> - The sorption of NO_3^- on calcined hydroxalcalite-type compounds at 550°C (HT550), 650°C (HT650), and 850°C (HT850) - The influence of temperature (10 to 40°C) for HT850 	Temperature was 25°C	<p>Sorption capacities were:</p> <ul style="list-style-type: none"> - 61.7 g/kg (HT550 at 25°C) - 147.0 g/kg (HT850 at 40°C). <p>Removal efficiency:</p> <ul style="list-style-type: none"> - 70.5% for HT550 at 25°C - 99.5% for HT850 at 40°C <p>At higher calcination temperature (850°C), the removal of NO_3^- was greater. The increase in the temperature led to increased sorption:</p> <ul style="list-style-type: none"> - at 10°C = 63.3 g/kg = 71.5% - at 40°C = 147 g/kg = 99.5% 	Socias-Viciana et al., 2008
III. Zeolite				
a. Zeolite coated with chitosan	Test the suitability of zeolite coated with chitosan to capture NO_3^- from water	<ul style="list-style-type: none"> - Surface modification of natural zeolite were performed by coating with a chitosan layer - The chitosan coated zeolite (Ch-Z) was protonated with: <ul style="list-style-type: none"> - sulfuric acid - hydrochloric acid - Temperature 20°C and 4°C 	<ul style="list-style-type: none"> - Protonation with hydrochloric acid resulted a higher maximum NO_3^- exchange capacity compared to sulfuric acid - Ch-Z has a comparable ion exchange capacity to other weak anion exchangers with NO_3^- ion exchange capacity of 0.74 mmol/g (protonated with HCl) 	Arora et al., 2010
b. Clinoptilolite and HDTMA- modified zeolite (SMZ)	Investigate the removal of nitrate and ammonium using SMZ	<ul style="list-style-type: none"> - The ions present in the solutions treated are: <ul style="list-style-type: none"> - Nitrates - Ammonium 	<ul style="list-style-type: none"> - Natural clinoptilolite had good affinity for ammonium, but low sorption ability for nitrate - The SMZ had removed 	Tao et al., 2015

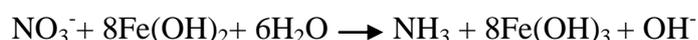
	and natural Clinoptilolite	- Phosphates	- ammonium at 93.6% - nitrate at 81.8%	
			- No phosphates – nitrates interaction - Langmuir isotherm - Electrostatic attraction existed between HDTMA and nitrate	
IV. Chitosan	Study the adsorption of NO ₃ ⁻ on Chitosan hydrobeads	Temperature was 30°C	- Maximum adsorption capacity : 92.1 mg/g - Adsorption increased with a decrease in the pH of the solution - Above pH 6.4, adsorption by chitosan beads indicated the involvement of physical forces - Adsorption capacity decreased by increasing the temperature from 20 to 30°C and from 30 to 50°C. - Desorption was done by increasing the pH of the solution to the alkaline range	Chatterjee and Woo, 2009
V. Agricultural wastes adsorbents				
a. Lignocellulosic agricultural waste materials, bagasse and rice hull	- Investigate the feasibility of lignocellulosic agricultural waste materials (LCM), sugarcane bagasse (BG) and rice hull (RH) conversion into weak base anion exchangers - Study their potential for NO ₃ ⁻ removal from water.	- Pure cellulose (PC) and pure alkaline lignin (PL) were used as reference materials - Epoxy and amino groups were introduced into BG, RH, PC and PL substrates after the reaction with epichlorohydrin and dimethylamine in the presence of pyridine and an organic solvent (DMF).	- Amino group incorporation decreased with the presence of water and increased with the reaction time and the presence of a catalyst (pyridine) - NO ₃ ⁻ exchange capacity: - PL (1.8 mmol/g = 412.5%) - BG (1.41 mmol/g = 300%) - PC (1.34 mmol/g = 166%) - RH (1.32 mmol/g = 180%)	Orlando et al., 2002
b. Pomegranate rind	Study the adsorption of nitrates using thermally treated carbons derived from pomegranate rind.	-Two types of activation: -Thermal at 200, 300, and 400°C - Boiling at 150°C - A control:untreated pomegranate rind (UPR)	- Maximum adsorption capacity : - thermally treated carbon 400°C. -Equilibrium time: 6 h - Adsorption capacity was higher at: - lower pH (2–3) - higher value of initial concentration of nitrate (200 mg/L) - Freundlich adsorption isotherm	Mishra et al., 2014

Treated Soybeans (SB)	Investigate nitrate and nitrite adsorption on treated SB	SB treated with CaCl ₂ and HCl, and calcination at 400, 600, 800, and 1000 °C to introduce chloride ions onto the SB surfaces	<ul style="list-style-type: none"> - SB calcined at 600°C (SB600), had the highest adsorption capacities - Nitrate and nitrite compete on the adsorption sites - Nitrate and nitrite adsorption decreased with higher chloride ions concentration - Langmuir and Freundlich adsorption isotherms - Nitrate adsorption increased with increasing temperature. - The adsorption equilibriums onto SB600: <ul style="list-style-type: none"> - For nitrate: 24 h - For nitrite: 16 h 	Ogata et al., 2015
VI. Industrial waste adsorbents	Study the removal of NO ₃ ⁻ from aqueous solution by using original and modified red mud	The red mud was: <ul style="list-style-type: none"> - washed with water - dried - washed with activated water - treated with HCl 	<ul style="list-style-type: none"> - NO₃⁻ adsorption capacity of activated red mud was found to be higher than that of the original form and decreased above pH 7 - Adsorption capacity was: <ul style="list-style-type: none"> - 1.9 mmol for original mud - 5.9 mmol/g for activated red mud - Equilibrium time: 60 min 	Cengeloglu et al., 2006
VII. Miscellaneous adsorbents	Study NO ₃ ⁻ adsorption on activated Sepiolite, slag and activated carbon	Sepiolite was activated by HCl	<ul style="list-style-type: none"> - Equilibrium time was 30 min for Sepiolite, 45 min for powdered activated carbon and 5 min for activated Sepiolite - pH 2 was better for powdered activated carbon, while pH did not affect adsorption with other adsorbents - Sepiolite activated by HCl showed the highest removal potential: 38.16 mg/g 	Ozturk et al., 2004

3.5. CHEMICAL DENITRIFICATION

Chemical denitrification (CD) corresponds to the reduction of nitrate by metals. Various metals have been used for nitrate reduction including aluminum and iron (both Fe⁰ and Fe²⁺); others such as copper under basic conditions (Eq. 3), palladium and rhodium were used as catalysts in nitrate reduction (Shrimali and Singh 2001). In this process, the nitrogen species are transformed rather than simply displaced to a concentrated waste stream that requires disposal. The reduction of nitrate beyond nitrogen gas to ammonia, partial denitrification, and insufficient nitrate removal (nitrite can be converted to nitrate with the use of chlorine in disinfection) are problematic issues that accompany this process.

The mechanism of denitrification involves the transfer of electrons from an electron donating metal to nitrate where the nitrogen in nitrate is often reduced to the least oxidized form, ammonium (Eq. 4)



Eq. 03

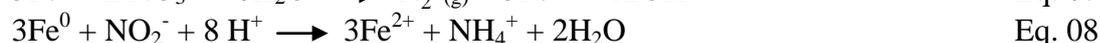
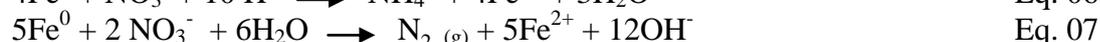
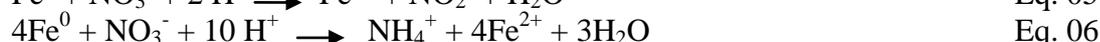


Eq. 04

Some drawbacks discouraged the use of this process, such as the production of huge amounts of iron sludge and ammonia which in turn needs to be treated by air stripping, in addition to its high cost (Kapoor and Viraraghavan, 1997).

3.5.1 ZERO VALENT IRON (ZVI)

Zero-valent iron has been recently used for denitrification purposes. Forms of application include powdered iron, stabilized iron as nanoparticles, iron filings, and permeable reactive barriers (PRBs). Using ZVI, nitrates are reduced progressively into nitrites (Eq. 5), ammonia (Eq. 6), or nitrogen gas (Eq. 7) (Huang et al., 1998; Hao et al., 2005; Xiong et al., 2009). Nitrite in its turn can be reduced to ammonia either easily as shown in Eq. 8) or by the hydrogen gas that is produced from corrosion reactions (Eq. 9) to ammonia (Eq. 10).



Some improvements to chemical denitrification were attained by further modifying the conventional chemical denitrification, summarized in Table 8. Moreover, the most important denitrification research studies done using chemical denitrification are cited in Table 9.

New process	New additions	References
Catalytic denitrification	- Metal reduction of nitrate in the presence of a catalyst - Applicable to potable water treatment	Sun et al., 2010
Sulfur-Modified Iron (SMI) media	- Nitrate reduced to ammonia by sulphur modified iron granular media as : $4\text{Fe}^0 + \text{NO}_3^- + 10 \text{H}^+ \rightarrow 4\text{Fe}^{2+} + \text{NH}_4^+ + 3\text{H}_2\text{O}$ - Used for potable water treatment - Multiple contaminant removal - Limited waste disposal costs - Significant effect of temperature (high temperature increases removal)	DSWA, 2010
Granular Clay Media	- Used for nitrate removal from potable water - Multiple contaminant removal - Cost effective - Green technology	MicroNose, 2010
Powdered Metal Media	- Metal iron-based powder (Cleanit®-LC) achieves 60%-90% nitrate removal from drinking water - Multiple contaminant removal - Density: 1800 – 2100 kg/m ³ - Particle size: 150-850 μm - Porosity: 60%	Lavis, 2010

Study aim	Results	References
Investigation of the use of powdered ZVI for the reduction of nitrate to ammonia.	<ul style="list-style-type: none"> - Highly pH dependent, nitrate reduction was favourable only at a pH below 4 - The minimum ratio of iron to nitrate was 120 m²/mol NO₃⁻ for complete reduction within 1 hour, - Pre-treatment of iron particles with high temperature exposure to hydrogen gas and deposition of copper resulted in improvement of nitrate reduction in neutral solutions - Hydrogen gas pre-treatment reduced the oxide layer, while deposited copper serves as a catalyst for the transfer of electrons - The end product of denitrification (nitrogen gas versus ammonia) could be controlled by the iron to nitrate ratio and the use of catalysts 	Huang et al., 1998 Liou et al., 2005 Xiong et al., 2009
	<ul style="list-style-type: none"> - Ammonium, found in great quantities, needed air stripping for removal - In combination with chlorine, ammonium produced chloramines, which can improve the stability of residual disinfection during water distribution - Optimum nitrate removal was found at pH range 9-10.5 	Murphy, 1991
Investigation of the efficiency of nitrate removal of nano-ZVI supported on polystyrene resins	<ul style="list-style-type: none"> - Initial nitrate concentration of 50 mg N/L was 97.2% eliminated using 2.4 g/L of nano-ZVI supported on polystyrene resins at an optimum pH 5 	Fu., et al. 2014
Investigation of the efficiency of nitrate removal of nano-ZVI supported on pillared clays	<ul style="list-style-type: none"> - Initial nitrate concentration of 50 mg N/L was nearly 100% eliminated using 0.5 g/L of nano-ZVI supported on pillared clays at an optimum pH 7 	Fu., et al. 2014
Nitrate reduction using liquid-phase hydrogenation using a bimetallic catalyst of Pd-Cu in drinking water.	<ul style="list-style-type: none"> - Efficiency of Cu/Pd nitrate reduction into nitrite was decreased due to atomic oxygen absorption on the catalytic surface blocking the active sites - 4 hours of batch treatment with a hydrogen/nitrogen mixture of 43% hydrogen showed a more than 50% denitrification yield 	Lecloux, 1999 Gasparovicova et al., 1999
Selective catalytic reduction of nitrate from the groundwater using palladium, platinum, and rhodium on carbon	<ul style="list-style-type: none"> - Palladium alumina catalysts were effective in reducing nitrite to nitrogen (98%) and ammonia in the presence of hydrogen - Compared to microbial denitrification, its activity was 30 times more efficient 	Kapoor and Viraraghavan, 1997
Chemical reduction of nitrates using a composite combination of nano-zero valent iron and granular activated carbon (GAC) into nitrogen with minimum by-products	<ul style="list-style-type: none"> - GAC intensified nitrate removal with nano-ZVI chemical reduction with improved dispersion and long-term reactivity of nano-ZVI - More than 80% of nitrate was removed at 10 g/L nano-ZVI/GAC composite within 90 min. The by-products of nitrite and ammonium formed in reduction process were below 0.008 and 0.04 mg/L, respectively, and nitrogen was the main end product 	Hu et al., 2015

3.6 ADVANCED OXIDATIVE PROCESSES

Advanced oxidative processes are successfully used to decompose many hazardous chemical compounds to acceptable levels, without producing additional hazardous by-products or sludge which require further handling. These processes are divided into four categories (Zaviska et al., 2009):

1. Homogeneous chemical oxidation processes in which hydrogen peroxide is used with either ferrous ions or ozone as catalysts;
2. Homogeneous or heterogeneous photocatalytic processes in the presence of $\text{H}_2\text{O}_2/\text{UV}$ and TiO_2/UV ;
3. Electrochemical oxidation processes;
4. Sonochemical oxidation processes.

These processes have a very high removal efficiency when it comes to eliminating refractory organic pollutants. Following the production of hydroxyl radicals with the organic pollutant during the advanced oxidative processes, two possible types of reactions could occur. These reactions are hydrogen substitution and electrophilic addition of hydrogen which would give oxidized and hydroxyl-organic products consecutively (Mazille, 2012). The latter then reacts with oxygen to start the oxidative degradation of the pollutant and finally its mineralization (Mazille, 2012). In practice, nitrates can only be reduced, need electron donors and must be included in an oxido-reduction cycle.

3.6.1 NITRATE DEPOLLUTION BY PHOTOLYSIS

Nitrate was found to absorb UV light at around 200 nm (Edwards et al., 2001). Depollution was performed using photolysis which leads to the formation of hydroxyl radicals that also aids in destroying other chemically stable organic substances (Torrents et al., 1997). However, the formation of other radicals along with nitrites and the consequent reactivity in face of light lead to the formation of highly cancerogenic polycyclic intermediates, such as aromatic polycyclic nitrated hydrocarbons.

Other more recent studies also showed the efficiency of photolysis on nitrate removal. For example, a combination of sodium dithionite as reducing agent with UV irradiation using medium pressure lamps (UV-M) as an activating method, leads to a complete removal of nitrate from aqueous solutions containing 25 mg NO_3^-/L using stoichiometric dose of dithionite of 68.8 mg/L at neutral pH conditions. NO_3^- ions were reduced to ammonium with formation of nitrite as intermediates in addition to the formation of small amounts of volatile species, mainly ammonia and nitrogen gas (Bensalah et al., 2014). The photolysis of NO_3^- was not only found efficient for its removal, but also for the production of HONO and NO_2 play important roles in tropospheric ozone and OH production (Scharko et al., 2014).

3.6.2 ELECTROCHEMICAL TREATMENTS

Electrochemical treatments have been applied for a long time for the degradation of nitrates, but are still under extensively studied. The simple concept of electrochemical reduction of nitrates at the cathode of an electrochemical cell makes it a theoretically simple technology for denitrification. Moreover, this method owns many advantages, as no sludge is generated, and can be used to treat larger concentrations of nitrate pollution (Burton et al., 2007).

When electrochemical techniques have to be employed for water denitrification, a key problem is the determination of adequate anodic and cathodic electrode materials. The objective is to enhance the selectivity of the electrode, but also its stability and its lifetime. The most common choice consists of using a monometallic electrodes such as Cu (Reyter et al., 2008), Bi (Dortsiou and Kyriacou, 2009), Ni (Bouzek, 2001), Sn (Katsounaros et al., 2006), Ti (Dash and Chaudhari, 2005), Pb, Zn, Pt, to obtain nitrite as intermediate and ammonia as the most common final product. Moreover, to enhance electrode efficiency and stability, bimetallic, ternary metallic or alloy electrodes were proposed, such as Cu–Zn (Macova et al., 2005), Cu–Ni (Mattarozzi et al., 2013), Rh–Ni (Verlato et al., 2013), Sn–Pd (Hossain et al., 2013), Ag–Pd and Ag–Pt–Pd (Hasnat et al., 2011), Pd–Co–Cu alloy (Szpyrkowicz et al., 2006), and stainless steel (Lacasa et al., 2012). The normal and modified non-metallic electrodes, such as graphite and silicon carbide (Lacasa et al., 2012), and Cu or Pd–Cu modified pyrolytic graphite (Ghodbane et al., 2008), have also been used as an alternative in the electrochemical reduction process. Table 10 summarizes the recent results on some electrochemical reduction studies on nitrates.

An alternative electrochemical treatment consists in using electrocoagulation in which a sacrificial anode is used to enhance nitrate adsorption on the oxyhydroxides formed by the metallic cations released at the anode. Electrocoagulation mainly uses Al and Fe cathodes. This will not be described, as it is one of the objectives of this work and will be detailed in the next chapter.

Electrode material	Study results	References
Graphite, diamond, stainless steel, silicon carbide and lead electrodes	Graphite exhibited the highest electroreduction removal of nitrate compared to other materials, like conductive diamond, stainless steel, silicon carbide and lead.	Lacasa et al., 2012
Titanium cathode	Titanium cathode showed higher reduction activity on nitrates than graphite.	Dash and Chaudhari, 2005
Copper and Cu–Ni alloy	Copper and Cu–Ni alloy showed a catalytic effect to enhance the adsorption of nitrate by limiting the adsorption of hydrogen onto the cathode surface.	Mattarozzi et al., 2013
Sn, Bi, and Pb metal electrodes	The reduction rates of several metal electrodes (Sn, Bi, Pb, etc.) were the same when performed at a definite rational potential (Er). To avoid the side reaction, the materials with a high overpotential for hydrogen evolution are usually preferred as cathodes for nitrate electroreduction.	Dortsiou et al., 2013
Cu and Cu–Ni alloy	Ammonium instead of the desired N ₂ was the main product during the electrochemical reduction of nitrate on Cu or Cu–Ni alloy.	Durivault et al., 2007
Zn and Pb cathodes	Nitrate reduction up to 90% was reached with Zn and Pb cathodes. Electrokinetics coupled to zero-valent iron (Fe ⁰) treatment wall, showed a 84-88% nitrate-nitrogen transformation in nitrate contaminated soil.	Chew and Zhang, 1998

Copper electrodeposited on a copper substrate (Cu/Cu) cathode	Nitrate removal efficiency up to 97% was achieved while treating high concentration (1 M) nitrate with the optimized Cu/Cu cathode. A 10% increase in nitrate removal efficiency on Cu/Cu system over bare Cu.	Rajmohan and Raghuram, 2014
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Depending on the treatment operational conditions, the reduction of nitrates can reach very high rates of elimination and sometimes complete ones. These conditions can cause different by-products of nitrate reduction such as nitrite, ammonium, and N₂ gas, where from an environmental concern, processes producing N₂ as final product are the most promising ones. Different studies have shown, however, that electrochemical treatments are not highly nitrate selective at industrial scale and thus their elimination yields are low (Dortsiou and Kyriacou, 2009). This, on the other hand, was not the case with the electrochemical treatment using copper electrodes where nitrates were selectively electroreduced into ammonium with high yields. This process, on the contrary, was not found environment friendly due to the risks of ammonium on health and aquatic life (Ghafari et al., 2008). In this case, further solutions and treatments for ammonium should be performed to render these treatment methods doable and non toxic at the environmental level. Consequently, the ammonium ions were chemically oxidized into N₂ in the presence of hypochlorite ions or used as fertilizers or a nutritive source for bacterial growth (Camargo et al., 2005). Due to problematic presence and to the possible uses of ammonium, electrocoagulation study was performed and 95% of nitrate reduction into ammonium, with nitrites being an intermediate of reduction at minimal concentrations, where the ammonium was found to be adsorbed on the flocs produced during electrocoagulation (Yehya et al., 2014, 2015).

For improving electrochemically-based nitrate removal, redox coupling reaction were proposed and shown to be efficient. The objective is to impose the anodic reaction, so that the effectiveness of nitrate reduction can be better controlled through cell potential. The most common method requires the presence of chloride ions as electron donors in the solution. Three different reactions take place at three different location in this situation (Table 11): namely, at the anode, the cathode and in the bulk.

Table 11: Different electrochemical redox-coupling reactions.	
Site of reaction	Reaction
Cathode	$\text{NO}_3^- + 10 \text{H}^+ + 8\text{e}^- \longrightarrow \text{NH}_4^+ + 3\text{H}_2\text{O}$
Anode	$2\text{Cl}^- \longrightarrow \text{Cl}_2 + 2\text{e}^-$ $\text{Cl}_2 + \text{H}_2\text{O} \longrightarrow \text{HOCl} + \text{H}^+ + \text{Cl}^-$ (dismutation reaction)
Solution	$\text{NO}_2^- + \text{HOCl} \longrightarrow \text{NO}_3^- + \text{Cl}^- + \text{H}_2\text{O}$ $2\text{NH}_4^+ + 3\text{HOCl} \longrightarrow \text{N}_2 + 5\text{H}^+ + 3\text{Cl}^- + 3\text{H}_2\text{O}$

4. BIOLOGICAL DENITRIFICATION

Biological denitrification is the process the most widely used for denitrification purpose and it has been more extensively studied in Europe than in the United States. It has been implemented in Europe since 1804 (Lenntech, 2009). The first European full-scale denitrification plant was constructed at Bucklesham (Great Britain) in 1982 (Kapoor and Viraraghavan, 1997). It can adjust more than ionic exchange processes to variations in water quality, such as natural organic matter, total dissolved solids (TDS), total suspended solids,

nitrites and sulfates levels. It is mostly and more efficiently used for the treatment of surface waters. It has the potential to address multiple contaminants including nitrites, chromates, perchlorates, and trace organic chemicals (Brown, 2008). Biological denitrification consists of the employment of some bacteria in the reduction of organic nitrogen compounds, such as nitrites to harmless elemental nitrogen gas (Eq. 11) by the act of respiration under anoxic conditions, where very low concentrations of O_2 (0.1-0.2 mg/L) could inhibit the process and bacteria would be preferentially reduce oxygen rather than nitrite, (Rittman and Huck, 1989), as follows:

When coupling the ion exchange and heterotrophic biological denitrification, it is found that each of them has a specificity in which one can draw out the respective drawbacks and the advantages in comparison to the others when used for nitrite removal, (Table 15). To circumvent their respective issues, hybrid processes coupling physicochemical and biological processes have been proposed. First, a coupled biological denitrification and ion exchange was established and proved efficient for nitrite removal up to 90% using an upflow fluidized bed in waste brine (Van der Hoek and Klapwijk, 1987). For example, complete denitrification using a combined ion exchange with a sequencing batch reactor for the biological denitrification of 0.5 N NaCl brine in 20 hrs., while using methanol to nitrite-nitrogen ratio of 2.2 (Clifford and Liu, 1993). Finally, the ion exchange combined with the biological denitrification resulted in a 50% reduction of regenerant consumption and a 90% reduction in the quantity of waste salt discharged (Kapoor and Viraraghavan, 1997). Similarly, a coupling between ion exchange and biological denitrification was performed for the denitrification of potable water (Clifford and Xiaosha, 1993). This process leads to an elimination of 95% of the initial NO_3^- concentration.

Other coupling processes used for denitrification are the membrane bioreactors. Two types of these membrane bioreactors exist: the external-loop membrane bioreactor, and the immersed membrane bioreactor. Different configurations of these membrane bioreactors were employed for the aim of denitrification. For example, in gas transfer membrane bioreactor of an external loop in which the membrane is microporous (0.02 μm), the denitrification driven by molecular diffusion leads to a denitrification efficiency of 92% when starting with 40 mg N/L (Mansell et al., 2002). Other configurations include as the extractive biological membranes and the ion exchange membrane bioreactor. In the extractive membrane bioreactor, the denitrifying bacteria forming an activated sludge work on denitrification, once the nitrites are filtered by the membrane and elimination could reach up to 60% when starting with 120 mg/L of nitrites (Fuchs et al., 1997) depending on the membrane material used. Ion exchange membrane bioreactors based on chloride ions as counter-ions and an activated sludge could reach up to 7 g $N/m^2/day$ when starting with 50 mg N/L (Fonseca et al., 2000).

5. COMPARISON OF DENITRIFICATION PROCESSES

As a conclusion, the respective advantages and disadvantages of all the hybrid denitrification processes used up to date are summarized in the Table 15. In addition, Table 16 sums up the overall denitrification efficiency of the major processes and depicts a clear comparison concerning the main characteristics of interest at the experimental and industrial levels.

Table 15: Advantages and disadvantages of physical and biological denitrification treatments.

	Advantages	Disadvantages
Ion exchange	<ul style="list-style-type: none"> - Regeneration of the resins (Aouina, 2010) - No temperature and pH effect (Bhatnagar and Sillanpaa, 2011) - Years of industry experience - Multiple contaminant removal - Selective nitrate removal - Financial feasibility - Use in small and large systems - The ability to automate - The disposal of waste brine - The need to address resin susceptibility to hardness, iron, manganese, suspended solids, organic matter, and chlorine - 90% efficiency can be achieved (Bhatnagar and Sillanpaa, 2011) 	<ul style="list-style-type: none"> - A corrosive produced water with the need for post treatment of effluents of regeneration (Reyter, et al., 2010) - The clogging of the membranes (Bhatnagar and Sillanpaa, 2011) - The higher affinity for sulphate than for nitrates, resulting in a great volume of water of disposal, with high nitrate content - Limited suitability to ground water relatively free of dissolved organic matter; not suitable for surface waters - Costly disposal of waste brine, the potential for nitrate dumping and resin fouling (Bhatnagar and Sillanpaa, 2011) - A potential for hazardous waste generation (<i>i.e.</i> co-contaminants like arsenic and chromium)
Reverse osmosis	<ul style="list-style-type: none"> - High quality product water - Multiple contaminant removal - Desalination (TDS removal) - Feasible automation - Less electrical and hydraulic complexity than other technologies 	<ul style="list-style-type: none"> - The disposal of waste concentrate - Typically high capital and O&M, costs and high pretreatment and energy demands, - The need to address membrane susceptibility to hardness - The lack of control over target constituents (complete demineralization) - Membrane fouling, and deterioration - Large waste volume requiring disposal

Electrodialysis	<ul style="list-style-type: none"> - Limited to no chemical usage - Long lasting membranes - Selective removal of target species - Flexibility in removal rate through voltage control - Better water recovery (lower waster volume) - Feasible automation - Multiple contaminant removal (WA DOH, 2005) - Dedicated to the following constituents: TDS, total chromium, chromium-6, arsenic, perchlorate, sodium, mercury, chloride, copper, sulfate, uranium, fluoride, nitrate/nitrite, iron, selenium, hardness, barium, bicarbonate, cadmium, and strontium (GE, 2010) - For waters with higher Silt Density Index, silica, and chlorine levels (Elyanow and Persechino, 2005) - Clogging widely reduced compared to ion exchange process (Mook et al., 2011) 	<ul style="list-style-type: none"> - The water requires pre-treatment systems and the treatment is limited to soft waters (Rautenbach et al., 1987) - The disposal of waste concentrate, and the need for post treatment of the polluted wastes (Strathmann, 2010) - The need to address membrane susceptibility to hardness, and suspended solids - High maintenance demands, and high costs, thus unused at industrial scale (Reddy and Lin, 2000) - The need to vent gaseous products - The potential for precipitation with high recovery - High system complexity - Dependence on conductivity - The possible need for pretreatment to prevent membrane scaling fouling, and waste disposal - Does not remove uncharged constituents in the water - Cations can only pass the cation exchange membranes and the anions pass the anions exchange membranes where the nitrate ions can only be transferred instead of being eliminated (Aouina, 2010)
Chemical denitrification	<ul style="list-style-type: none"> - Conversion of nitrate to other nitrogen species (no brine or concentrate waste stream) - The potential for more sustainable treatment - High water recovery - Multiple contaminant removal 	<ul style="list-style-type: none"> - The possible reduction of nitrate beyond nitrogen gas to ammonia, or partial denitrification, and the associated production of green house gases GHGs - The possible dependence of performance on pH and temperature - The possible need for iron removal - Unknown reliability, costs, and operational complications due to the lack of full-scale chemical denitrification
Advanced oxidative processes	<ul style="list-style-type: none"> - Rapid reaction rates - Potential to reduce toxicity of organic compounds - Mineralization of organic pollutants - No concentration of waste for further treatment (as in the case of membranes) - No production of "spent carbon" such activated carbon absorption - Easily automated and controlled - No created sludge (as with physicochemical or biological processes (wasted biological sludge) (Spartan water treatment) 	<ul style="list-style-type: none"> - Highly costly, thus are only doable at the laboratory scale except the homogeneous chemical oxidation processes with hydrogen peroxide and ozone (Galey and Paslawski, 1993) - Quenching of excess peroxide is required (Spartan water treatment)

Adsorption on activated carbon	<ul style="list-style-type: none"> - The high thermal stability of the activated carbon - Insensitivity to toxic substances (Mook et al., 2011) - Its weak reactivity in acidic or basic conditions (Monsalvo et al., 2011) 	<ul style="list-style-type: none"> - High cost - Low nitrate adsorption efficiency (Mook et al., 2011) - Getting rid of post-adsorption-adsorbent and production of "spent carbon"
Biological denitrification	<ul style="list-style-type: none"> - High water recovery - No brine or concentrate waste stream (nitrate reduction into nitrogen gas rather than removal to waste stream) - Low sludge waste - Less expensive operation - Limited chemical input - Increased sustainability - Multiple contaminant removal - Suitable for surface waters - > 99% efficiency can be achieved (Bhatnagar and Sillanpaa, 2011) - Medium operational cost 	<ul style="list-style-type: none"> - The need for substrate and nutrient addition - High monitoring needs for intermittent operation (Talhi, 2010) - Significant post-treatment requirements due to bacterial contamination (Feleke et al., 2002) and pollution with organic substrates (Mansell et al., 1999) - High capital costs - Sensitivity to environmental conditions - High system complexity - The possibility of partial denitrification - Permitting and piloting requirements - Slower initial start-up of up to 6 weeks - Little sludge is produced (>0.1% by volume of treated water) - Temperature-sensitive (low at cool temperatures) (Rantanen et al., 2000) - Efficient for treating nitrate concentrations inferior to 1000 mg/L (Badea, 2009) - Nitrite formation when old sludge is used

Table 16 : Comparison of the main characteristics of some nitrate removal techniques.

Task, characteristics, and limitations	IX	RO	ED	BD	CD
Denitrification or nitrate removal efficiency	+	+	+	++	N/A
Industrial use	++	++	+	-	-
Ease of post treatment waste management	-	-	+	++	++
Efficiency and reliability	++	++	++	+	-
Ease of application	++	++	++	+	N/A
Wide range of treated water	-	-	+	++	N/A
Average total annualized cost	+++	++++	++	+	N/A
Average O & M annualized cost	+++	++++	++	+	N/A
Average capital annualized cost	+	+++	++++	++	N/A
++++: very high ; +++: high ; ++: medium ; +: low N/A : Data not found ; O & M : Operational and Maintenance					

6. CONCLUSION

This review chapter highlights nitrates as a potent pollutant in water with its different hazardous effect on health and environment. It also reviews all the different treatment methods destined for its removal from water. Developments and adjustments of each method for enhancing nitrate removal process were discussed and detailed throughout this review. Each discussed treatment method was found to have its own advantages and limitations, in terms of implementation simplicity, efficiency and cost. For example, nitrate ions are concentrated by physico-chemical and/or partially destroyed or transformed by biological denitrification process. In terms of removal efficiency, biological denitrification was found to be the most efficient. This treatment is widely used at industrial scale for wastewater treatment despite its bacterial growth requirements and limitations because of its low cost, but it cannot be used at high nitrate content and/or when compounds toxic to the microorganisms are present. Whereas, in terms of cost, reverse osmosis process was found to be highly costly, but is also used at the industrial scale due to its reliability and ease of application for example for the production of purified water. Generally speaking, ion exchange was found as the best treatment method to use industrially since ages, although it needs excessive-post treatments. In conclusion, no process sounds to be perfect practically and economically for nitrate removal from water. Consequently, nitrates are still cycling in the environment with yearly increasing concentrations. This calls out the necessity of finding new cost effective, easy to handle, and highly efficient treatment methods of denitrification of least requirements possible.

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CHAPTER II: EXPERIMENTAL ANALYSIS AND MODELING OF DENITRIFICATION USING ELECTROCOAGULATION PROCESS

This article is published online on 2 June 2014 in Separation and Purification Technology. Consequently, this chapter follows the guidelines of this journal.

T. Yehya, M. Chafi, W. Balla, Ch. Vial, A. Essadki, B. Gourich, 2014. Experimental analysis and modeling of denitrification using electrocoagulation process, Separation and Purification Technology, 132, 644–654.

ABSTRACT

Electrocoagulation (EC) has been studied to assess its applicability as a denitrification process for drinking water. The objective was to investigate the mechanisms of nitrate removal. Electrolysis has been driven in the discontinuous mode with aluminum electrodes, using a synthetic water representative of drinking water. The respective effects of mixing, initial nitrate concentration, current and initial pH have also been analyzed. Experimental results have shown that EC removes effectively the nitrate anions, following first-order kinetics. The rate of denitrification is proportional to current. The removal of nitrate anions results primarily from their electroreduction into ammonium, but total nitrogen decreases simultaneously in water and follows zero-order kinetics. Nitrogen mass balance has shown that the formation of N₂ gas is negligible and that the secondary depollution mechanism is adsorption onto the flocs.

Adsorption experiments on preformed flocs highlight a preferential adsorption of ammonium. A numerical model able to simulate nitrate removal has been established. The analysis of operating costs has Water treatment shown, however, that EC is an expensive method, except for waters exhibiting very high nitrate contents. Consequently, EC should be preferentially used as a pretreatment step for biological denitrification when implemented to eliminate simultaneously other types of pollution.

1. INTRODUCTION

Nitrate is considered as an undesirable substance in surface and ground water. It is responsible for eutrophication in surface water. For human health, its intrinsic toxicity is still subject to discussion, but nitrates are likely to turn into nitrites and ammonia, both toxic, and also into carcinogenic nitroso-derivatives [1]. For example, nitrites combine to hemoglobin to form methaemoglobin in the human body, which can be fatal to neonates [2]. WHO drinking water guidelines are 50 mg/L for NO₃⁻ [1], but the maximum concentration is 45 mg/L in India [3] and lower values are suggested for infants in many countries of the world, such as EU, USA and India. Naturally present at low concentrations in surface water and groundwater, nitrate content is constantly increasing in aquatic systems in the last decades. This is mainly due to human activities, including agriculture and urban practices. Fertilizers and animal waste strongly contribute to the discharge of inorganic nitrogen. Nitrate can also be found in industrial wastewater, such as food or metal industries [4,5]. However, even in 2013, the EU Nitrates Directive [6] that protects the water resources, in particular from agricultural sources, is applied imperfectly in many European countries. Denitrification treatments are, therefore, necessary both for drinking water and wastewater. Nitrate is a stable and highly soluble anionic compound with low potential for co-precipitation or adsorption. Even though emerging

adsorbents have been proposed, their use is not yet assessed for nitrate removal under industrial conditions [7]. Many popular processes, such as chemical coagulation, lime softening, and oxidation processes, are effective for removing most of the pollutants including heavy metals, but fail for nitrate. In practice, biological denitrification remains the most common method because it is environmentally-friendly and cost-effective [8]. This mainly consists of anaerobic digestion which reduces nitrate anions into nitrogen gas, using either heterotrophic or autotrophic microorganisms [9]. The main drawback is that microbial denitrification is slow and highly temperature-dependent. Heterotrophic processes also require the addition of organic substrates, which is compulsory at low C/N ratio, strongly affects denitrification yield and requires purification post-treatments for organic by-products and dead bacteria. Autotrophic bacteria denitrification requires hydrogen, thiosulfate or sulfide anions as electron donors, which imposes other additional constraints. Advanced physicochemical treatments have been proposed as an alternative. These include ion exchange process, reverse osmosis, electrodialysis, chemical and catalytic reduction, electroreduction and electrocoagulation (EC). Ion exchange is the most attractive alternative for small and medium-size industrial applications (see, e.g., [10]), but it suffers from a limited selectivity in the presence of competing anions and remains fairly high in capital and operating costs in comparison to biological treatments. Membrane cost and fouling are the main limitations of reverse osmosis and electrodialysis [11]. Another disadvantage is that all these treatments cannot convert nitrate into harmless compounds but only transfer nitrate from water to brine waste, which should be circumvented by chemical and electrochemical reduction processes.

Chemical reduction presents the advantage to be also cost-effective. The applicability of zero-valent aluminum or iron powder [12,13] has been studied. Recent advances mainly involve iron nanoparticles [14]. However, nitrate is mainly converted into ammonia, which requires a downstream stripping system. Heterogeneous catalytic reduction has also been investigated, for example by Fe(II) cations even though it remains slow, or by hydrogen [15], but this cannot prevent the accumulation of nitrite. For enhancing nitrate reduction, electrochemical process has been applied for nitrate removal, using an inert anode and a metal electrode such as copper, stainless steel, or a semiconductor material cathode such as boron-doped diamond and silicon carbide [16,17]. Depending on cathode material, nitrite, ammonium (adsorbed and dissolved), and soluble gaseous NO_x (mainly NO, but also NO_2 , N_2O ...) were the main reaction products. Gaseous nitrogen was only significant with aluminum and tin cathodes [18]. This is mainly due to the complex mechanisms of nitrogen oxydoreduction that strongly depend on pH and can be summarized schematically in Fig. 1. In practice, selectivity is a major issue in the presence of other reducible pollutants that has often been disregarded in the literature. When a sacrificial anode is used, electrochemical treatment proceeds as electrocoagulation, which presents the advantage to be able to circumvent partially this issue.

Electrocoagulation (EC) is a non-specific electrochemical water treatment technology that can be applied to both drinking water and wastewater. It consists of the controlled corrosion of a sacrificial anode (usually in iron or aluminum) under the effect of a constant current or voltage. The metal cations released in situ by metal dissolution then act as coagulants, adsorbents or coprecipitating agents when they react with hydroxide anion under neutral or alkaline conditions to form metal oxyhydroxides. It differs therefore from conventional coagulation in which the coagulant is added locally at once because metal cations are produced continuously and in situ [19]. Its main advantage is that it is able to treat simultaneously almost all types of pollution, such as organic pollutants and turbidity [20], dyes [21], pharmaceuticals [22], heavy metals [3,23], inorganic anions including sulfide [24], fluoride [25] and nitrate that will be discussed further. This explains why this technology regained interest in the last decade.

Another advantage is that H_2 generated at the cathode resulting from the reduction of water promotes the separation of flocs formed by flotation. Its main potential applications have been summarized by Emamjomeh and Sivakumar [26] and specific applications on nitrate removal with aluminum electrodes were described by Koparal and Ögütveren [27], Emamjomeh and Sivakumar [28] and Lacasa et al. [29]. These authors established that EC is an effective method to remove nitrate ions, but their conclusions differed on the depollution mechanisms. Koparal and Ögütveren [27] and Emamjomeh and Sivakumar [28] suggested an electroreduction of nitrate anions into ammonium cations, and then into gaseous N_2 , as Murphy [12] who had reported the direct reduction of NO_3^- into N_2 . Conversely, Lacasa et al. [29] proposed a mechanism based on the adsorption on precipitated oxyhydroxides.

As a result, the objective of this work is to investigate nitrate removal using EC so as to better understand the underlying mechanisms and to show the potentiality but also the limits of the application of electrocoagulation process for nitrate elimination. Experimental results will also be used to estimate the operating costs, while the description of the mechanisms will be used to establish a modeling approach.

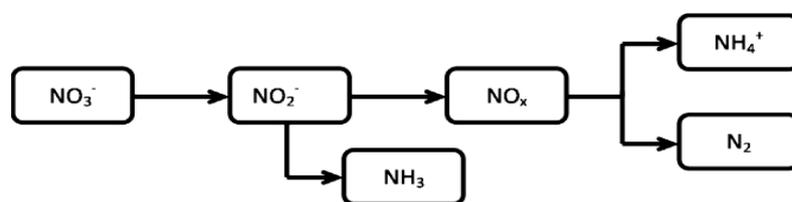


Figure 1: Simplified reduction pathway of nitrate anions.

2. MATERIALS AND METHODS

In this study, electrocoagulation was applied to synthetic water, representative of the composition of drinking water. This gives access to more reproducible experimental conditions than real drinking water and is required to get reliable kinetic data. The composition of the synthetic water is reported in Table 1. Nitrate anions were added to vary the initial concentration (C_0) between 50 and 200 mg NO_3^-/L by the addition of sodium nitrate $NaNO_3$ (Sigma–Aldrich, UK), which increases also the Na^+ content of water in comparison to Table 1. The initial pH (pH_i) was adjusted between 3.8 and 10.2 by a minute addition of 0.1 M HCl or NaOH solutions. This did not modify significantly water conductivity (K). Electrocoagulation was carried out in a 4-L cylindrical tank ($V = 4$ L) equipped with a Rushton turbine for mixing purpose. EC was conducted in the galvanostatic mode using a 30 V-10 A power supply (ELC, France), while the cell voltage (U) was recorded by means of a VC950 voltmeter (Votcraft, France) in order to derive the electric power input. The respective influences of rotation speed of the turbine (100–400 rpm), current (I) between 0.5 and 4.5 A and initial pH were also studied. Aluminum metal was used for cathode and anode despite its higher cost than iron because it remains affordable for drinking water treatment. Electrodes were rinsed with acetone and a 0.01 N HCl solution to remove organic and inorganic deposits, and then weighed before use. Planar rectangular electrodes of identical surface area (S), 102 cm^2 , were used as anode and cathode. For all the runs, the inter-electrode gap (e) was maintained at 1 cm. Operation time (t) was varied between 30 and 120 min. Experiments were carried out at room temperature and atmospheric pressure, but temperature was recorded over time. During EC, samples were taken out at different time intervals and filtered through $0.45\ \mu\text{m}$ filters; the filtrates were then used for subsequent chemical analysis. The ionic composition over time was obtained using ion chromatography (*Metrohm AG, Switzerland*) both for cations (Na^+ , NH_4^+ ,

K^+) and anions (Cl^- , NO_2^- , NO_3^- , SO_4^{2-}). Concentrations were derived from peak area using the addition of an internal standard, Li^+ for cations and Br^- for anions, respectively. Total nitrogen was measured in each sample using a TNM-1 analyzer (*Shimadzu*, Japan). Nitrogen speciation in the liquid phase was deduced from these measurements. pH and conductivity of water were monitored over time using a HI-213 pH meter (*Hanna Instruments*, USA) and a CDM210 conductimeter (*Radiometer Analytical*, France) using data acquisition. Other measurements were carried only at the end of EC operation. Electrode mass loss was measured after rinsing by comparing electrode weight at the beginning and at the end of EC, so that the actual metal consumption could be deduced and the faradic yield of the electrodisolution (ϕ) derived from Faraday's law could be estimated. Floccs recovered by sedimentation/flotation were filtered, washed and dried at 120°C overnight before being weighed to quantify the mass of dry sludge. The TNM-1 total nitrogen analyzer was also used to estimate the amount of nitrogen in the solid phase. The possible formation of N_2 gas during EC was deduced from the mass balance on the solid and the liquid phases. The solid phase was also characterized by X-ray diffraction (*XRD D501*, *Siemens*, Germany) and by nitrogen BET surface area analysis with nitrogen adsorption (*Tristar II*, *Micromeritics Instr.*, USA). The floccs and the electrode surface were also observed by scanning electron microscopy SEM (*JSM820*, *Jeol Ltd.*, Japan). A sketch of the experimental setup of this work is shown in Fig. 2a, while a picture of the setup and of the electrodes is shown in Fig. 2b.

Property	Value
Cl^-	60 mg/L
SO_4^{2-}	1090 mg/L
HCO_3^-	107 mg/L
Na^+	78 mg/L
K^+	835 mg/L
pH	8.2
κ	2.8 mS/cm

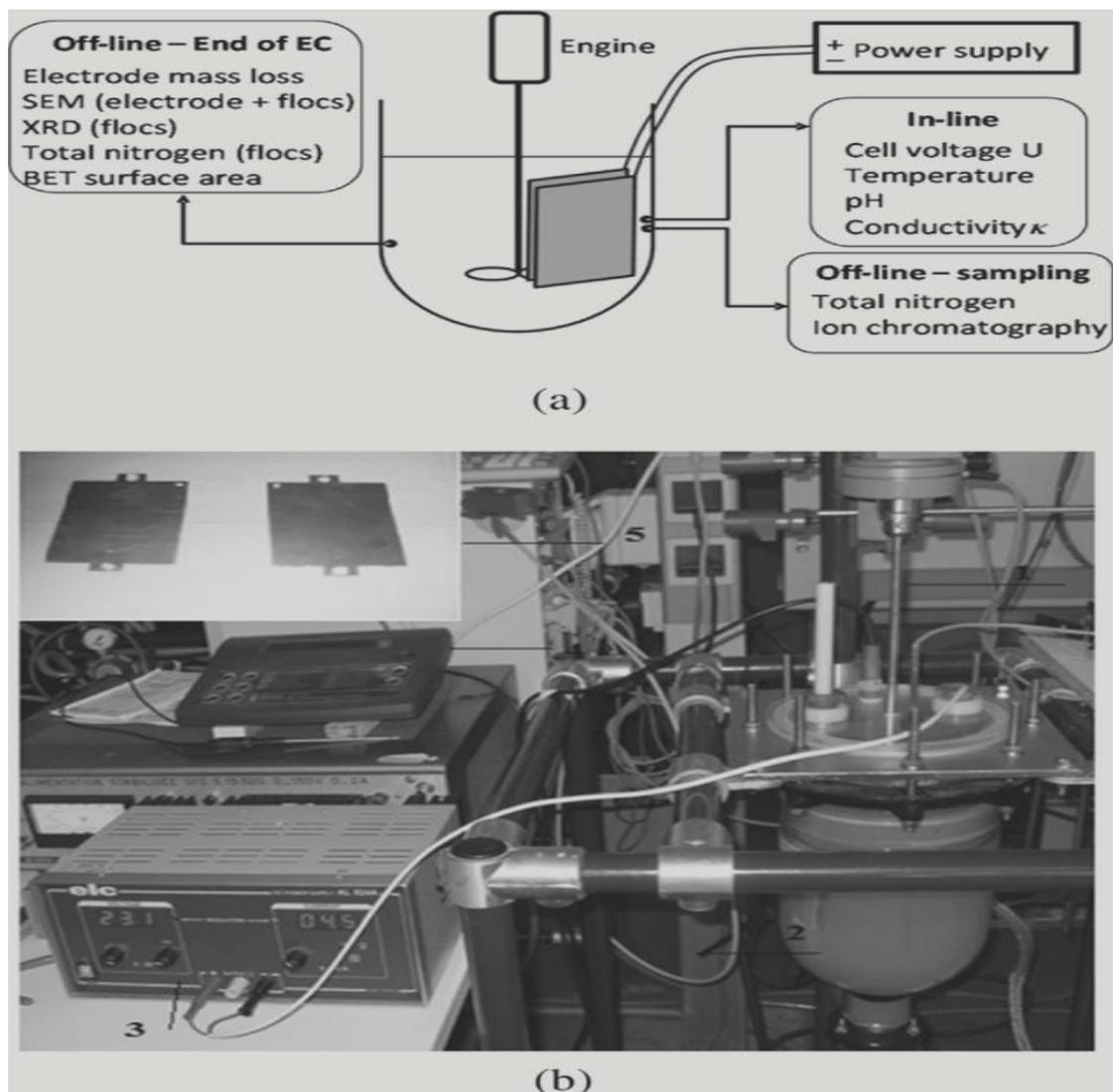


Figure 2: (a) Sketch of the experimental setup with the associated measuring techniques; (b) picture of the experimental setup: 1: Stirrer; 2: Electrochemical cell; 3: DC power supply; 4: pH-meter; 5: Aluminum electrodes.

EC experiments were conducted in duplicate and each measurement was repeated three times. Each concentration is, therefore, the average of three values. The good reproducibility of the experiments is illustrated in Fig. 3a for pHi 3.8 and pHi 8.2.

3. RESULTS

3.1. INFLUENCE OF MIXING AND INITIAL PH

The experimental study started with an analysis of the influence of mixing conditions, varying rotation speed of the turbine between 100 and 400 rpm. This had no apparent effect on nitrate removal (data not shown). This means that regardless of the mechanism of depollution (electroreduction at the cathode or adsorption onto the flocs), there is no apparent limitation due to mass transfer in the EC process. This is a key point because both electroreduction and adsorption may be controlled by mass transfer, which leads to pseudo-first order kinetics [17].

A speed of 200 rpm has finally been retained, so as to prevent swirl, while reducing the power input for mixing purpose.

Unlike the effect of the stirring rate which can be easily overcome, pH is typically a key parameter affecting EC both in terms of effectiveness and operating cost [30]. In accordance with the work of Emamjomeh and Sivakumar [28], experimental results obtained by ion chromatography showed a weak dependence of the reduction of NO_3^- anions as a function of initial pH (Fig. 3a).

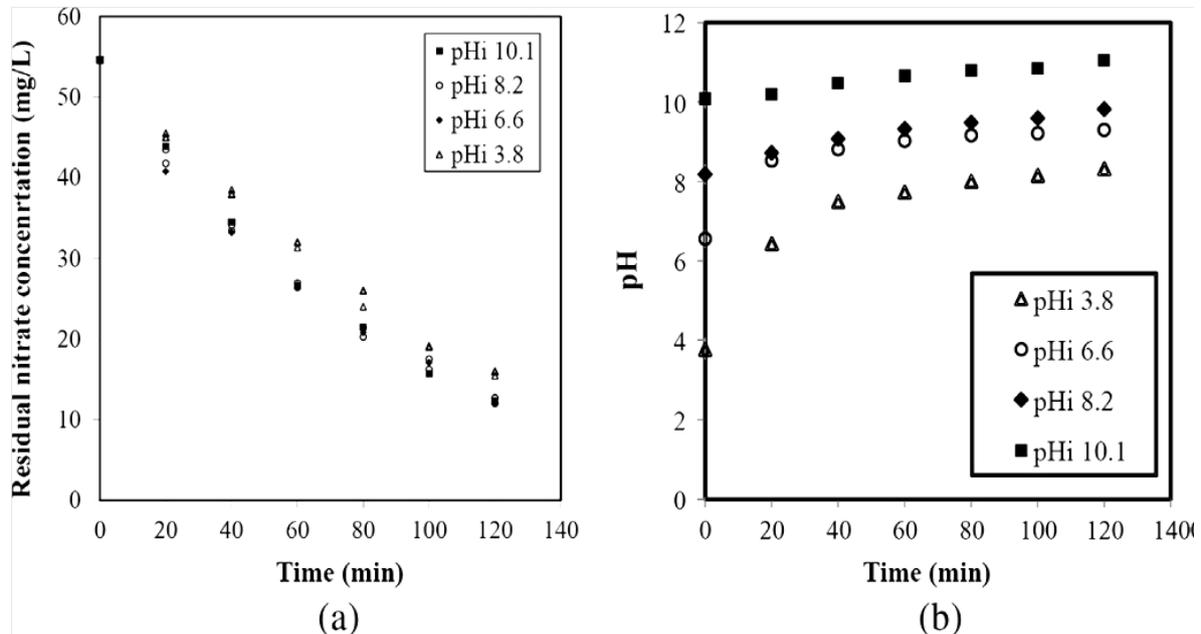


Figure 3: (a) Effect of the initial pH on the evolution of nitrate concentration $C(t)$ in $\text{mg NO}_3^-/\text{L}$ over time ($C_0 = 54\text{mg NO}_3^-/\text{L}$; $I = 4.5\text{ A}$); (b) influence of initial pH (pH_i) on pH evolution during EC ($C_0 = 54\text{mg NO}_3^-/\text{L}$, $I = 4.5\text{ A}$).

Nitrate removal seems, in a first period, a little faster between pH_i 6 and 8, but similar removal yield has been achieved at the end of electrolysis with pH_i 10.1 at a fixed current I . Only highly acidic initial conditions seem to delay nitrate elimination, without inhibiting pollution abatement. One possible reason is that pH varies strongly during EC and tends rapidly to alkaline values at the end of operation, even for an initial acid pH (Fig. 3b). This contrasts with the amount of floc formed during EC that varies from 4.5, 6.1, 9.0 to 6.5 g when the initial pH increases from 3.8, 6.6, 8.2 to 10.1 after 120 min electrolysis, respectively. These results agree with the speciation of aluminum: Al^{3+} cations dominate at low pH, aluminate $\text{Al}(\text{OH})_4^-$ anions prevail at pH higher than 10 and the insoluble $\text{Al}(\text{OH})_3$ hydroxides at intermediate pH. This explains, first, the delay to achieve similar yield when EC starts under highly acidic conditions. This result also suggests that adsorption is not the predominant mechanism because the amount of nitrate removed is not obviously correlated to the amount of floc formed. In addition, the behavior observed in Fig. 3b is atypical for EC conducted with Al electrodes. This operation classically tends to act as a pH buffer around 7 since the oxidation of Al to $\text{Al}(\text{OH})_3$ at the anode and the reduction of water at the cathode produce and consume the same amount of H^+ cations [30]. The trend observed in Fig. 3b may indicate the presence of another chemical or electrochemical reaction that shifts the pH towards alkaline values, such as the reduction of nitrate ion into $\text{NH}_4^+/\text{NH}_3$, or into gaseous NO and N_2 (Eqs. (1)– (3)), except nitrate to nitrite electroreduction that does not affect pH (Eq. (4)):



3.2. INFLUENCE OF CURRENT AND INITIAL NITRATE CONCENTRATION

The second set of experimental runs was dedicated to the study of the respective influences of the initial nitrate concentration C_0 and of current I on nitrate elimination. The results show that an increase of current results in an acceleration of nitrate removal (Fig. 4a), so that the nitrate concentration falls below the guideline value of 50 mg NO_3^- more rapidly. The shape of the curves shows an evolution of removal efficiency that looks like a decreasing exponential pattern, which seems to correspond to first-order kinetics. This is confirmed in Fig. 4b: this highlights that the nitrate concentration over time can be related to $\ln(C)$ at time t with coefficients of determination R^2 always above 99%, regardless of C_0 , by the classical expression:

$$\ln(C_0/C) = k.t \quad (5)$$

This figure also shows that the rate constant k (min^{-1}) is almost proportional to I , which is consistent with the data from Emamjomeh and Sivakumar [28]. This is also consistent with those from Lacasa et al. [29] who showed that the removal yield of nitrate anions depended only on the amount of aluminum released from the anode. In parallel, Fig. 5a confirms that the kinetics of nitrate removal is independent of the initial concentration C_0 , in accordance with ‘‘real’’ first-order kinetics, and not with a pseudo-first order as observed for fluoride anions removal using EC by Essadki et al. [25]. As shown in Section 3.1, this cannot be explained by mass transfer control, as mass transfer coefficient is sensitive to mixing conditions even at current density as high as 44 mA/cm² ($I = 4.5$ A). A re-analysis of the data from Emamjomeh and Sivakumar [28] provides a k/I ratio close to $2 \times 10^4 \text{ C}^{-1}$, while k/I approaches $4.5 \times 10^5 \text{ C}^{-1}$ in this work. This difference may stem from their EC cell based on five monopolar electrodes that strongly modifies the S/V ratio, but it may also result from their synthetic water that has a simpler composition (only sodium nitrate and sodium bicarbonate). Indeed, it is well known that the presence of other co-anions than chlorides can impair the pollution abatement, especially sulfates, as shown by Hu et al. [31] in the case of fluoride elimination by EC. The high sulfate content of our synthetic water may, therefore, explain the lower rate constant of nitrate removal in this work (Table 1).

As a conclusion, an electrolysis time of about 5, 70 and 120 min, respectively, was necessary to reach nitrate concentrations lower than the guideline value of 50 mg NO_3^-/L using $I = 4.5$ A for C_0 values of 54, 110 and 203 $\text{NO}_3^- \text{ mg/L}$ in Fig. 5b. This means that EC was always efficient enough to achieve nitrate removal, both for high C_0 values (Fig. 5) or when the nitrate concentration was lower than 50 mg NO_3^-/L (Fig. 4). As a result, EC seems to be able to reach any desired nitrate level, for example guidelines for infants. Eq. (5) gives access to the electrolysis time required to achieve this objective, provided the k and C_0 values are known and the current I is chosen. The advantage is that this time is nearly independent from pH_i (Fig. 3). This confirms the results from the literature that had already highlighted the high performance of EC for nitrate removal, but using in this paper the synthetic water described in Table 1 with a more complex composition than reported in previous works.

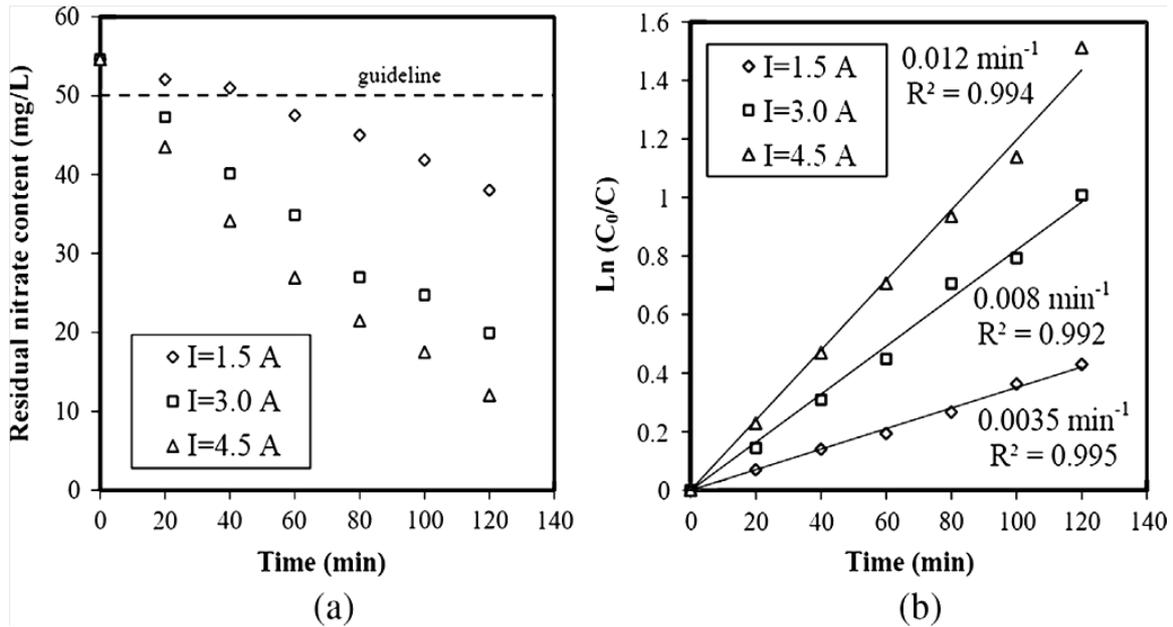


Figure 4: Validation of first-order kinetics for nitrate removal and current dependence at initial pH 8.8 with $C_0 = 55 \text{ mg NO}_3^-/\text{L}$ as a function of current I (C in $\text{mg NO}_3^-/\text{L}$).

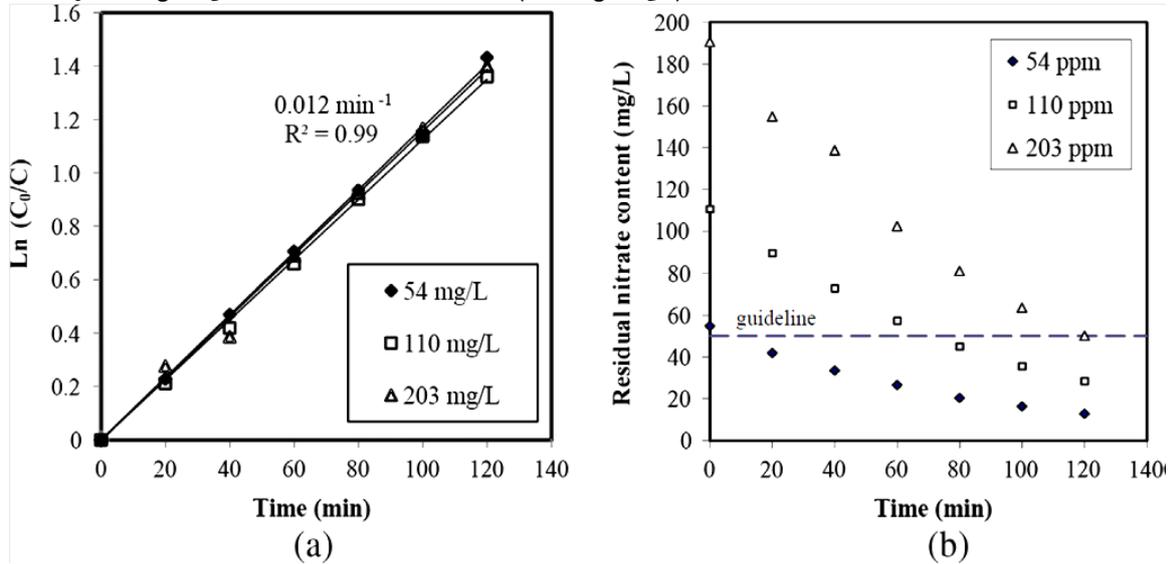


Figure 5: Validation of first-order kinetics for nitrate removal and concentration dependence at initial pH 8.8 with $I = 4.5 \text{ A}$ as a function of initial concentration C_0 (nitrate concentrations C and C_0 in $\text{mg NO}_3^-/\text{L}$).

3.3. SPECIATION OF NITROGEN AND SOLUBLE SPECIES

To carry out mass balance on nitrogen, all the concentrations involving nitrogenous species will be delivered in mg N/L in this section and the next one (which corresponds to a guideline value of $10 \text{ mg N- NO}_3^-/\text{L}$), except the initial nitrate concentration C_0 that will always be expressed in $\text{mg NO}_3^-/\text{L}$. The analysis of nitrogen compounds in water over time revealed the formation of ammonium ions, and to a much lesser extent, nitrite. This is consistent with the literature on electroreduction in Section 1. An example of the evolution of nitrogen speciation in the liquid phase is shown in Fig. 6a. The formation of ammonium ions had already been reported by Emamjomeh and Sivakumar [28] and Lacasa et al. [29], but in different proportions. However, the nitrogen content of the solid phase had never been studied so far:

- Emamjomeh and Sivakumar [28] assumed that the difference between the initial amount of soluble nitrogen and the amount present at time t as NO_3^- anions and NH_4^+

was totally converted into gaseous N_2 , advocating the absence of nitrate compounds on the X-ray diffractograms of the flocs. However, this neglects adsorption or physical capture by a solid phase.

- Lacasa et al. [29] assumed that nitrates are adsorbed onto the solid under thermodynamic control, following a Freundlich isotherm, but only the liquid phase was analyzed and this does not explain the high ammonium concentration found with aluminium electrodes in Fig. 6a.

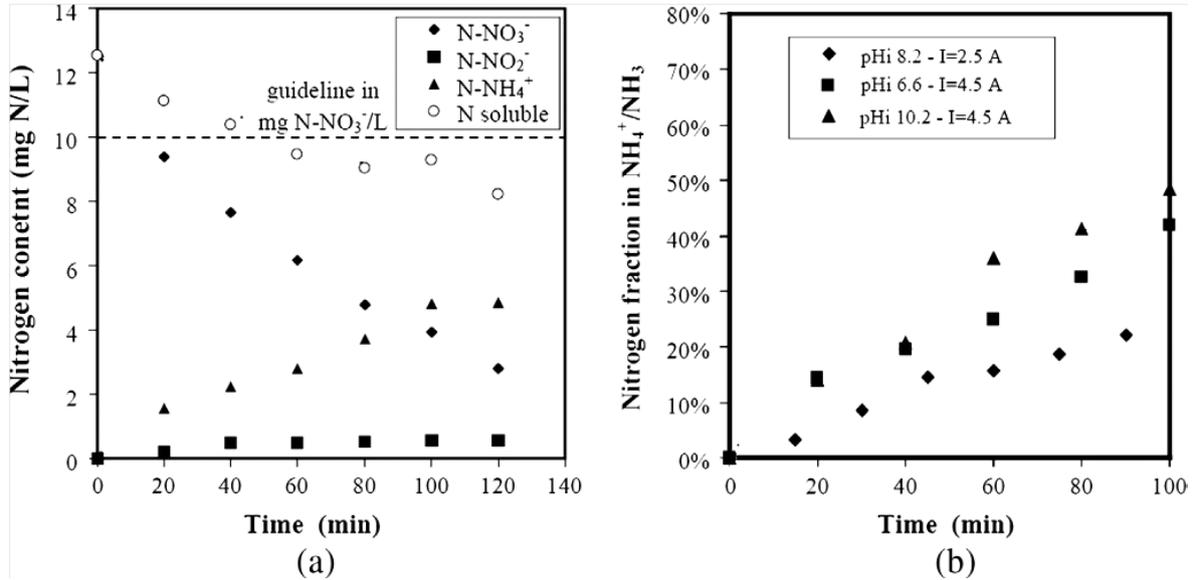


Figure 6: (a) Evolution of the speciation of the soluble nitrogen with species concentrations expressed in mg N/L for $C_0 = 54 \text{ mg NO}_3^-/\text{L}$, $\text{pH}_i = 7.0$ and $I = 4.5 \text{ A}$; (b) fraction of nitrogen present as ammonium ions based on total nitrogen for $C_0 = 54 \text{ mg NO}_3^-/\text{L}$, various pH_i and current.

In this work, XRD analysis highlights that the flocs are amorphous and that it is not possible to identify nitrate or ammonium solid compounds (data not shown). However, the total nitrogen analyzer shows on the one hand that the solid phase contains nitrogen compounds and, on the other hand, that total nitrogen content in the liquid phase decreases at the end of EC when the current increases. In the solids, the average nitrogen content is usually about 1.5 mg N/g floc, which corresponds roughly to 5 mg N/g dissolved Al. Typical examples of nitrogen mass balance at the end of EC operation are reported in Table 2. The “undefined” fraction of nitrogen mass balance in Table 2, *i.e.* not found in the liquid and the solid phases, is always lower than 10%. This can be considered to be overestimated due to a loss of solid during filtration and washing operations, but another reason explaining this undefined fraction may be ammonia desorption: when pH is about 9 or higher, NH_3 becomes the dominant species in place of NH_4^+ ; this occurs at the end of EC when pH_i is 6 or higher in Fig. 3b, and although NH_3 is a highly soluble gas, its desorption may be enhanced by H_2 desorption. In addition, if measuring error is also accounted for, especially if we consider the various measuring techniques involved in the analysis of nitrogen speciation, it seems that the probability of formation of gaseous NO and N_2 compounds remains low in comparison to that of ammonium cations. Accordingly, the mechanisms proposed by Murphy [12] and adopted by Emamjomeh and Sivakumar [28] do not seem to be able to explain our experimental results. It is worth of note that the amount of nitrogen that is not in the form of NO_3^- , NO_2^- and NH_4^+ ions in Fig. 6a never exceeds 30% of the initial nitrogen, which means that the capture by the solid phase is never the dominant phenomenon during EC. This can also be seen in the examples of Table 2, but it remains also true for all EC runs after 120 min.

Considering the dependence of the amount of NH_4^+ ions formed on I (Fig. 6b), the most probable mechanism of denitrification is the electroreduction of NO_3^- ions on the aluminum cathode. The main product corresponds to ammonium cations, but neutral NH_3 may prevail when the pH becomes strongly alkaline (Fig. 3b). Electroreduction probably proceeds through an intermediate reduction into nitrite anions, but their concentration always remains low. The nitrite content seems to vary with pH_i and current; the maximum was reported for $I = 4.5 \text{ A}$, *i.e.* for high current (Fig. 7 and Table 2) at about 5% of total nitrogen, which is in accordance with their role of intermediate in the nitrate reduction into ammonium (Fig. 1). As a result, it seems that the increase in current enhances simultaneously the rate of formation of NO_2^- ions and the rate of their reduction into NH_4^+ , but a bit less the rate of formation of NH_4^+ . However, at low C_0 and current, nitrite concentration was often close to the detection limit at low current, which agrees with literature data [29]. Consequently, it is not possible to correlate accurately the evolution of the nitrite concentration and the operating conditions in this work. Table 2 also highlights the presence of other “soluble” species, usually about 5–10% of total nitrogen. Although the difference between the sum of nitrogen content in NO_3^- , NO_2^- and NH_4^+ and total nitrogen may be partly attributed to experimental error, the presence of other soluble species is consistent with the literature on the electroreduction of nitrate ions, such as soluble NO , NO^- , NH_2OH [18]. Their amount is, however, too low, to be considered. As a conclusion, only NH_4^+ and NO_3^- concentrations in water are robust enough for modeling purpose in this work. But contrary to nitrate, ammonium concentration varies not only with current, but also with pH_i : Fig. 6b highlights that ammonium concentration seems independent of pH_i at earlier times of EC, but it increases faster for higher pH_i values after 60 min. This is in agreement with the results from [28]. The consequence is that the estimation of kinetic data on ammonium formation is difficult during EC, as pH changes with time (Fig. 3b).

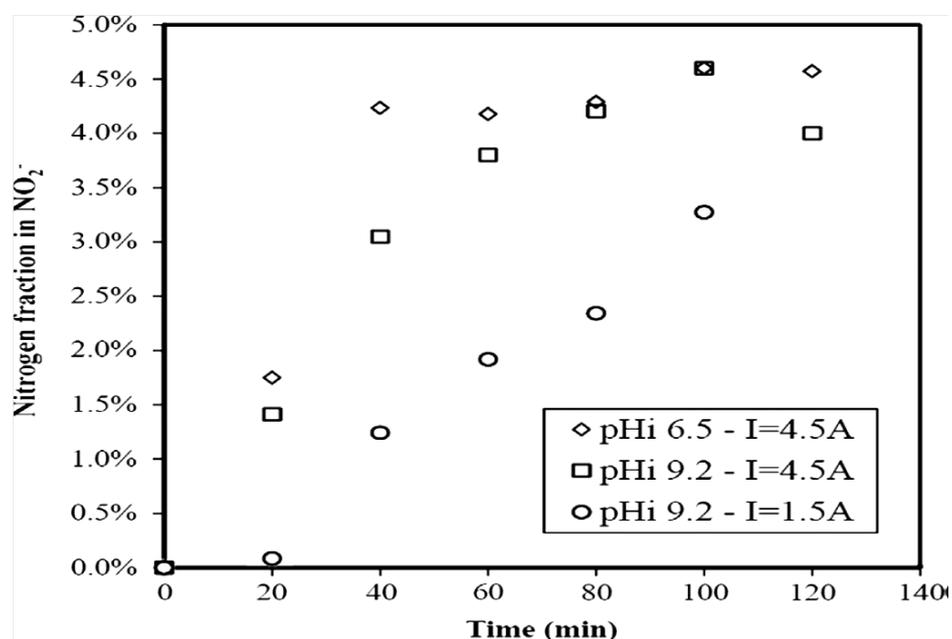


Figure 7: Evolution of nitrogen fraction in nitrite anions based on total nitrogen as a function of pH_i and current.

Table 2: Example of nitrogen mass balance at the end of EC operation on the liquid and solid phases for pH _i 7.							
Initial nitrogen content: 11.4 mg/L; I = 2.5 A; t = 120 min				Initial nitrogen content: 42 mg/L; I = 4.5 A; t = 90 min			
Soluble ions	NO ₃ ⁻ 39.4%	NO ₂ ⁻ 3.8%	NH ₄ ⁺ 24.0%	Soluble ions	NO ₃ ⁻ 28.9%	NO ₂ ⁻ 5.2%	NH ₄ ⁺ 32.6%
Soluble nitrogen	Total ion 67.3%	Total soluble 73.1%	Other soluble 5.8%	Soluble nitrogen	Total ion 66.7%	Total soluble 79.1%	Other soluble 12.4%
Total nitrogen	N in solids 20.2%	Total detected 93.3%	Undefined 6.7%	Total nitrogen	N in solids 10.9%	Total detected 90.0%	Undefined 10.0%

3.4. NITROGEN REMOVAL BY THE SOLID PHASE

Table 2 highlights that 10–20% of total nitrogen can be found in the solid phase. Several mechanisms can be involved: namely, coprecipitation, physical capture and adsorption. As nitrate compounds are highly soluble, only the last two mechanisms will be considered. First, the solid phase is mainly formed by the precipitation of aluminum oxyhydroxides. The mass balance on aluminium electrodes demonstrates that the faradic yield of aluminium dissolution ϕ was higher than 100%, but decreased when the current increased, for example from 152% to 132% and to 127% at initial pH 9.2 when I was increased from 1.5 A to 3.0 A and to 4.5 A, respectively. The mass loss of the cathode is due to pitting corrosion, both on the cathode and the anode, highlighted by SEM (Fig. 8), which explains faradic yield values higher than 100% due to the additional chemical attack of aluminum electrodes superimposed on electrodisolution: the presence of Cl⁻ ions results in the depassivation of the electrodes and the possibility of a chemical oxidation of Al by hydroxide anions generated at the cathode (Eq. (6)).

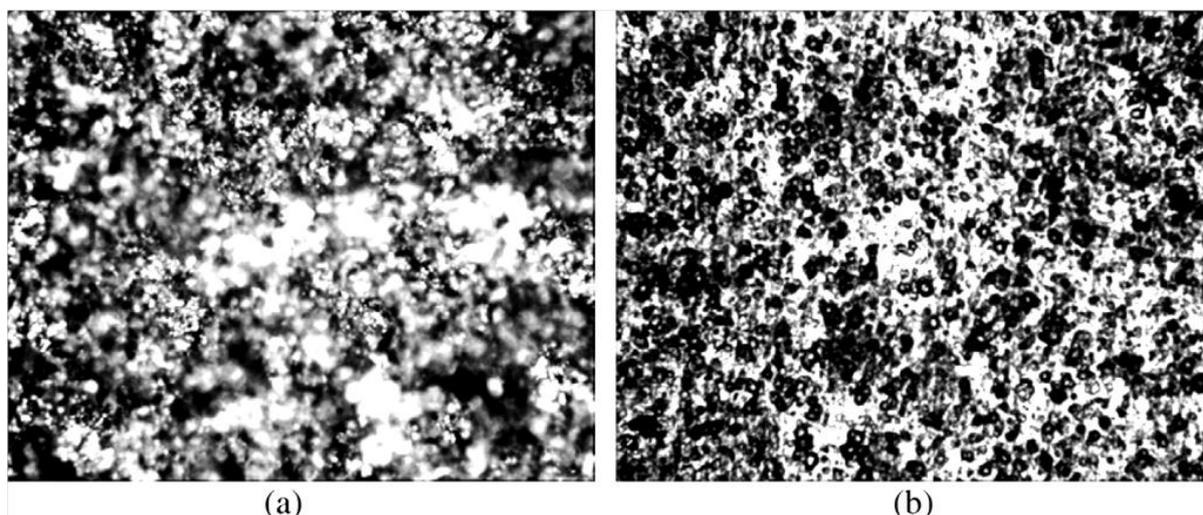


Figure 7: Surface of aluminum electrodes at the end of EC: (a) anode; (b) cathode.



This agrees with the literature data in the presence of chloride anions [32]. This effect is also clearly demonstrated by the current–voltage curves in the presence and absence of chloride ions in the synthetic water which highlights the pitting potential (Fig. 9a).

In contrast, ion chromatography indicates that there was no significant oxidation of Cl^- into Cl_2 , contrary to expectations, as Cl^- concentration did not vary [19]. The concentration of sulphate anions also remained constant during EC operation. As chloride anions, sulfates do not participate in nitrate removal and are not electrochemically active (Fig. 9b), but are known to slow down EC [31]. A consequence of these results is that nitrate removal cannot proceed through physical capture of ions during aluminium precipitation because this would affect similarly all the anionic species. A mechanism based on physical capture would, obviously, contradict the chromatographic analysis of Cl^- and SO_4^{2-} anions in Fig. 9b. As a result, the only denitrification mechanism involved in the solid phase seems to be adsorption. This opinion is reinforced by the high specific surface area of the flocs: the BET method provides reproducible values between 200 and 250 m^2/g floc.

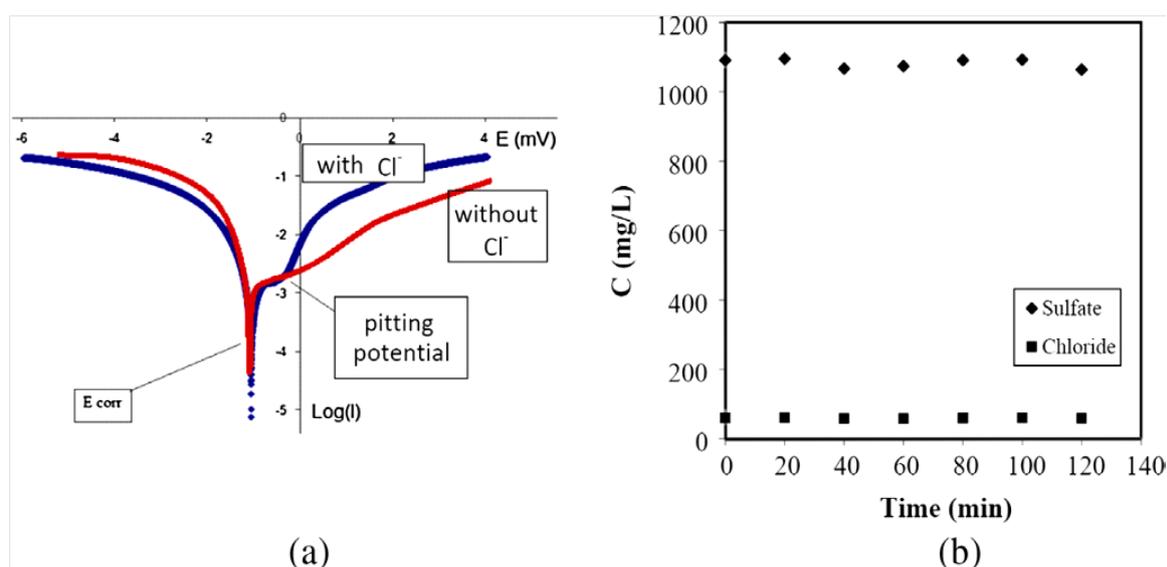


Figure 9: (a) Current–potential curve using the synthetic water without nitrates, with and without chloride ions; (b) evolution of co-anion concentrations vs. time during EC.

Even though the sum of “total soluble nitrogen” and “N in solids” does not exactly achieve 100% in Table 2, only total soluble nitrogen in water could be monitored during EC. Typical data are reported in Fig. 10a. The curves show a linear decrease. A deviation from this trend emerges only at late times and high current, *i.e.* when the nitrate concentration becomes low (for $I = 4.5$ A in Fig. 10a) and, at the same time, when pH becomes high. At earlier times, this behavior reflects zero-order kinetics. Zero-order kinetics is quite rare and usually describes adsorption or heterogeneous catalysis. In EC, it must be pointed out that the electrodisolution of aluminum also follows a zero-order kinetics defined by Faraday’s law and that the mass of flocs formed by precipitation is roughly proportional to the amount of aluminum cations released into the water. In this work, this behavior could be interpreted as an immediate saturation of adsorption sites under thermodynamic control, for example when it follows a Langmuir adsorption isotherm. A first confirmation of this analysis emerges from Fig. 10a which shows that the decrease of total soluble nitrogen in water is proportional to current, at least when the amount of floc does not become too high in comparison to soluble nitrogen or when pH is not too high (*i.e.* when EC still produces $\text{Al}(\text{OH})_3$ particles). A slight dependence on pH_i also emerges from Fig. 10a, but remains limited.

Finally, Fig. 6a, 6b and 10a confirm that both mechanisms, the formation of ammonium ions and the decrease of soluble nitrogen, take place simultaneously. A key point is, now, the identification of adsorbed compounds. As total desorption was never ensured and the

chemical analysis of dissolved flocs was tricky, an alternative procedure was developed. Flocs were produced in the synthetic water described in Table 1 without nitrates. Then, adsorption experiments with various concentrations of nitrate, nitrite and ammonium ions were carried out. Experimental results are summarized in Fig. 10b. This shows that nitrate and nitrite do not adsorb significantly, while ammonium anions present a significant adsorption level. This may be due to the positive charge of ammonium cations. Consequently, the most probable denitrification mechanism is nitrate electroreduction into ammonium, followed by adsorption of ammonium by aluminum oxyhydroxides. This explains why, at early times of EC, adsorption plays a limited role on the denitrification efficiency: the amount of solid formed is low, while the rate of electroreduction of nitrate is maximized. At late times of EC, the amount of flocs still increases linearly, while the amount of nitrates in water becomes low, which means that electroreduction rate becomes slower. Finally, adsorption should become dominant, which is however not the case in our study, limited to two hours; but this is in agreement with the data of [29] which shows an increase followed by a decrease of ammonium concentration until it becomes negligible in water. This last trend occurs when adsorption kinetics is controlled by electroreduction rate. As a result, beyond a certain ratio between the nitrate concentration and the mass of aluminum hydroxides, adsorption should no longer follow a zero-order kinetics, which could explain the deviation observed in Fig. 10a at 60 min for $I = 4.5$ A. In addition, current should play a role similar to time: increasing I enhances both adsorption and electroreduction, but electroreduction is favored by high current density, as in this work or in [28], while adsorption prevails at low current [29]. This analysis also agrees with data from Table 2.

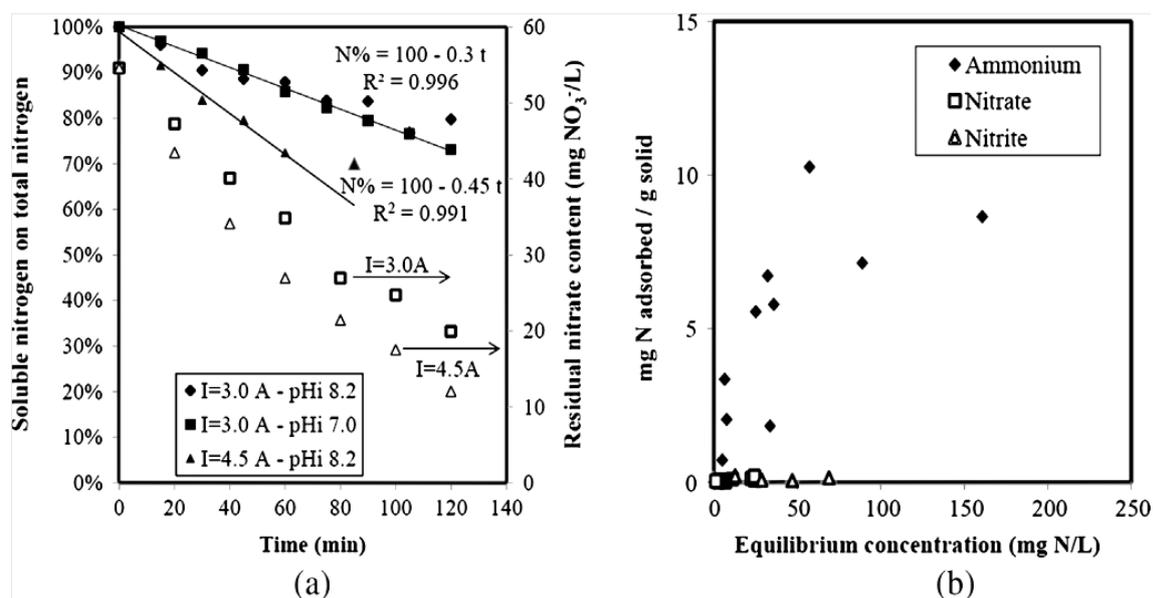


Figure 10: (a) Evolution of the soluble nitrogen fraction based on total nitrogen for various initial pH and current; (b) isotherms of nitrate, nitrite and ammonium adsorption on EC flocs.

3.5. DENITRIFICATION MODELING

To validate these assumptions, a simple model combining the zero-order kinetics of ammonium adsorption and the first-order kinetics of electroreduction valid for earlier times of EC operation has been established with the parameters obtained previously. No mass transfer limitation is taken into account. Nitrite and soluble species other than ammonium and nitrates are neglected. pH evolution is also not accounted for, which is valid for nitrate electroreduction, but is only a rough assumption for ammonium formation (see Section 3.2).

The equations describing the evolution of the nitrogen concentrations of ionic compounds expressed in mg N/L (N-NO_3^- and N-NH_4^+ for nitrates and ammonium, respectively) can be summarized as follows:

$\frac{d[\text{NNO}_3^-]}{dt} = -k_{red} \cdot [\text{NNO}_3^-] \cdot I$	(7a)
$\frac{d[\text{N-NH}_4^+]}{dt} = k_{red} I [\text{NNO}_3^-] - k_{ads} I$ with the constraint $[\text{N-NH}_4^+] \geq 0$	(7b)
$\frac{d[\text{N-NH}_4^+]}{dt} = 0$ if a negative $[\text{N-NH}_4^+]$ value is found numerically	(7c)

As nitrite content is neglected, the concentration of soluble nitrogen may be estimated by adding $[\text{N-NH}_4^+]$ and $[\text{NNO}_3^-]$. Eq. (7b) is valid only when the amount of ammonium cations is in large excess in comparison to flocs. For the solid phase, the amount of adsorbed nitrogen (q_N) in mg N/g of floc is deduced from the following mass balance, knowing the mass m of flocs (supposed to be $\text{Al}(\text{OH})_3$) deduced from Faraday's law:

$$q_N \cdot \frac{m}{v} = \frac{7}{31} C_0 - [\text{N-NH}_4^+] - [\text{NNO}_3^-] \quad (8a)$$

$$\frac{dm}{dt} = 2.9 \cdot \phi \cdot 27 \cdot 10^{-3} \frac{I}{3F} \quad (8b)$$

Calculations have been driven, assuming $k_{red} = 4.5 \cdot 10^{-5} \text{ C}^{-1}$ and $k_{ads} = 2.1 \cdot 10^{-4} \text{ mg L}^{-1} \text{ C}^{-1}$. Typical results obtained from the simulations are presented in Fig. 11a. This figure highlights the key role of ammonium adsorption on the evolution of soluble nitrogen, as the hypothetical N-NH_3 content without adsorption is plotted for comparison with experimental and simulated data. The simulations with adsorption show a good agreement with the experiments for nitrates, but also for ammonium when the effect of pH is negligible. As pH_i is 9.2 in Fig. 11a, this remains true below pH 10.5, *i.e.* as far as $\text{Al}(\text{OH})_3$ is the main product of aluminium reduction in the first 60 min. At higher pH, it could be considered that ammonium cations are released in water and do not adsorb anymore. This assumption seems to predict correctly ammonium concentration in Fig. 11a, but the model is not able to estimate pH change at the moment; consequently, the onset of the transition between adsorption/non-adsorption had to be determined empirically in Fig. 11a about 62 min. It is worth of note that this result also agrees with Emamjomeh and Sivakumar [28] who observed higher ammonia content in water when pH_i was alkaline. As expected, the effect of pH_i is less accurately accounted for by the model than that of current. This is particularly true for the prediction of ammonium concentrations, as illustrated by Fig. 11b.

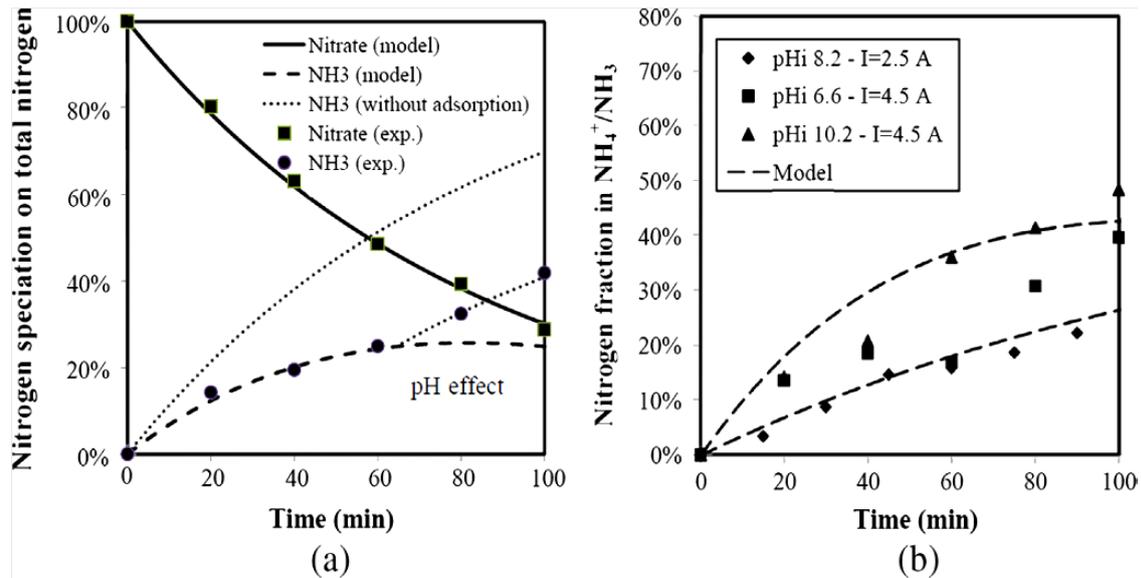


Figure 11: Comparison between simulations and experiments: (a) on nitrate and ammonium fractions based on total nitrogen species for $C_0 = 54 \text{ mg NO}_3^-/\text{L}$, $\text{pH}_i 9.2$ and $I = 4.5 \text{ A}$; (b) on ammonium fraction based on total nitrogen vs. time in the conditions of Fig. 6b.

Finally, the simulations confirm that nitrate electroreduction is favored by short electrolysis which generally corresponds to high current and leads to the presence of a small amount of adsorbent with the maximum amount of nitrates: this situation is illustrated by Fig. 12a. Our experiments also correspond to this case: Eq. (8) predict that q_N is always higher than 5 mg N/g floc in this work.

The opposite conditions favor adsorption, as shown in Fig. 12b. A comparison with the literature shows that the works of Emamjomeh and Sivakumar [28] and Kopal and Ögütveren [27] correspond to the first situation, while that of Lacasa et al. [29] corresponds to the second, but so far, none of them had identified the specific adsorption of the electrogenerated $\text{NH}_4^+/\text{NH}_3$ species. In particular, the peak of ammonium concentration that emerges from Fig. 12b perfectly agrees with the experimental trends observed in [29]. This analysis reconciliates the data from these different contributions that lead to different conclusions and explains why chemical coagulation with aluminum salts is ineffective for nitrate removal, as this process is not able to promote nitrate reduction into ammonium.

However, some issues remain to be clarified. The first one is that nitrogen content in the solid phase at the end of EC is about 5 mg-N/g dissolved Al, while Lacasa et al. [29] suggested values between 15 and 20 mg N/mg dissolved Al for their data operating at lower current density, which tend to assume that the structure of the flocs and their adsorption capacity depends on the current. Similarly, the simple set of equations used in this work could be coupled to a more detailed model able to predict pH. These points will be the subject of future works.

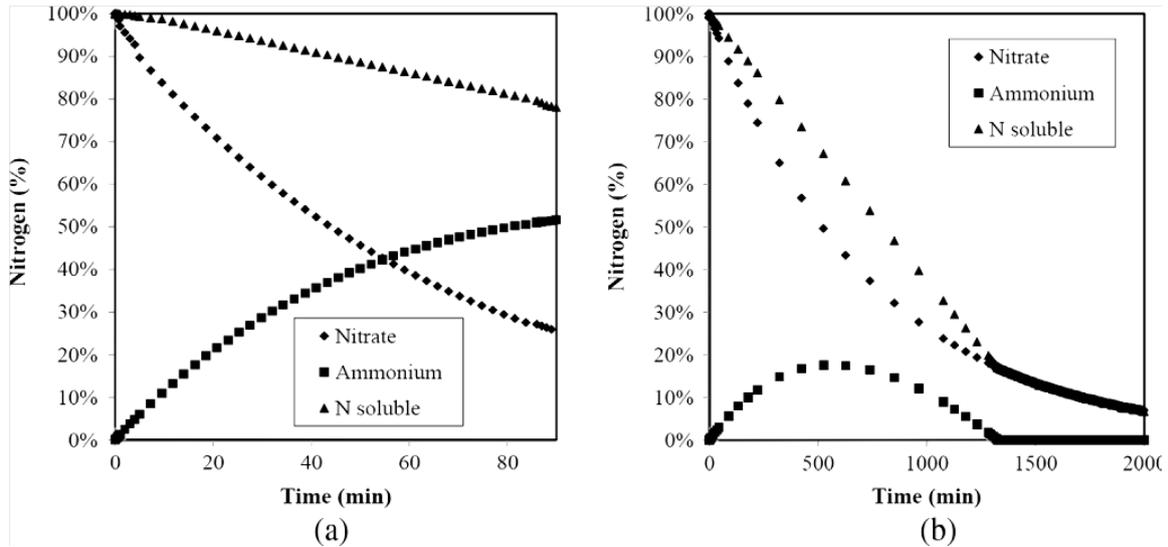


Figure 12: Typical predictions of nitrogen speciation based on total nitrogen: (a) for $I = 2.5$ A and electrolysis time $t = 90$ min; (b) at lower current $I = 0.5$ A and longer electrolysis time $t = 2000$ min.

3.6. ANALYSIS OF OPERATING COSTS

Although this work was conducted on synthetic water, an estimate of operating costs of EC is necessary to estimate at least approximately its economic viability. This especially includes the cost of the metal related to electrodisolution of aluminum, which is relatively expensive, and also the energy cost due to electricity consumption. Mechanical power for mixing purpose is neglected. Power input has been investigated first. Fig. 13 shows that the evolution of the cell voltage is perfectly linear as a function of current and follows Eq. (9).

$$U = 5.0I + 2.4 \quad (9)$$

A comparison with Chen et al. [33] shows that the Tafel term is negligible and the resistive term dominates at pH about 7, contrary to Chafi et al. [30]. This seems due to the lower water conductivity in comparison to Chafi's wastewater. The resistance of about 5Ω measured under EC is, however, slightly higher than expected if it was estimated as a function of inter-electrode gap (e), electrode surface area (S) and conductivity (K): $e/(KS) = 3.5 \Omega$. This value depends slightly on pH_i (Fig. 13). As already mentioned, conductivity slightly increases during EC, which cannot explain the discrepancy between resistance values. The higher resistance may be due to the presence of hydrogen gas in the gap. Now, this equation can be used to estimate energy consumption using the product $U \cdot I$, which highlights that electric power input varies nearly as I^2 .

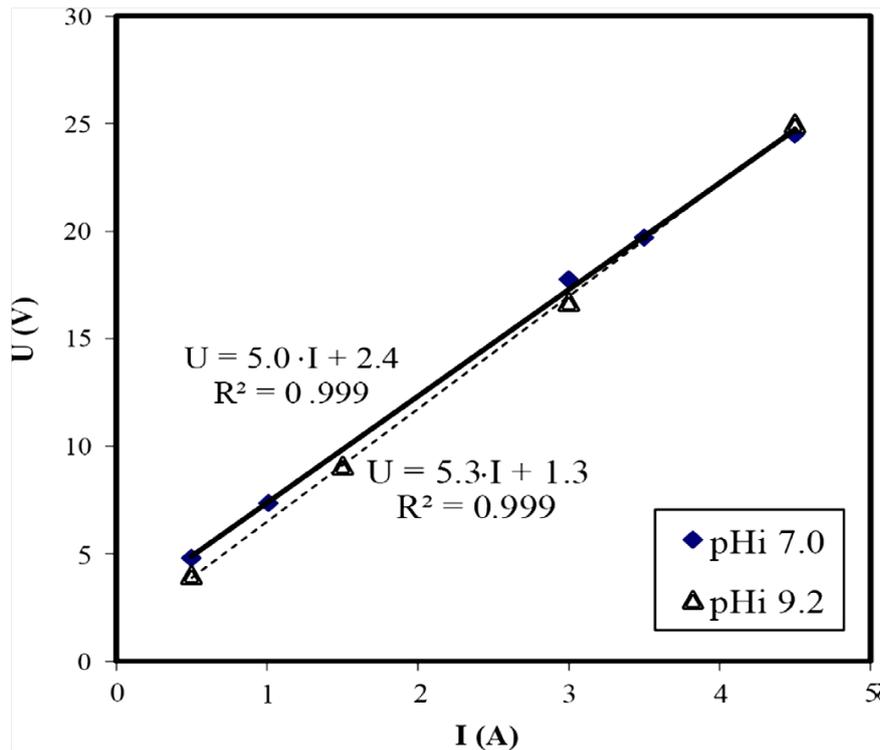


Figure 13: Cell voltage (U) as a function of current (I) for several pH_i values.

For aluminum consumption, the data on electrode mass loss was used, but it can also be deduced from Faraday's law, which implies that this value is proportional to $\phi \cdot I \cdot t$. The results in terms of material and energy consumption are summarized in Table 3. As expected, the specific energy input (*i.e.* per mg NO_3^-) increases with the current, as well as the specific consumption of aluminum for fixed operation time. However, if the amount of aluminium required increases with the initial concentration of NO_3^- , the specific energy required decreases. Using 0.12 €/kW h for electricity cost and 4.0 €/kg Al for electrode material, Table 3 shows a high energy cost per gram of nitrates, about 50% of the EC cost, higher than typically found for EC using Al electrodes (see, e.g., [30]). Conversely, this agrees with the cost estimation of Lacasa et al. [34] in which the difference between nitrate removal by iron and aluminium is slight, despite the lower price of iron metal. This constitutes an additional indication which highlights a different mechanism for nitrate removal from the common adsorption or coprecipitation. Electricity plays a key role because the first step is the electroreduction of nitrate.

As a consequence, it emerges from Table 3 that EC is an expensive technique, with an operating cost between 0.12 and 0.20 €/g NO_3^- , that should only be used for water heavily loaded with nitrates. Therefore, EC remains costly for drinking water exhibiting C_0 values between 50 and 100 mg/L NO_3^- . This cost corresponds to about 1.0–1.5 €/m³ for nitrogen removal, which agrees roughly with the order of magnitude found in [34]. As a result, EC cannot replace cheap biological treatments. The only opportunity to reduce the operating cost is to conduct EC with a very low current, as in [29], since energy consumption varies proportionally to t , but as I^2 . However, the consequence is a very high operation time, about 50–100 h in order to release the necessary amount of metal. If energy saving is expected, this may be partially counterbalanced by the increase of the volume of the EC cell or of buffer tanks and the higher capital costs induced. Finally, our results confirm that EC is a method able to remove efficiently nitrate anions, but rather as a pretreatment in the case of very high nitrate concentrations, or when EC enables to remove at the same time other types of pollution

for which the efficiency of EC is well established.

Table 3: Cost analysis of EC operation for nitrate removal as a function of current and initial nitrate concentration C_0 .			
Current (A) for $C_0= 55$ mg/L and 120 min EC	1.5	3.0	4.5
Specific energy (kWh/g NO_3^- eliminated)	0.42	0.73	1.36
Specific Al mass (g Al/g NO_3^- eliminated)	18	21	21
Cost (€/g NO_3^- eliminated)	0.12	0.17	0.25
% cost due to energy	41%	51%	66%
C_0 (mg/L) for $I= 4.5\text{A}$	55	104	203
Duration of $C(t) < 50^a$ or $C(t) < 25^b$ mg/L (h)	1 ^b	2 ^a -1 ^b	2.1 ^a
Specific energy (kWh/g NO_3^- eliminated)	1.0 ^b	1.1 ^a -0.42 ^b	0.48 ^a
Specific Al mass (g Al/g NO_3^- eliminated)	18 ^b	23 ^a -18 ^b	25 ^a
Cost (€/g NO_3^- eliminated)	0.19 ^b	0.23 ^a -0.12 ^b	0.16 ^a
% cost due to energy	62% ^b	59% ^a -42% ^b	37% ^a

4. CONCLUSIONS AND PERSPECTIVES

As a conclusion, electrocoagulation process has been shown to be able to remove efficiently nitrate, whatever the initial concentration, and to reach nitrate concentrations far below the guideline value, while maintaining in agreement with previous works of the literature. However, these did not correctly describe the mechanisms involved in nitrate elimination. Many previous works had assumed an electroreduction of nitrates into ammonia and finally into N_2 gas and disregarded adsorption. However, only the first step is clearly observed in this work. Conversely, a recent contribution suggested the adsorption of nitrogen compounds onto the flocs, while electroreduction was negligible. During EC, it has been demonstrated in this work that the formation of ammonium ions results from the electroreduction of nitrates and not from the chemical reduction of the metal. As a consequence, the results from Murphy [12] on aluminum metal do not apply during EC. Another finding is that only ammonium cations adsorb on the aluminium hydroxide particles formed during EC. Our conclusions are that both phenomena, namely the electroreduction of NO_3^- into NH_4^+ and the NH_4^+ adsorption, are consecutive mechanisms that proceed at the same time, following first-order and zero-order kinetics, respectively. As a result, a simple model has been defined for the prediction of nitrate removal and nitrogen speciation during EC. This agrees relatively well with experimental data. Regarding techno-economic analysis, the nitrate removal by EC appears to be a process expensive to operate that requires either high current or high operation time; EC is not competitive against the conventional biological process. EC can, however, be cost-effective when applied as a pretreatment able to promote the abatement of nitrates and other types of pollution at the same time. The perspectives of the present work will aim at implementing the influence of the pH in the model and to better estimate the dependence on pH of NH_4^+ formation and adsorption. In addition, further work is needed to investigate how other co-anions among those that have not been studied in this work (e.g., phosphates...) could interfere and also the possible interactions with organic and colloidal compounds before conducting tests on real river water or ground water.

NOMENCLATURE

C	concentration (kg m^{-3})
C_0	initial nitrate concentration expressed in kg NO_3^- (kg m^{-3})
e	electrode gap (m)
EC	electrocoagulation
F	Faraday's constant (C mol^{-1})
I	current (A)
k	rate constant of a first-order process (s^{-1})
k_{ads}	adsorption rate constant ($\text{kg m}^{-3}\text{C}^{-1}$)
k_{red}	electroreduction rate constant (C^{-1})
m	mass of flocs (kg)
pH_i	initial pH (-)
R^2	determination coefficient (-)
S	electrode surface area (m^2)
t	electrolysis time (s)
U	cell voltage (V)
V	cell volume (m^3)

Greek letters

κ	water conductivity (S/m)
ϕ	faradaic yield (-)

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CHAPTER III: ASSESSMENT OF DENITRIFICATION USING ELECTROCOAGULATION PROCESS

This article is published online on February 2015 in The Canadian Journal of Chemical Engineering. Consequently, it follows the guidelines of this journal.

Tania Yehya, Wafaa Balla, Mohammed Chafi, Fabrice Audonnet, Christophe Vial, Abdelhafid Essadki and Bouchaib Gourich, 2015. Assessment of denitrification using electrocoagulation process, The Canadian Journal of Chemical Engineering 93, February 2015.

ABSTRACT

The objectives were to study the applicability of electrocoagulation (EC) for the denitrification of drinking water and to determine the main mechanisms of pollution removal. Electrolysis in the intensiostatic mode was applied to a synthetic water representative of potable water in which the concentration of nitrate ions was varied up to 200 mg/L. The respective influences of process parameters, initial concentration of nitrates, and initial pH were investigated. Experimental results show that EC removes efficiently nitrates, following first-order kinetics. A two-step mechanism was established: it consisted of the electroreduction of nitrates into ammonium on the cathode, followed by adsorption of ammonium on the precipitated oxyhydroxides. Adsorption exhibited a zero-order mechanism. The rates of the two mechanisms were proportional to electrical charge loading and to the total amount of aluminium released in water, as current did not modify significantly the surface area of precipitates. However, adsorption was impaired by the increase of pH resulting from the electroreduction of nitrates, whereas the electrochemical step was insensitive to pH. While the electroreduction of nitrates is known to be far more expensive than biological denitrification, aluminium hydroxides formed during EC present interesting adsorption properties for ammonium removal.

1. INTRODUCTION

Maintaining the quality of drinking water resource is a major concern for the future due to the combined effects of population increase, industrialization and intensive agriculture. This requires more efficient wastewater treatments that prevent the pollution of the ecosystems by human activities. Nitrates, for example, used as a fertilizer, correspond to a major pollution source in the regions of intensive agricultural activities. Being themselves non-toxic, nitrates are present as undesirable molecules in water. Their transformation, however, into toxic nitrites and ammoniac on one side, and other nitrosated carcinogenic compounds (nitrosamines and nitrosamides) on the other side, cause problematic health effects. The guide level set by the European Union for nitrates is 25 mg/L; it is consistent with the recommendation of the WHO with a maximal daily intake of 50 mg/L [1]. Naturally present at low concentrations in surface and underground waters, their presence is in constant increase in the last few years about 0.5 to 1 mg/L/year. Although issued during 1991, the EU Directive n°91/676/CEE (12/12/91) named "Nitrates Directive" destined to protect the water resources from nitrates of agricultural sources is not perfectly applied in many countries in the European Union. Thus, a denitrification step has to be considered and integrated both by the drinking water and wastewater treatment plants. The removal of nitrate anions can be

achieved by different treatment methods. First, physicochemical treatments can be applied, such as ion exchange (the most common physicochemical treatment used at industrial scale), membrane processes (like electrodialysis, reverse osmosis, nanofiltration). These present the drawback to transfer or concentrate the pollution instead of eliminating it [2]. Other physicochemical treatments consist of the chemical reduction using aluminium [3], and electroreduction processes, *e.g.* on metal [4], or boron-doped-diamond [5], but these can form secondary species that are toxic, such as ammonia or nitrites. As a result, the most common method to remove nitrates in water consists of biological treatments in which denitrifying bacteria, heterotrophs and autotrophs, are employed to reduce nitrates into N₂ gas. This is, however, limited to waters exhibiting low C:N ratios, or containing pollutants toxic to microorganisms.

In the recent years, electrocoagulation (EC) has been reported to be able to remove efficiently nitrates [6, 7]. EC is a nonspecific electrochemical technique in which direct current is applied between a sacrificial anode, usually made of iron or aluminum, and a cathode. During the electrolytic process, cationic species from the sacrificial anode dissolve in-situ. First, current is able to destabilize any dissolved ionic or electrostatically suspended pollutant species, but the electrogenerated cations, playing the role of coagulants, form metal oxides and hydroxides which precipitate and can react with the soluble or suspended pollutants or adsorb them. As a result, EC differs from a conventional electrochemical treatment with an inert electrode in which only the electrooxidation or electroreduction of pollutants is desired, but this makes it differ also from conventional chemical coagulation in which chemicals are added at once for pollution removal. EC also presents many advantages over conventional coagulation: versatility, energy efficiency, safety, lower pH change, reduced amount of sludge, environmental compatibility, and better amenability to automation, among others [8, 9]. Also, the production of hydrogen gas at the cathode, resulting from water reduction, can help floc separation by flotation [10]. However, EC is a complex process involving a multitude of pollutant removal mechanisms operating synergistically. The advantage is that it can cope with various polluting species: from organic materials, heavy metals, microorganisms, to color and turbidity [6]. A key drawback is, consequently, that cell geometry and scale-up procedures are not clearly established [11]. This is particularly the case for nitrate removal using EC, as different outcomes on the depollution mechanisms of EC on aluminium electrodes have been reported in the literature. These include the electroreduction of the nitrate anions [12, 13], and the adsorption of nitrogen species on metal hydroxides [7].

In this work, the objective is to assess the effectiveness of EC using aluminium electrodes for nitrate removal by the identification of the key mechanisms of denitrification and the determination of the speciation of nitrogen in both phases: treated water and flocs. The respective influences of process parameters, initial concentration of nitrates, and initial pH will be studied. Conclusions will emphasize the pros and cons of EC process.

2. MATERIALS AND METHODS

In this study, EC was used to study nitrate removal from synthetic water representing potabilizing water in which the initial concentration C_0 of nitrates NO₃⁻ is varied between 50 and 200 mg/L by adding NaNO₃. The composition of the synthetic water also includes cations (sodium: 78 mg/L; potassium: 835 mg/L), and anions (chlorides: 60 mg/L; sulfates: 1.09 g/L; bicarbonates: 107 mg/L). The initial conductivity of water is 2.8 mS/cm and pH is 8.2. Initial pH is then adjusted between 3.8 and 10.2 by the minute addition of either 0.1 M hydrochloric acid or sodium hydroxide solutions.

2.1 EXPERIMENTAL SET-UP

For EC process, two rectangular aluminum electrodes were used as the anode and the cathode, of surface area $S=102\text{ cm}^2$ each, with an inter-electrode distance of 1 cm. The EC cell consisted of a batch cylindrical reactor of volume $V=4.0\text{ L}$, mechanically stirred using a standard Rushton turbine. EC was carried out in an intensiostatic mode by means of a *BK-Precision* (USA) generator with a current intensity j ranging between 5 and 45 mA/cm². A recording voltmeter (*Voltcraft VC 950*, France) was used to deduce the electric power consumed. The electrolysis time of each run was between 30 and 120 minutes. The respective effects of mixing speed (from 100 to 400 rpm), current, initial pH pH_i and initial nitrate concentration C_0 were investigated. The conductivity and the pH of the solution were recorded online.

2.2 ANALYTICAL METHODS

The concentrations of soluble anions and cations were obtained using ion chromatography (*Metrohm AG*, Switzerland). The electrode mass loss was used to evaluate the rate of metal dissolution and to deduce the faradic yield ϕ of the electrolysis. Total nitrogen in the liquid phase was measured using a total nitrogen analyzer (*TNM-1*, *Shimadzu*, Japan). At the end of EC, the flocs recovered by decantation or flotation were filtered, washed, and dried at 105°C overnight before being weighted. The solid phase was characterized by X-ray diffraction (*XRD D501*, *Siemens*, Germany), and the BET surface area of the flocs was estimated using nitrogen adsorption (*Tristar II*, *Micromeritics Instr.*, USA). To establish the mass balance on nitrogen, the solid phase was analyzed using the total nitrogen analyzer described above. To check for adsorbed nitrogen species flocs were obtained with the same synthetic water in the absence of nitrogen compounds, and adsorption experiments with different concentrations of nitrate, nitrite, and ammonium were performed.

3. RESULTS

3.1 ANALYSIS OF THE RATE OF NITRATE REMOVAL

Preliminary experiments showed that the rotation speed of the impeller studied at 100, 200, and 400 rpm, had no significant effect on nitrate removal above 100 rpm. This means that there was no mass transfer limitation, whatever the prevailing mechanism of denitrification, including reduction on the aluminium electrodes [3], adsorption onto the flocs [7], or electroreduction on the cathode [13]. Subsequent experiments have been driven at 200 rpm, which prevents vortex in the tank, and at the same time does not hinder flotation. In addition, power consumption for mixing purpose remains negligible in comparison to the power requirements of electrolysis.

Preliminary results also highlighted a first-order mechanism, in agreement with literature data [7,13], which can be checked by plotting $\ln(C_0/C)$ vs. time in which C is the nitrate concentration at time t . To account for the influence of current, an elegant way consists of plotting $\ln(C_0/C)$ as a function of the theoretical concentration of total aluminium released by the anode C_{Al} . This can be derived from Faraday's law (Eq. 1) using current I , the molar mass of Al, M_{Al} , and Faraday's constant, F .

$$C_{Al} = \frac{I \cdot t}{3F} \cdot \frac{M_{Al}}{V} \quad (1)$$

This plot is reported in Figure 1 which confirms a first-order mechanism for nitrate removal as a function of time, electrical charge loading and the mass loss from the cathode. The kinetic constant k can be deduced from Eq. 2:

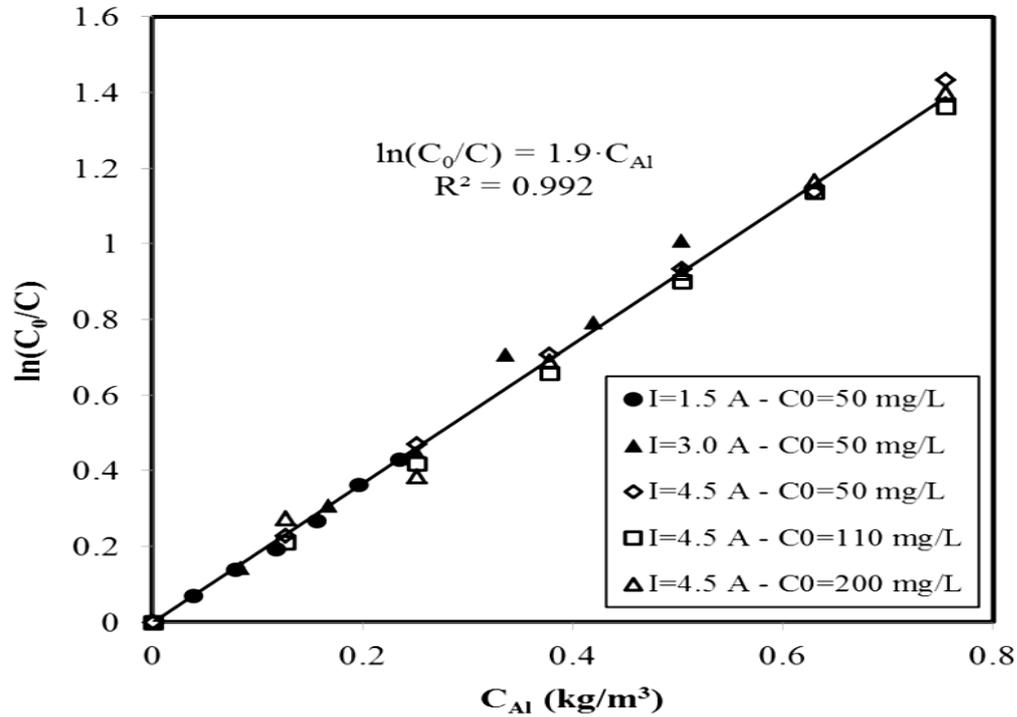


Figure 1: Validity of the first-order kinetics of nitrate removal vs. dissolved aluminium concentration C_{Al} as a function of current I and initial nitrate concentration C_0 .

$$\ln(C_0/C) = k \cdot C_{Al} \quad (2)$$

k is equal to $1.9 \pm 0.1 \text{ m}^3/\text{kg Al}$. This value is also independent from initial pH when pH_i is higher than 5, as shown in Figure 2: a decrease of 15% of k is observed when pH_i is 3.8, but nitrate removal is not inhibited.

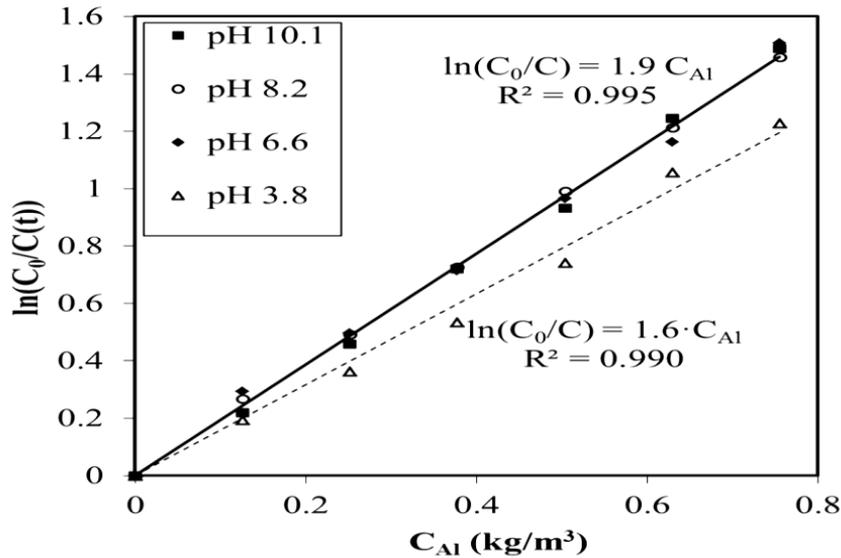


Figure 2: Validity of the first-order kinetics of nitrate removal vs. dissolved aluminium concentration C_{Al} as a function of initial pH ($C_0=50$ mg/L; $I=4.5$ A).

As a conclusion, the kinetics of denitrification using EC process appears to be nearly independent of pH_i , C_0 , and of the rotation speed of the impeller at the same time. It depends only on the amount of aluminium released in water, *i.e.* on the ratio between current and water volume, which denotes a robust process for operation and scale-up. It could be advocated that C_{Al} is not the real amount of total aluminium in water because the faradic yield ϕ was always higher than 100%. This value is typical of EC with aluminium electrodes [14]. It results from the possible secondary chemical reactions and the chemical corrosion of the electrodes enhanced by chloride anions that acts as an additional dissolution mechanism on the cathode and the anode whose influence decreases when current increases. ϕ , estimated on EC operation decreased, as expected, when I was increased. It also increased slightly with pH_i : the maximum, 150%, was reported for $I=1.5A$ and pH_i 9.2, while the minimum, 110%, was observed for $I=4.5A$ and pH_i 6.6. In practice, ϕ was typically between 120-130% and correcting C_{Al} estimation is unnecessary. In addition, ϕ may vary with time, which means accounting for the amount of aluminium released ϕ does not ensure that aluminium release vs. time is more accurately predicted.

A possible explanation of the small effect of pH_i on nitrate removal is that pH varies during EC. Aluminium electrodes are known to exhibit a buffer effect with a final pH close to neutrality [15], when only water reduction and aluminium dissolution proceed at the cathode and the anode, respectively: the anodic oxidation of Al into $Al(OH)_3$ and the cathodic reduction of water provide and consume the same amount of H^+ cations, thus attaining the neutral pH.



However, this does not fit experimental results reported in Figure 3 which shows that pH tends to alkaline values.

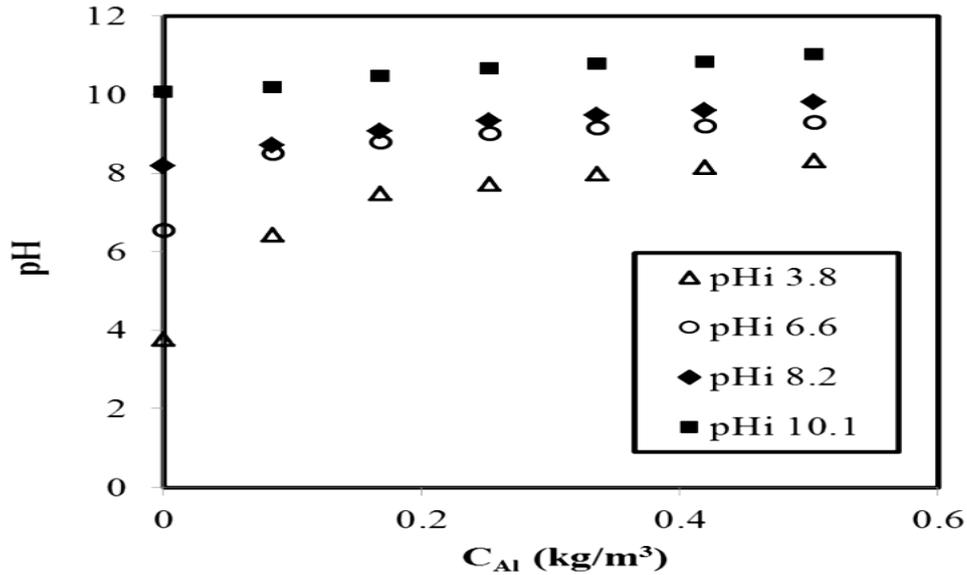


Figure 3: Influence of pH_i on the evolution pH as a function of C_{Al} ($C_0=50$ mg/L, $I=3.0$ A).

This is justified by an additional mechanism of nitrate reduction on the cathode into ammonium (Eq. 4), as the latter's concentration corresponded to 50% of total nitrogen detected using ion chromatography (see section 3.2).



As pH does not apparently change when $I=0$, the chemical reduction of NO_3^- by Al metal is negligible and only the electroreduction mechanism on the cathode can be accounted for. While this mechanism shifts pH to higher values, it also modifies the amount of floc (Cf. Figure 4a); this passes through a maximum when pH_i is about 8, which is consistent with the speciation of aluminium described by Pourbaix diagrams: at low pH, Al^{3+} cations dominate, $Al(OH)_4^-$ prevail at pH higher than 10, while insoluble hydroxides $Al(OH)_3$ that precipitate predominate at intermediate pH values.

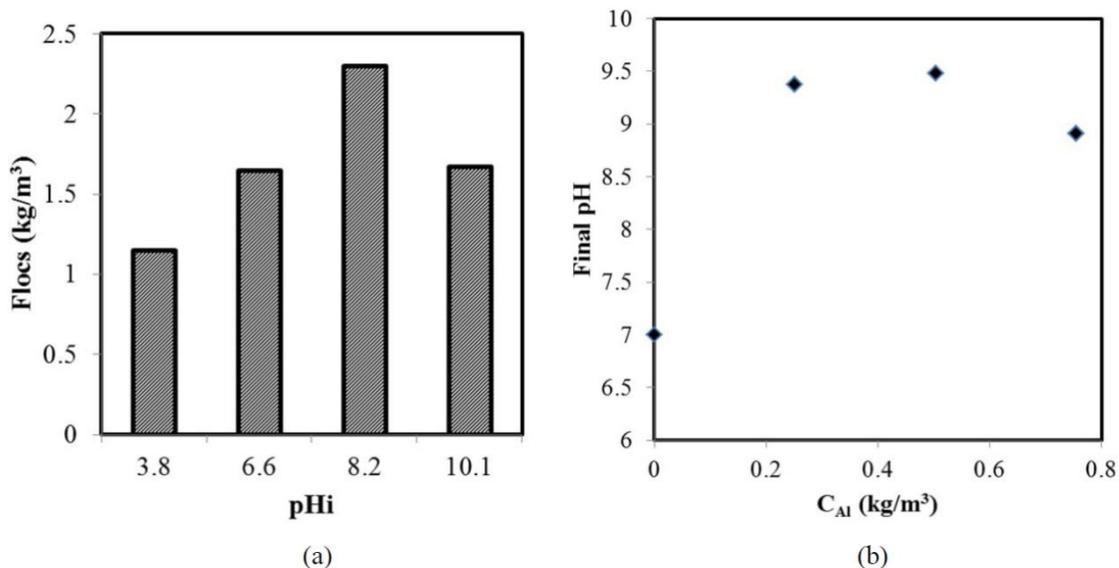


Figure 4: (a) Influence of pH_i on the final mass of flocs ($C_0=50$ mg/L, $I=4.5$ A, $t=120$ min); (b) Influence of the amount of Al released varying I on the final pH ($C_0=50$ mg/L, $t=120$ min, pH_i 7).

On the contrary, when most nitrate anions have been consumed, pH can decrease again, which results from:



This occurs at high electrical charge loading, as seen in Figure 4b. Finally, all these results highlight that nitrate removal in Figure 1 is nearly independent of the amount of floc, which is in agreement with a nitrate removal mechanism based only on electroreduction, while other mechanisms, such as ammonium adsorption on the flocs, always played a secondary role in this work.

3.2 NITROGEN SPECIATION IN THE LIQUID PHASE

Ion chromatography gives access to nitrogen speciation. As already mentioned, ammonium cations appeared to be the main product of nitrate electroreduction, but nitrite anions were also found (Cf. Figure 5).

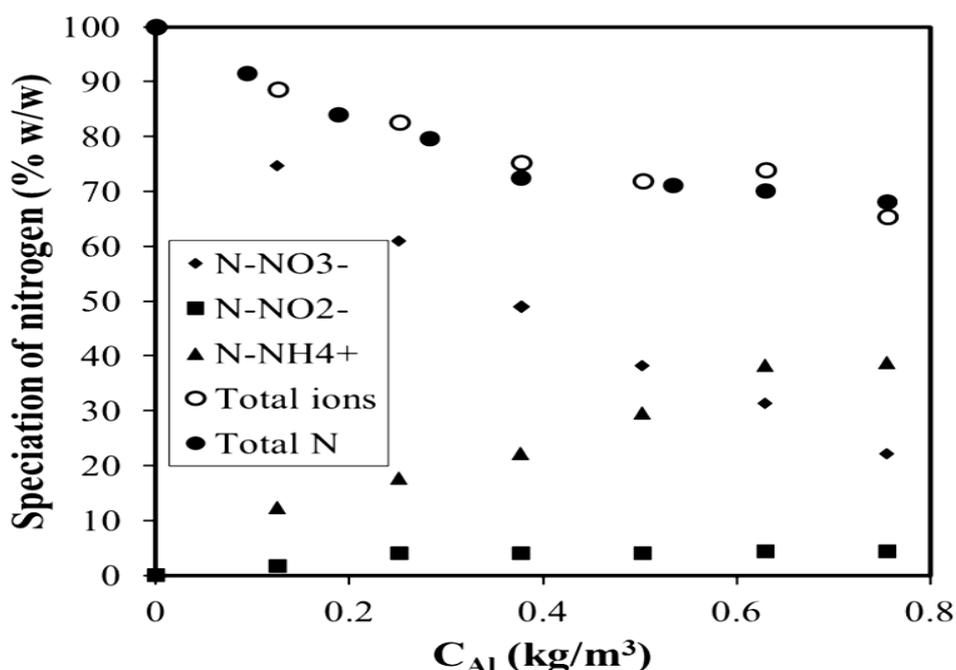


Figure 5: Speciation of nitrogen compounds in the liquid phase ($C_0=50$ mg/L, $I=4.5$ A, $t=120$ min): *total ions* corresponds to the sum of NO_3^- , NO_2^- and NH_4^+ concentrations, *total N* is measured using the total nitrogen analyzer.

This result agrees perfectly with the conclusions of Emamjomeh et al. [13] It also agrees with Lacasa et al. [7] but only to a lesser extent because in their work, these authors found that adsorption on the flocs was the mechanism responsible for nitrate removal. This seems, apparently, to differ strongly from our own data. As in Emamjomeh et al. [13] Figure 5 shows that the sum of nitrogen content in ionic species does not achieve the initial nitrogen content in nitrates when EC proceeds and that it decreases with time. These authors suggested that nitrogen that could not be found in the liquid phase was converted into gaseous species, such as NO and N₂ as on Al metal [3], because they could not identify nitrogenous compounds in

the solid phase using XRD, but this could also be explained by nitrogen adsorption on the solid phase [7] or even by physical capture in the precipitates. Figure 5 also shows that the amount of total ions (nitrogen in $\text{NO}_3^- + \text{NO}_2^- + \text{NH}_4^+$) is very close to total nitrogen in the liquid phase obtained using the nitrogen analyzer. This means that other soluble species than ammonium, nitrates and nitrites can be neglected in the nitrogen mass balance.

As a result, a focus on the evolution of nitrites and ammonium cations are presented on Figure 6 as a function of C_{Al} . As already mentioned, ammonium is the main product of nitrate reduction. Ammonium content seems to be independent of pH_i and current when C_{Al} is low, but when $C_{Al} > 0.4 \text{ kg/m}^3$, the amount of soluble NH_4^+ increases with pH_i (Cf. Figure 6a). This corresponds to pH values higher than 10 in EC operation. Nitrites are known to be an intermediate in the reduction of nitrates, both in electrochemical and biological processes [4,16]. Their amount is however, small in comparison to ammonium cations, always lower than 5% of total nitrogen (Cf. Figure. 6b). Nitrite content seems to tend to a plateau value, or even to exhibit a maximum. From this figure, it is difficult to conclude on the respective influences of pH_i and current, as these can probably not be distinguished from experimental error. As a conclusion, nitrate, nitrite and ammonium constitute the only soluble species including nitrogen and the nitrite concentration becomes rapidly negligible in comparison to ammonium.

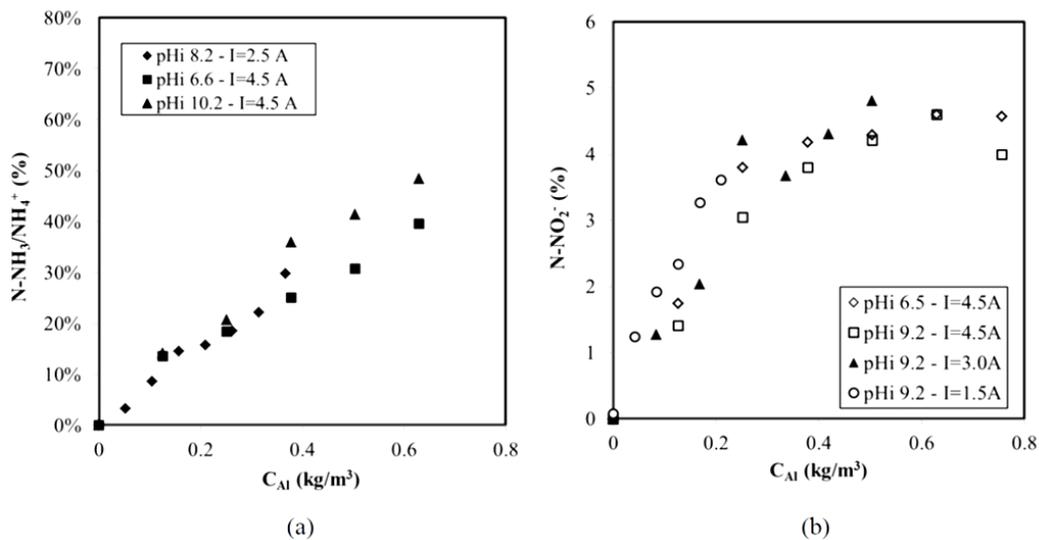


Figure 6: Evolution of nitrogen content in ammonium/ammonia (a) and nitrites (b) as a function of pH_i and current ($C_0=50 \text{ mg/L}$, $t=120 \text{ min}$).

By monitoring the amount of total dissolved nitrogen in water (C_N , expressed in mg N/L), a linear decrease was detected at early electrolysis time in Figure 5. A deviation from linearity appeared only at late times of the EC, *i.e.* when the nitrate concentration became low and when pH became high. At the beginning of the EC run, this behavior can therefore be fitted by zero-order kinetics. This analysis as a function of pH_i and current is confirmed in Figure 7, as the rate of total nitrogen removal from the liquid phase expressed in NO_3^- (using the 4.42 factor between the respective molar mass of NO_3^- and N) is proportional to the current, as follows:

$$\frac{C_0 - 4.42C_N}{C_0} = 0.66 \cdot C_{Al} \quad (6)$$

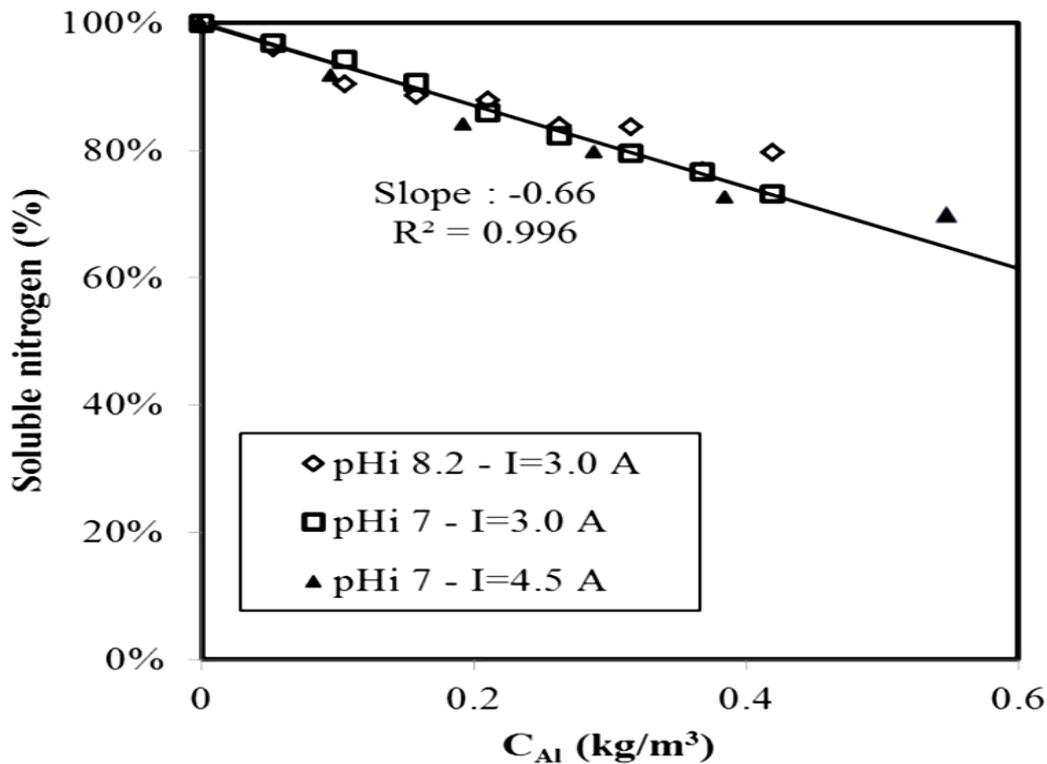


Figure 7: Evolution of total soluble nitrogen vs. total aluminum concentration released in water as a function of pH_i and current ($C_0=50$ mg/L).

As already mentioned, the decrease of C_N in Figure 7 proportional to the current could result from three possible mechanisms. The first one is the physical capture of nitrogen by the solid phase: this is unlikely to occur because ion chromatography highlighted that concentration of other anions (Cl^- and SO_4^{2-}) and cations (Na^+ , K^+) were unaffected by EC, although their concentration is far higher than that of nitrates. Now, it is necessary to distinguish between the two other: the formation of gaseous NO_x and N_2 species on the one hand, and the adsorption of nitrogen on the flocs on the other hand, using a specific analysis of the solid phase. It must, however, be reminded that these mechanisms play only a secondary role in comparison to nitrate electroreduction, although they cannot be neglected in this work.

3.3 EFFECT OF THE SOLID PHASE

Up to now, the nitrogen content in the solid phase during nitrate removal using EC process has received little attention. First, XRD data showed that the dried flocs were amorphous and it was impossible to detect any species involving nitrates or ammonium. However, by analyzing the same solid phase using the total nitrogen analyzer, it was found that these solid flocs contained nitrogen, although this technique did not allow the identification of the nitrogen species. At whatever current and pH_i, about 90% of nitrogen from the nitrates present in water at the beginning of EC could be found either in the liquid or in the solid phase at the end of EC operation. In the solid phase, the only remarkable effect that was observed is that the amount of nitrogen in g/g solid decreased when I increased. This means that the kinetics of nitrogen capture is a bit slower than that of oxyhydroxide precipitation. However, it also appeared that a non negligible fraction of the flocs was lost during recovery and drying operations. This means that the amount of gaseous nitrogen compounds, among which NO is the most probable species, remains low, probably less than 5%. If the

uncertainty on the various analytical tools is cumulated, the formation of gaseous species cannot be ascertained. As a conclusion, the mass balance on nitrogen shows that total nitrogen in the liquid phase decreases mainly because it is captured by the flocs.

Now, either nitrogenous compounds may be amorphous, or nitrate, nitrite and ammonium may be adsorbed on the flocs. In practice, ammonium and nitrate compounds are highly soluble and only adsorption can be supposed to occur. First, dried flocs were shown to exhibit a very high BET surface area, about $250 \pm 40 \text{ m}^2/\text{g}$. This could favor adsorption. It seemed that the value of floc surface area did not vary significantly with pH_i between 6 and 10. This value varied only slightly with current, as shown in Figure 8 for I between 0.7 and 4.5 A: it increased slightly at low current and then it tended to a plateau value. This is also in line with Figure 7 that highlighted a rate proportional to the current. The zero-order mechanism reported in section 3.2 is also in accordance with an adsorption mechanism: it is usually observed with a Langmuir isotherm when adsorption is thermodynamically favored. But in EC, the situation is more complex than in conventional adsorption, as the amount of solid increases with time: it must be pointed out that the electrodisolution of aluminum also follows zero-order kinetics defined by Faraday's law, and it can be considered that the mass of the flocs formed by precipitation is roughly proportional to the amount of aluminum cations released into water. These trends of Figure 7 could be interpreted as a rapid saturation of the solid adsorbent by nitrogen compounds under thermodynamic control which follows the rate of floc formation.

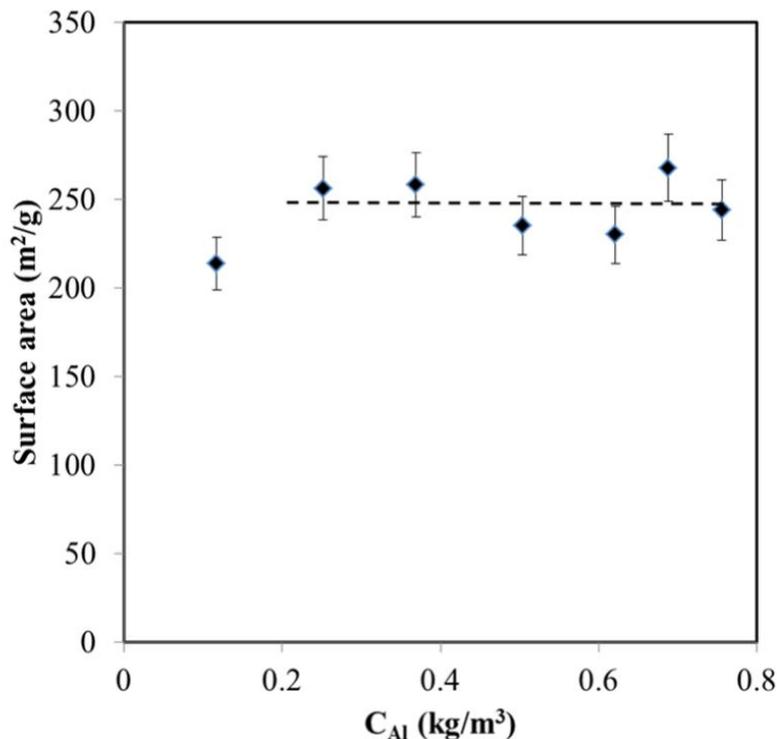


Figure 8: Evolution of the BET surface area of dried flocs vs. C_{Al} ($t=120 \text{ min}$, data averaged from various pH_i).

As total desorption was never ensured when the samples were immersed in pure water, an alternative method to study the species adsorbed was developed. Flocs were obtained with the same synthetic water in the absence of nitrogen compounds, and adsorption experiments with different concentrations of nitrate, nitrite, and ammonium were performed. The experimental results showed that both nitrites and nitrates do not adsorb, while there is a significant adsorption of ammonium (Cf. Figure 9). The isotherm curve of Figure 9 cannot, however, be

used to predict adsorption during EC operation, as the conditions are different: the precipitation of aluminium oxyhydroxides proceeds at the same time of NH_4^+ formation and adsorption during EC, which means that both the amount of adsorbent and adsorbate vary at the same time. However, a comparison with the amount of mg N/g solid obtained with EC under similar operating conditions is consistent with the adsorption experiments (Cf. Figure 9).

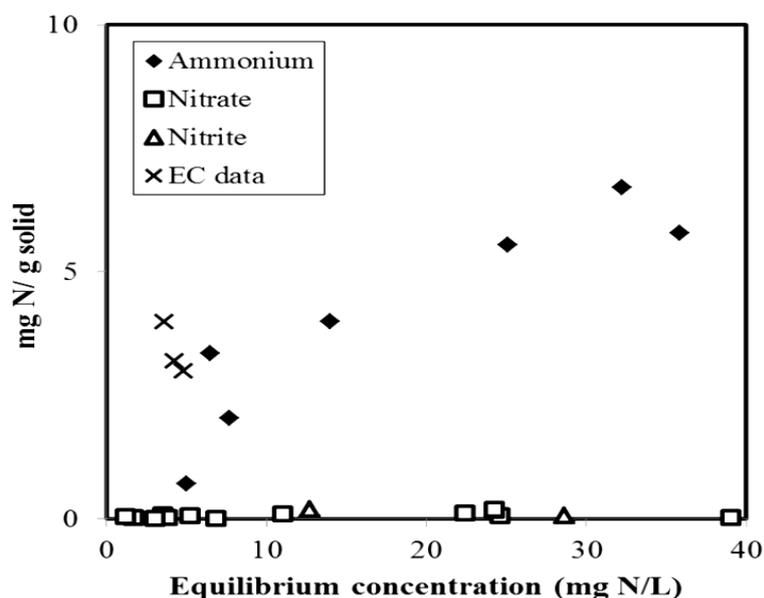


Figure 9: Adsorption isotherm of nitrate, nitrite and ammonium ions on flocs formed in the synthetic water without nitrates.

As a conclusion, denitrification during EC on aluminium electrodes appears to be a two-step mechanism: first, the electroreduction of nitrates occurs on the aluminium cathode; then, ammonium cations are adsorbed on the flocs. The consecutive mechanism proceed in parallel at the same time, as their respective rates are roughly proportional to the electrical charge loading, *i.e.* to the amount of aluminium released in water as far as the pH shift does not prevent the precipitation of $\text{Al}(\text{OH})_3$. High current seems, however, to enhance the electroreduction step in comparison to adsorption: nitrogen content was 3 mg N/g solid for $I=4.5$ A and about 4 mg N/g solid for $I=2.5$ A. This is in line with literature data: using very low current, Lacasa et al. [7] found that ammonium cations could be detected only at short time and that adsorption was the key mechanism of denitrification. This is consistent with our own data if it is considered that adsorption rate becomes more rapid than electrocoagulation rate and that electrocoagulation is, consequently, the limiting step of the two-step mechanism. In the present work, as in Emamjomeh et al. [13], adsorption is the slowest step; this does not slow down the electroreduction of nitrates, but causes the accumulation of ammonium cations in water. This two-step mechanism explains all the data on EC applied to nitrate removal in the literature (see, e.g., [7,12-13]). Finally, another key result is that oxyhydroxides precipitated during EC are not only very effective adsorbents for ammonia/ammonium removal when produced in situ, but also when used as a conventional adsorbent, which had already been shown for organic dyes [17].

4. DISCUSSION ON EC EFFICIENCY FOR NITRATE AND NITROGEN REMOVAL

Electrocoagulation appears to be an effective process for nitrate removal, as it combines electroreduction and adsorption. In addition, the electrogeneration of H₂ gas promotes the flotation of the flocs. A key point is that if the electroreduction is the limiting step, the denitrification process can be described by Eq. 1 and nitrogen compounds are adsorbed onto the flocs, which corresponds to Lacasa et al. [7]. When adsorption is the limiting step, Eq. 1 describes nitrate removal, but ammonium cations remain mainly in water, which requires a subsequent treatment for ammonium removal. This can be done within a longer EC operation [7], or using an alternative way (ion exchange, membrane processes, adsorption, biological treatment... [19-21]). As already mentioned, the other key advantage of EC is that it can remove at the same time many other types of pollutants. However, EC for nitrate removal may be a costly process in account to power requirements and metal consumption. Specific power requirements E (kWh/m³ water) can be derived from cell voltage U . This varies linearly with I and can be combined easily to Eq. 1 and Eq. 2 to give:

$$E = \frac{U \cdot I \cdot t}{V} = [5.0 \cdot I + 2.4] \frac{3F}{k \cdot M_{Al}} \cdot \ln\left(\frac{C_0}{C}\right) \quad (7)$$

This expression describes not only E for nitrate removal, but also for nitrogen removal from water if the adsorption of ammonium becomes rapid in comparison to nitrate electroreduction. As Eq. (7) does ensure the validity of this assumption, it can be also considered as the minimum specific power requirements for nitrogen removal using EC. Equation 7 can be illustrated by the case $C_0=50$ mg/L and $C=25$ mg/L (guideline value for nitrate concentration in water), which is presented in Figure 10a as a function of current and confronted to experimental data. This is expressed, as usual, as a function of current density $j=I/S$ for scale-up purpose. This figure shows the good agreement between experimental data and predicted values: it is obviously linear and varies theoretically as U , as shown by Eq. 7. In this case, specific metal consumption C_{Al} (Eq. 1) should be a constant. Another example consists of plotting Eq. 7 and Eq. 1, varying arbitrarily C_0 at constant j with the same objective: $C=25$ mg/L: predictions of specific power and metal consumptions are shown in Figure 10b and follow the logarithm plot that tends to 0 when C tends to C_0 . In Figure 10 (a and b), the most interesting points are the E values and, to a lesser extent, the C_{Al} data: E is particularly high in comparison to values reported for example by Chafi et al. [15] for the removal of organic dyes. This probably results from the difference of mechanisms that prevail in the EC process: Chafi et al. [15] had highlighted that iron was less effective than aluminium to remove orange II dye because the dye was only adsorbed on the flocs with Al, while it reacted electrochemically on Fe. In this work, electroreduction is the first step of denitrification, which increases drastically power requirements.

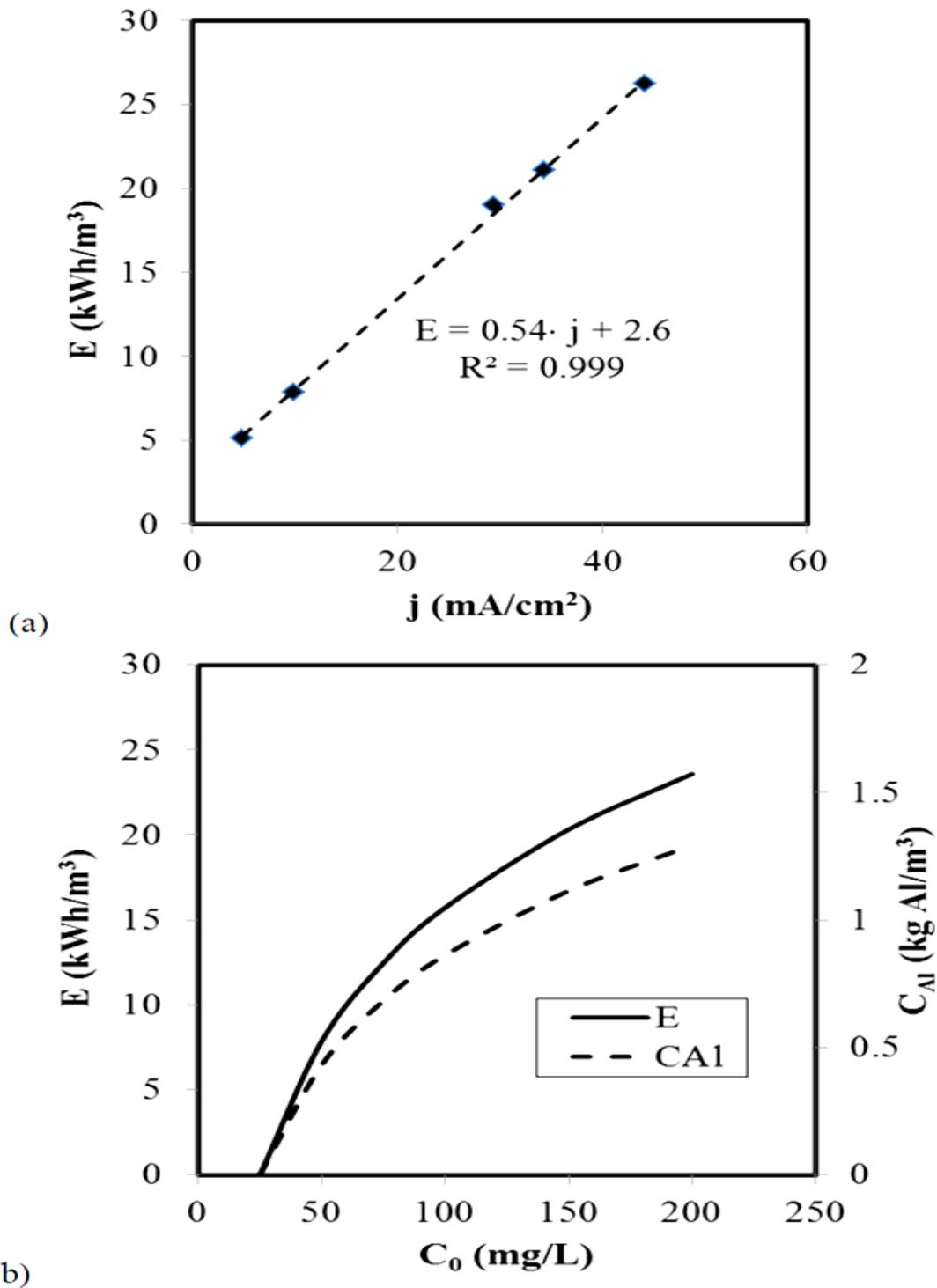


Figure 10: Estimation of specific energy (E) and total aluminium released in water (C_{Al}) to achieve a final nitrate content of 25 mg/L: (a) using $C_0=50$ mg/L as a function of current density j ; (b) using $j=10$ mA/cm² vs. C_0 .

As a conclusion, Eq. 7 provides a very easy way for estimating the minimum power and metal requirements with the assumption of rapid adsorption of ammonium for nitrogen removal from water using EC. The estimated values are, however, high in comparison to the low cost of biological treatments [18]. As a result, EC should be applied only when various types of pollutants can be removed at the same time, nitrates being one of them. For nitrates, EC may also appear as an interesting pretreatment when the initial concentration is high because it

corresponds to the most favorable conditions: high removal rate (Eq. 1), and low E from Eq. 7 by increasing the objective value C . In addition, aluminium oxyhydroxides formed using EC may constitute interesting adsorbents for ammonium removal.

5. CONCLUSION

The objectives of this work were to study the applicability of electrocoagulation (EC) for the denitrification of drinking water and to determine the main mechanisms of pollution removal. These have been achieved: experimental results confirm that EC may remove efficiently nitrates in agreement with literature data. However, an original two-step mechanism was established: nitrates undergo first an electroreduction on aluminium electrodes into ammonium cations with nitrites as an intermediate; then, only ammonium can be adsorbed on the aluminium precipitates. Other mechanisms are negligible: namely, physical capture by the solid phase, chemical reduction by aluminium, reduction or electroreduction of nitrates into gaseous nitrogen compounds. Both steps have different kinetics which depend strongly on current. The electroreduction is first-order, weakly dependent on pH, and gives access to the minimum time for the removal of nitrogen compounds from water when adsorption is rapid. Its drawback is to shift pH to alkaline values. The second step is zero-order, at least when the amount of ammonium cations is high in comparison to that of the flocs. Current does not seem to modify significantly the surface area of precipitates, but adsorption is more sensitive to pH and is impaired by alkaline pH. While the estimation of power requirements shows that EC is a costly method in terms of power requirements in comparison to biological denitrification, aluminium hydroxides formed during EC exhibit interesting adsorption properties for ammonium removal.

NOMENCLATURE

EC	electrocoagulation
C	nitrate concentration (km/m^3)
C_{Al}	total aluminum concentration released in water (kg/m^3)
C_N	total dissolved nitrogen (kg/m^3)
C_0	initial nitrate concentration (km/m^3)
E	specific energy input (kWh/m^3)
F	Faraday's constant (C/mol)
I	current (A)
j	current density (A/m^2)
k	kinetic constant of denitrification (m^3/kg)
M_{Al}	molar mass of Aluminum (g/mol)
pH_i	initial pH (-)
R^2	determination coefficient (-)
S	electrode surface area (m^2)
t	electrolysis time (min)

U cell voltage (V)
 V reactor volume (m³)
Greek letters
 ϕ faradaic yield (-)

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CHAPTER IV: CARBMAZEPINE, A POLLUTANT IN WATER AND ITS TREATMENT

This article is submitted online in Environmental Science and Pollution research. Consequently, it follows the guidelines of this journal.

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ABSTRACT

The tricyclic anticonvulsant carbamazepine is being increasingly used to treat a variety of neuropsychiatric disorders and, consequently, it is found to increasingly pollute the aquatic environment. In this review chapter, the different physiological medical uses of Carbamazepine and its side effects are explained. Then, the occurrence of this molecule and its metabolites are examined in various water bodies including wastewater treatment plants (WWTP) effluents, surface waters, groundwater and drinking water, soil and in human body fluids and tissues, are treated in details in this review. At its environmental concentrations, carbamazepine is supposed to have a harmful effect on human health and the ecosystem, even though any acute toxicity has not been proved clearly yet. However, the accumulation of this synthetic molecule is still growing in aquatic ecosystems. Consequently, various treatment methods have been studied to remove carbamazepine from domestic and hospital wastewater. These will be discussed in detail. Similarly, as carbamazepine is a micropollutant in the environment, the analytical techniques applied for its detection at very low concentrations will be summarized.

1. INTRODUCTION

Carbamazepine (CBZ) is a global pharmaceutical product classified as a class 2 drug under the biopharmaceutical classification system (Rahman et al., 2011). It is an anticonvulsant and mood stabilizing drug discovered by Walter Schindler in 1953 (Tolou-Ghamariet et al., 2013). It is registered as a pharmaceutical in 1962 (Tolou-Ghamariet et al., 2013) and was first approved for the US market by the FDA in 1968 (Rahman et al., 2011). CBZ is commercialized under the following brands: *Biston*, *Calepsin*, *Cabama* or *Carbamaze*, *Carbatrol*, *Epimaz*, *Epitol*, *Equetro*, *Finlepsin*, *Hermolepsin*, *Degranol*, *Sirtal*, *Stazepine*, *Tegretol*, *Telesmin*, *Timonil* (Zhang et al., 2008). It is an iminostilbene derivative (Mohapatra et al., 2014a), lipophilic neutral tricyclic compound (Atkins et al., 2013). Its efficacy and safety profiles have made it the first choice epileptic drug for adults and children, and has replaced both phenytoin and phenobarbitone for a number of pediatric seizure disorders (Miao et al., 2003). Table 1 represents the general physicochemical and pharmacokinetic description of CBZ.

Table 1: Physico-chemical and pharmacokinetic properties of CBZ.	
Therapeutic class	Antiepileptic, analgesic
Physico-Chemical Properties	
Molecular weight	236.268558 (NCBI)
Chemical formula	C ₁₅ H ₁₂ N ₂ O
Elemental composition	C 76.25%, H 5.12%, N 11.86% , O 6.77%
Monoisotopic mass	236.094963 g.mol ⁻¹ (NCBI)
Topological Polar Surface Area	46.3 Å ² (NCBI)
Color	Crystals from absolute ethanol and benzene (NCBI) White to off-white powder (NCBI)
Solubility in water at 25°C	0.33 mmol/L (Celiz et al., 2009), 17.66 mg/L (drugbank.ca)
Soluble in acetone and propylene glycol, chloroform, dimethyl-formamide, ethylene glycol monomethyl-ether, and methanol; slightly soluble in ethanol	(NCBI)
Storage temperature	2-8°C (lookchem.com)
pKa at 25 °C	-0.49, 13.9 (Hai et al., 2011)
Density	1.266 g.cm ⁻³ (lookchem.com)
Melting point	187-193°C (Abou-Enein and Al-Badr, 1980); 190.2 °C (NCBI)
Boiling Temperature	411°C at 760 mmHg (lookchem.com)
Pharmacokinetics	
Half-life of elimination	37.7 h (Abou-Enein and Al-Badr, 1980)
Half-life of elimination after repeated consumption	21 h (Abou-Enein and Al-Badr, 1980)

1.1. MEDICAL USES OF CBZ

CBZ is an anticonvulsant antiepileptic psychotropic mood stabilizing agent that controls the convulsions (fits) suffered by patients of epilepsy. It mainly works on reducing the activity in the brain (Mohapatra et al., 2014a), *i.e.* decreasing the potentiation of the synaptic transmission in the affected area (Rahman et al., 2011). It is used to treat schizophrenia (Miao et al., 2003), and trigeminal neuralgia (severe burning or stabbing pains in the face (Gonzalez et al., 2006) that is afflicting 1.2% of adults in the U.S (Miao et al., 2003). It is also prescribed for the treatment of post traumatic stress disorder, restless leg syndrome, diabetes insipidus, chorea (a disease that affects children) (Mohapatra et al., 2014a), generalized tonic-clonic seizures, complex seizures (Atkins et al., 2013), and partial seizures (Zhang et al., 2008). For instance, episodes of mania (irritated mood) or mixed episodes (depression that affects patients with bipolar disorder), can be treated with CBZ extended release capsule (Equetro brand) (Mohapatra et al., 2014a). In addition, combining it with other medications, CBZ is used to treat cases of alcohol withdrawal (Miao et al., 2003).

1.2. THERAPEUTIC ROLES OF CBZ

The exact mechanisms of action of CBZ in the human body is yet not well understood. However, some hypotheses are set to explain the roles of CBZ in the treated body. For instance, CBZ is known as an anticonvulsant antiepileptic agent that may act postsynaptically by enhancing the inactivation of sodium channels or presynaptically by blocking the sodium channels and, thus, decreasing the neurotransmitter transmission in

the synaptic cleft. Moreover, the influence of CBZ on decreasing the release of glutamate and the stabilization of neuron membranes can essentially explain the anti-epileptic effects (Houeto et al., 2012).

1.3. SIDE EFFECTS AND PRESCRIPTION DOSAGES

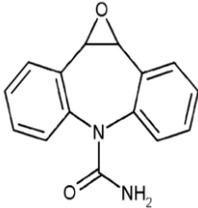
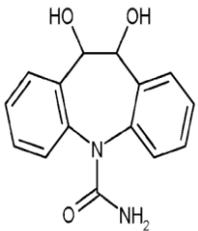
CBZ is usually prescribed at high dosages of 100-2000 mg/day. Consequently, its annual production is considerably high (Kosjek et al., 2009). Germany, for instance, is known as the country of world's highest CBZ consumption of 87 tons sold yearly, corresponding to the highest prescribed dose per capita (DPC), reaching 1061 mg per day. CBZ is commercially sold as either orally taken tablets, capsules or suspension forms with 85% of compound availability with some other synthesis related organic impurities (Atkins et al., 2013). The side effects accompanying the intake of CBZ could affect 50% of patients. These could be varied starting from sleepiness, mild unsteadiness of walking, dizziness, double vision, nausea, vomiting, allergic rash, effects on the liver or the blood (blood tests are required in this case), and thinning of the bones with age where taking vitamin D and calcium supplements is a must to prevent this. CBZ also reduces the amount of contraception pills in the body, and delays the mental development of the baby, where 1% of newborns are born with spina bifida for mothers taking CBZ (Cambridge University Hospitals, patient information).

1.4. CBZ BIOLOGICAL METABOLISM AND FATE IN THE HUMAN BODY

Clinical studies have shown different absorption profiles of the different commercial forms of CBZ, *i.e.* the suspension was the most rapid to be absorbed, followed by the conventional tablet, and the slowest form was the extended release capsule (NCBI). The peak concentration of CBZ is attained in 1.5 hours. in blood after the intake of suspension form, 4-5 hrs. for conventional tablets and 3-12 hours. for extended release tablets (NCBI). In general, once ingested, CBZ peaks about 4 to 8 hours. after ingestion in the blood plasma. A clear medical effect, however, could take place only after 26 hrs. of intake with an elimination half-life of 25-65 hrs. post administration (Zhang et al., 2008). The different metabolic pathways are summarized in Table 2, as follows:

1. CBZ is highly permeable at the level of the intestines and thus easily gets into the blood (Rahman et al., 2011);
2. In the liver, CBZ is usually prone to extensive hepatic metabolism by the cytochrome P450 (CYP) system.

Thirty three metabolites of CBZ have been identified in human and rat urine. Moreover, it was found that an induced radioactive ¹⁴C-CBZ was absorbed and then found in the feces. It was also found that between less than 1% (according to Zhang et al., 2008) and 3% (according to Miao et al., 2005) of the administered CBZ was excreted in its unaltered form with a pharmacologically active compound 10,11-epoxycarbamazepine, as the major metabolite (Miao et al., 2005). Other metabolites could be also formed, such as 10,11-dihydro-10,11-expoxy carbamazepine (CBZ-epoxide) that has similar antiepileptic properties to CBZ and trans-10,11-dihydro-10,11-dihydroxycarbamazepine (DiOH-CBZ) not pharmaceutically active (Miao et al., 2003; Zhang et al., 2008).

Table 2: CYP metabolic agents and the respective metabolites of CBZ hepatic metabolism.			
Parent molecule	Agent of the CYP system	Metabolites	Chemical Structures
CBZ	CYP3A4 and CYP2C8	CBZ-epoxide (Zhang et al., 2008)	
CBZ	Microsomal epoxide hydrolase	DiOH-CBZ 10,11-dihydro-10,11-dihydroCBZ (Zhang et al., 2008)	
CBZ	CYP1A2 (a less taken pathway) + oxidation	DiOH-CBZ (Zhang et al., 2008)	

2. OCCURRENCE OF CBZ

Due to the worldwide excessive use of CBZ, this molecule is found and detected in different sites in the world. The presence of CBZ was studied in water, soil, and in human body.

2.1. OCCURRENCE OF CBZ IN WATER BODIES

Occurrence and behavior of pharmaceuticals in aquatic ecosystems are more and more taken into consideration by the research community, as well as by regulatory authorities and water suppliers. As pharmaceuticals represent a potential risk for drinking water supply, knowledge about their fate is a necessity. The concentration of these pharmaceuticals change among different countries depending on their annual production, their human usage, the amount disposed in water, and their transport behavior. Generally speaking, once a pharmaceutical enters the aquatic environment it can redirect itself into three possible fates.

- it is either mineralized into carbon dioxide and water,
- or partially retained in the sedimentation sludge (as it is hard to degrade due to its lipophilic nature),
- or if it metabolizes into a more hydrophilic molecule, it then passes untreated in the wastewater plant and ends up in the receiving water (Klavarioti et al., 2009).

Because of the direct disposal from hospital wastewaters and the presence of CBZ and its metabolites in biological fluids, there is a reason to suspect its presence in domestic sewage and in the aquatic environment. Environmental studies confirm the presence of CBZ as one of the most frequently detected pharmaceuticals in sewage effluents, in river

water and in sea water (Miao et al., 2003). For instance, CBZ presence is reported in wastewaters (up to $6.37 \mu\text{g}\cdot\text{L}^{-1}$), in surface waters (up to $1.1 \mu\text{g}\cdot\text{L}^{-1}$), and in drinking water ($30 \text{ ng}\cdot\text{L}^{-1}$) (Kosjek et al., 2009). Researchers considered that CBZ could be a "witness molecule" confirming the presence and persistence of other drugs in water bodies (Mohapatra et al., 2014a), thus evaluating the efficiency of removal of pharmaceuticals in sewage treatment plants (STP) (Miao et al., 2003).

Due to CBZ high stability, many treatment methods studied for its removal and it emerged that most of these treatments are not efficient. For example, the conventional treatment of CBZ in the WWTPs which is mainly based on the *activated sludge* biological process is limited to below than 10% removal. This leads CBZ to finally find its way in the WWTPs effluents which are important gateways from which it can enter the water cycle (Hata et al., 2010). Consequently, CBZ was found in the wastewater treatment plants effluents at $6300 \text{ ng}\cdot\text{L}^{-1}$, which is the highest among the other pharmaceuticals studied (Zhang et al., 2008).

In general, pharmaceuticals worldwide average consumption reaches up to 15 g per year and per capita. In the developed countries, however, this value is expected to be between 50 and 150 g (Dominguez et al., 2010). The world annual consumed volume of CBZ is 1014 tons (Klavarioti et al., 2009), which is in accordance with the intercontinental marketing services (IMS) Health Data (Mohapatra et al., 2014a). The yearly consumption of CBZ can even differ between the industrial countries. For instance, in 1999, the annual consumption of CBZ in Germany was 87 tons. In 2001, it was 28, 40, 40, and 43 tons in Canada, France, England, and USA respectively. In 2004, it was found to be 10 tons in Australia. In 2007, however, 942 tons were sold in 76 major countries which are believed to account for 96% of the global pharmaceutical market. Consequently, CBZ can be found in the water resources in different concentrations depending on the amount produced, used and disposed. In comparison to other pharmaceuticals, CBZ is found in most water bodies of Europe, America, and Asia in the WWTP effluents at high concentrations ($30\text{-}1100 \text{ ng/L}$) (Zhang et al., 2008). Table 3 summarizes the different occurrences of CBZ in different countries of the world and shows that WWTP effluents may exhibit either high CBZ content or high contents of CBZ metabolites, for example in Canada.

1. In Europe		
	Site	CBZ or metabolite concentration
Germany-Berlin (highest concentration in the world)	Surface waters	CBZ: 1075 ng/L (Heberer et al., 2002)
Germany	Sea water	CBZ: 2 ng/L (Weigel et al., 2001)
	Elbe river	CBZ: $1.2 \mu\text{g/L}$ (Miao et al., 2005)
Germany and Portugal	46 samples of influent and effluent of WWTPs	CBZ: $5 \mu\text{g/L}$ DiOH-CBZ : $4.8 \mu\text{g/L}$ 10,11-dihydro 10-hydroxy-CBZ (10-OH-CBZ) : $1.1 \mu\text{g/L}$ (Bahlmann et al., 2014).
Spain-Seville (Santos et al., 2007)	WWTPs influents and effluents	CBZ in influents: $0.28 - 0.36 \mu\text{g/L}$ CBZ in effluents: $0.29 - 0.50 \mu\text{g/L}$

France (Anses, 2011)	Water for human consumption	CBZ: 33 ng/L 10,11 epoxy carbamazepine: 6 ng/L
Switzerland (Tixier et al., 2003)	Greifeuse lake (with residence time of 408 days)	29.2 g/day with CBZ half-life of 63 days in the epilimnion
Luxembourg (Banzhaf et al., 2012)	Surface waters of river banks in a hyporheic zone	CBZ: 600 ng/L
Mediterranean region	2 out of 7 wells in a watershed	CBZ in the 2 wells: 43.2 and 13.9 ng/L (Rabiet et al., 2006)
	Coast influents and effluents (Gomez et al., 2007)	CBZ in the influents : 0.12-0.31 µg/L CBZ in the effluents : 0.13 µg/L
2. In America		
USA	Surface waters	CBZ: 1.2 µg/L (Weigel et al., 2004) CBZ: 60 ng/L (Thacker et al., 2005)
USA	44 rivers	CBZ: 60 ng/L (Thacker et al., 2005)
USA	Huron river	CBZ: 9 ng/L (Skadsen et al., 2004)
USA	Detroit river	CBZ: 0.3-0.8 ng/L (Hua et al., 2006b)
USA- New York	Jamaica Bay	CBZ: 5-35 ng/L (Benotti and Brownwell, 2007)
Canada	Effluents of WWTPs	CBZ: 2.3 µg/L (Mohapatra et al., 2014a) - 29% of CBZ removed by treatment (Miao et al., 2005), but DiOH-CBZ not effectively removed and found at higher concentrations (Hummel et al., 2006)

2.2. OCCURRENCE IN THE SOIL SEDIMENTS

Pharmaceuticals are found in the soil sediments via many sources. For example, the disposal of those pharmaceutical compounds used in agriculture, industry, common households, medical treatment, hospitals contributes to 26% of total CBZ found in municipal WWTPs (Heberer and Feldman, 2005), and veterinary pharmaceuticals greatly contribute to CBZ entry into fresh bodies (Klavarioti et al., 2009). Moreover, spreading wastewater on soil during reuse, especially in arid and semi-arid countries for irrigation may result in the transfer of some pharmaceutical active compounds including CBZ and its metabolites (Mohapatra et al., 2014) to the soil, and consequently to the ground and drinking water. Another reason is that the removal of CBZ or its metabolites in the activated sludge process in WWTPs could be partially explained by biosorption/bioaccumulation, which could also contribute as a secondary pathway for soil pollution.

In general, most pharmaceuticals are relatively polar, which renders their adsorption to soil or particulates of little importance. Hence, most of these compounds are mobile in the environment (Celiz et al., 2009). CBZ is found to be a small a mobile molecule, not very polar and not very apolar (so that it does not adsorb efficiently neither on hydrophilic nor on hydrophobic compounds), used as an anthropogenic indicator (Banzhaf et al., 2012). Being found in wastewater-irrigated soil at 0.02 to 15 ng/g of dry matter (Mohapatra et al., 2014a), CBZ can enter the aquifer when surface water and ground water are connected hydraulically (Banzhaf et al., 2012). Knowing that it has a high ability to pass through an unsaturated ground zone, CBZ reaches aquifers without being subjected to any degradation or adsorption during its underground or ground water passage (Clara et al., 2004). As a result, CBZ was detected at high concentrations of 100 ng/L in the ground water near a river bank in Grand Duchy of Luxembourg (Strauch et al., 2008). Other studies have shown that CBZ adsorption and transport behavior depend not only on CBZ, but also on the soil content, *i.e.* adsorption of CBZ was greater on soils of higher organic content (Stamatelatou et al., 2003). Other sand column transport experiments revealed that CBZ was not adsorbed (Scheytt et al., 2006), and that when passing in a saturated column experiment, CBZ showed a significant retardation factor of Rf about 2.8 (Mersmann et al., 2002), where retardation factor is the distance covered by a molecule in a fluid at a certain time.

The travel of the stable CBZ molecule was also explained by a study in which CBZ was found in a well at 90 ng/L after 6 years travel time in the subsurface originating from an effluent comprising 155 ng/L (Drewes et al., 2002). CBZ was also found at 20 ng/L in an abandoned drinking water well located 100 m away from a lake measuring 135 ng/L (Heberer et al., 2001). In fact, the behavior or trend followed by CBZ and its metabolites in the subsurfaces differs strongly between regions due to the differences in soil types and hydrophobicity (Mohapatra et al., 2014a). In Germany, for example, the presence of CBZ was found to be mainly due to river infiltration at a maximum concentration of 83 ng/L where this concentration decreased with an increasing distance from the river (Osenbruck et al., 2007). Moreover, in Canada, it was found that the mass concentration of CBZ and its metabolites increased in biosolids after sludge treatment in a wastewater treatment plant as shown in Table 4 (Miao et al., 2005) where a mass balance showed that the majority of CBZ and its metabolites exist in wastewater (Miao et al., 2005).

In the treated solids	In the untreated solids
CBZ 258.1 µg/kg	CBZ 69.6 µg/ kg
2-hydroxycarbamazepine (CBZ-2OH) 3.4 µg/kg	CBZ-2OH 1.9 µg/kg
3- hydroxycarbamazepine (CBZ-3OH) 4.3 µg/kg	CBZ-3OH 1.6 µg/kg
CBZ-DiOH 15.4 µg/kg	CBZ-DiOH 7.5 µg/kg

2.3. OCCURRENCE OF CBZ AND ITS METABOLITES IN THE HUMAN BODY

CBZ occurrence was also studied in the human body in patients, in the post mortal phase, and during pregnancy. Upon studying the occurrence of CBZ and its metabolites CBZ-10,11-epoxide and iminostilbene in body fluids and organ tissues, the analysis on GC-MS

of five autopsies have shown higher CBZ concentrations in the organ tissues than in the blood and urine, and was higher in the liver than in the lungs in three cases of the five autopsies (Takayasu et al., 2010). Moreover, the concentrations of both CBZ and its metabolites were compared to each other. For example, the occurrence of 10-11, epoxide and iminostilbene were lower than that of CBZ in body fluids and organ tissue specimens. The iminostilbene concentration, however, was higher than the CBZ epoxide concentrations in four cases. Both metabolite concentrations were fluctuating, being higher or lower than each other at relatively important levels in the lungs and liver (Takayasu et al., 2010). The analysis of a *post mortem* fluid and tissue specimens has shown that OXCBZ was mostly found in the kidneys at 2.539 µg/mL and less present in the muscle tissues. However, 10,11-dihydro-10-hydroxyCBZ (DiCBZ) was mostly found in the liver 38.741 µg/mL and less in the blood at 9.848 µg/mL. CBZ, on the other hand, was the least found in the body compared to its metabolites and mostly found in the liver and less in the heart and spleen (Johnson et al., 2010). CBZ was also investigated in a study during pregnancy and it was found out that CBZ, CBZ-epoxide, CBZ-DiOH, CBZ-2OH, CBZ-3OH accounted for 0.5, 1.5, 35, 2.7 and 4% of total concentrations in urine samples respectively (Bernus et al., 1995).

3. TOXICITY

The environmental effects of pharmaceuticals and antibiotics in water are, among others, the development of antibiotic resistant microbes in water treatment processes and in the aquatic environment, nitrate oxidation retardation and methanogenesis, and the potential increased toxicity of chemical combinations and metabolites (Omatoyo et al., 2007). Pharmaceuticals are fabricated in a way to have a physiological effect on humans and animals even when existing at trace concentrations (Klavarioti et al., 2009). They are persistent against biological degradation and, thus, keep their biological activity and their chemical structure long enough to do their therapeutic work once retaken. They could remain for a long time in the aquatic ecosystem, due to the fact that they are continuously added to the environment and thus, are considered potentially dangerous at both low and high concentrations (Klavarioti et al., 2009). One of these potentially dangerous pharmaceuticals is CBZ that is continuously introduced into the environment and prevalent at low concentrations (Mohapatra et al., 2012). The presence of CBZ affects water quality and strongly influences water supplies ecosystem and human health (Heberer et al., 2002).

The ecotoxicological studies on CBZ imply that this pharmaceutical does not clearly cause acute toxic consequences at its environmental concentration, though its chronic effects need cautious attention (Zhang et al., 2008). Moreover, considering the results of research on CBZ toxicity and the present European legislation on the classification and labeling of chemicals (92/93/EEC), CBZ is classified as R52/53 harmful to aquatic organisms and may cause adverse effects in the aquatic environment (Zhang et al., 2008). However, the molecule and its metabolites are not classified as carcinogenic or mutagenic *in vitro* and *in vivo* even at high concentration (Glatt et al., 1975; Königstein et al., 1984; Schaumann et al., 1985; Margaretten et al., 1987; Flejter et al., 1989; Celik, 2006) by the National Toxicological Program (NTP), the International Agency for Research on Cancer (IRAC), and the Food and Drug Administration (FDA). A single carcinogenesis study has been carried out: it is a two-year study on Sprague–Dawley rats (male and female) at doses of 25, 75 and 250 mg/kg/day. An increase in the incidence of hepatocellular tumors in females and benign testicular interstitial cell adenomas in males

has been observed starting at doses of 25 mg/kg/day (United States Pharmacopeia, 2008; Houeto et al., 2012).

Many assessment studies on CBZ have been done on different species. A study performed in the Netherlands showed that the CBZ concentration found in water is much less than the hazardous concentration to human health (Sémran et al. 1997, 1999). More precisely, the drinking water equivalent level (DWEL) calculated from the toxicity reference value of the toxicological approach is 99.10^3 ng/L for adults and 55.10^3 ng/L for children. These values are much higher than the maximal concentration of 33 ng/L of CBZ found during the sampling survey, and thus the presence of CBZ residues in drinking water at these concentration is not dangerous on human health (Houeto et al., 2012). As for humans, two set values for the toxicity of CBZ are found on the fauna and the flora. The first one explains, for example, the toxicity due to fishery products consumption and is equal to $2000 \mu\text{g}/\text{kg}_{\text{biota}}$, and the second one is the corresponding concentration of CBZ in water and is equal 115 $\mu\text{g}/\text{L}$ (INERIS, 2012). The influence of CBZ on reproduction in rats, mice and human was also tested. The observed effects were weight loss during lactation, decrease in the number of fetuses and fertility, increase in resorptions, bilateral twisting of ribs, increase in neural tube and urinary tract defects, cleft palate, and cardiovascular disorder (Houeto et al., 2012). Other studies showed that CBZ had a limited acute ecotoxicity on lower organisms, as bacteria, algae, microcrustacean and fish. Chronic tests displayed higher toxicity than acute tests. (Zhang et al., 2008). Conversely, it was found out that CBZ did not have any effect on the algae *Ankistrodesmus braunii* and that CBZ concentration progressively decreased in the culture of algae (Andreozzi et al., 2002). As a conclusion, the toxicity of CBZ remains controversial. Accordingly, other values were proposed for water quality standards involving CBZ, including the mean and maximum values. On the one side, for fresh water, a mean annual concentration in water is set equal to 2.5 $\mu\text{g}/\text{L}$ with a maximal acceptable concentration at 310 $\mu\text{g}/\text{L}$. On the other side, values set for marine water are 0.25 $\mu\text{g}/\text{L}$ as the mean concentration and 31 $\mu\text{g}/\text{L}$ as a maximal acceptable concentration in water (INERIS, 2012).

4. TREATMENT METHODS OF CBZ ELIMINATION FROM WATER

Water treatments for CBZ removal can be divided into two main categories: physicochemical and biological processes. Physicochemical treatments involve, in practice, many subcategories (such as oxidation, photochemical processes...) that will be detailed. Electrochemical treatments that will be studied in this Ph.D. thesis will be studied in Chapter V, as already mentioned.

4.1. BIOLOGICAL TREATMENT

The fear of the influence of the increasing concentration of pharmaceuticals in water drove researchers to find different possible treatment methods of pharmaceuticals and employ them in wastewater treatment. Biological treatment, for example, is a very vital step of the conventional wastewater treatments. The advantages of the biological treatment of WWTPs settle in its low operating cost compared to other treatments such as chemical oxidation, thermal oxidation, etc. (Arun, 2011). Depending on their structural

differences, many pharmaceuticals, such as ibuprofen, are readily degraded under most studied conditions; however, CBZ tends to be refractory and recalcitrant in face of the different methods applied for its treatment (Kagle et al., 2009). As a result, many projects have addressed the potentiality of the activated sludge process to remove CBZ from wastewater in the world (e.g. in France the AMEPERE project from 2006 to 2009, the ARMISTIQ and the EchiBioTEB projects from 2010 to 2013...). Even though the results of all these projects in the world show a large discrepancy of conclusions, which can be partly explained by the difference in analytical tools and the variability of the composition of activated sludge and influent quality, it emerges that CBZ remains one of the most difficult pharmaceuticals to biodegrade in WWTPs (Liu et al., 2012).

As a result, if the objective is to avoid the use of any additional physicochemical downstream treatment, two main strategies have been proposed. The first one consists of using *bioaugmentation* and *bioacclimatation* (Semrany et al., 2012) of the activated sludge to enhance so as to enhance CBZ removal. The second one is to use an additional biological treatment step involving other microorganisms. These strategies are not contradictory, as the second may be used to identify microorganisms able to remove CBZ that could be then, used in conventional WWTPs through a bioaugmentation pathway. As a consequence, various microorganisms have been investigated to treat CBZ removal using pure cultures. The literature show that the best of all microorganisms employed are the white rot fungi. These are basidiomycetes that present a weak substrate specificity and a varied enzymatic activity capable of undergoing an aerobic depolymerization of lignin. A combination of these enzymes with the cytochrome P450 system is currently employed in the degradation of many personal pharmaceutical products, such as CBZ, clofibrac acid, ibuprofen, and other endocrine-perturbating chemical products (Marco-Urrea et al., 2009). The inhibition experiments showed that the cytochrome P540 system of these enzymes plays an important role in degrading CBZ (Marco-Urrea et al., 2009; Golan-Rozen et al., 2011). A new developed method was based on the induction of hydroxyl radicals using the redox cycle of the quinone has caused a higher degradation of CBZ of up to 80% during 6 hours (Marco-Urrea et al., 2010).

Other white rot fungi (WRF) enzymes comprise manganese peroxidase (MnP), lignin peroxidase (LiP), versatile peroxidase (VP) and laccase (Tanaka et al., 1999; Duran and Esposito, 2000). Many studies were performed to check the efficiency of each of these enzymes singly in degrading the CBZ. For example, the LiP enzyme taken from *Phanerochaete chrysosporium* led to a limited degradation of less than 10% of CBZ (Zhang and Geiben, 2010). However, when the fungi were grown on polyether foam with a sufficient nutrient supply, and a preferred acidic medium (Gao et al. 2008), CBZ was highly eliminated up to 60–80% by adsorption on the foam during 100 days (Zhang and Geiben, 2012). Other studies in which a single enzyme is employed showed that only 22% of CBZ was eliminated upon single treatment with laccase taken from *Trametes versicolor* and a redox mediator 1-hydroxybenzotriazole (HBT), while repeated treatments of CBZ led to a 60% degradation after 48 hours. This treatment, however, also led to the formation of two by-products namely, 10,11-dihydro-10,11-epoxycarbamazepine and 9(10H)-acridone (Hata et al., 2010). Other enzymes like MnP and VP taken from by *Pleurotus ostreatus* have shown an elevated percentage (98%) of CBZ degradation (Golan-Rozen et al., 2011; Semrany, 2014).

As a conclusion, there is no definite answer on the possibility to apply bioaugmentation to the conventional activated sludge process at the moment, so that CBZ can be

effectively removed and not biosorbed on the sludge. This remains, however, an attractive way of research because it could minimize the equipment modifications necessary to define WWTPs able to remove CBZ from wastewater.

4.2. ADVANCED OXIDATION PROCESSES

Advanced oxidation processes (AOPs) are aqueous chemical oxidation processes that are based on in-situ generation of powerful oxidizing agent, hydroxyl radicals ($\cdot\text{OH}$). The latter are produced by means of one of the conventional primary oxidant: namely ozone, hydrogen peroxide and oxygen and, in the most of time, in the presence of energy source, such as UV light and catalysts, such as titanium dioxide or ferrous ions. The highly reactive hydroxyl radicals generated through AOPs oxidize organic compounds and, from the properties of pollutants to be removed and that of matrix, two cases may be encountered: oxidation of pollutants by AOPs may either lead to the utter destruction that ends up with total mineralization, or make secondary products that can be sometimes less toxic and sometimes more toxic than the pollutant. It is expected that these are, in both cases, more biodegradable where the conventional processes, such as physicochemical and biological processes could be applied as post-treatment process with success. Having proven their effectiveness to treat all kinds of persistent pollutants such as pharmaceuticals, biorecalcitrant and refractory compounds, AOPs allow equally to reduce considerably the concentration of pollutants from several-hundred ppm to less than 5 ppb. On the basis of the aforementioned reasons, AOPs are known as *water treatment processes of the 21st century* (Munter, 2001). CBZ has been recognized as being one of the most persistent pharmaceuticals and has been detected at the highest frequency in industrialized countries (Mohapatra et al., 2014b; Tran et al., 2013). Due to CBZ low abatement yield by the conventional methods, AOPs have been suggested and investigated as emerging methods to treat CBZ by many researchers, given the high efficiency of AOPs to treat the persistent pollutants even when they are present in trace concentrations. AOPs include various processes among which one can cite the most widely used to treat CBZ: namely ozonation, UV/hydrogen peroxide, Fenton, photo-Fenton and heterogeneous photocatalytic processes. Electrochemical treatments, that are one of the objectives of this paper, will be treated in Chapter V.

4.2.1. OZONATION

Ozonation is a promising oxidative process that has been widely used in the treatment of water, wastewater and sludge from wastewater treatment plants and has proven its high efficiency to treat all the types of organic pollutants. For fresh water, it is now a current and established tertiary treatment. Unlike other conventional water/wastewater treatment processes, ozonation has made difference by its significant effectiveness in extirpating organic micropollutants and pharmaceuticals (Ternes et al., 2003; Hua et al., 2006a; Esplugas et al., 2007; Schaar et al., 2010). Throughout the ozonation process, oxidation of organic compounds takes place due to a combination with hydroxyl radicals and reactions with molecular ozone. Some conditions conducive to *in-situ* generation of hydroxyl radicals must be met. Ozone may lead to hydroxyl radicals once combined with hydrogen peroxide, in presence of ultraviolet light or catalysts and when ozonation proceeds at elevated pH (Munter, 2001).

Abatement of CBZ by ozonation has led to satisfying results. It has been found out that

CBZ can be straightforwardly and completely removed from drinking water by ozonation even from wastewater, once ozonation is performed under optimum operating conditions, *i.e.* when the suitable ozone dosage and the other factors that trigger or expedite the production of free hydroxyl radicals are accurately adjusted. For instance, some experiments were run to treat drinking water collected from the upper Detroit River (Ontario, Canada), on the one hand using the conventional treatment process for potabilization and on the other hand using the same conventional treatment process with ozonation. Thereafter, it was revealed that CBZ was utterly insensitive to the conventional process, while the CBZ removal was achieved up to 99% as a function of the annual season of sample collection with only an input of 1.5-2 mg/L of O₃ (Hua et al., 2006a). In the work performed by Schaar et al. (2010), CBZ withstood completely to the biological treatment of wastewater, but a subsequent step of ozonation turned out to be efficient to deplete CBZ for an initial concentration of 900 ng/L; the removal yield was also above 99% for all operating conditions used.

Up to now, the mechanisms taking place during the oxidation of organic species by hydroxyl radicals are still badly known. Consequently, the specific mechanisms of CBZ removal by molecular hydroxyl radicals are still undefined. However, these involved when CBZ reacts with ozone have been dissected in many studies, which is mentioned in plenty of published papers (Andreozzi et al., 2002; Hübner et al., 2014). It was corroborated that the molecular ozone attacks first the double bond of non-aromatic carbons. However, intermediate by-products formed from ozonation of CBZ that could lead to mineralization of the carbon and ammonia are not explicitly defined. Andreozzi et al. (2002) suggested that oxidation of CBZ by molecular ozone led to glyoxal, glyoxilic acid, oxalic acid, oxamic and ketomalonic acids, carbon dioxide and an intermediate compound. The latter subsequently underwent hydrolytic and oxidation reactions, and it turned into anthranilic acid, ammonia, carbon dioxide and glyoxilic and oxalic acid. For initial CBZ concentration of $5 \cdot 10^{-4}$ mol/L with a dosage factor of 10 with respect to ozone, 30% mineralization was achieved and the amount of ammonia produced turned out to be a function of ozonation time: for example, 68.9% and 100% of initial nitrogen contained in CBZ were converted into ammonia in 60 and 90 minutes ozonation, respectively. Recently, Hübner et al. (2014) attempted to precisely determine and identify ozonation products of CBZ. As an outcome of their study, four main by-products and fourteen additional by-products were identified. These four main by-products were already known: 1-(2-benzaldehyde)-4-hydro-(1H,3H)-quinazoline-2-one, 1-(2-benzaldehyde)-(1H,3H)-quinazoline-2,4-one, 1-(2-benzoic acid)-(1H,3H)-quinazoline-2,4-one, 1-(2-benzoic acid)-4-hydro-(1H,3H)-quinazoline-2-one. The first three by-products had been previously identified by McDowell et al. (2005) and it was the first time that 1-(2-benzoic acid)-4-hydro-(1H,3H)-quinazoline-2-one was suggested as an ozonation product of CBZ. The fourteen additional by-products have been detected only when the initial concentration of CBZ was high and nine among them had been found before in other oxidation methods of CBZ. It is worth mentioning that almost all these by-products were found biodegradable. Thereafter, this explains why these minor by-products are obtained at trace level.

As a conclusion, ozonation seems able to remove efficiently CBZ. A key problem is, however, the operating cost of ozonation that is, for this reason, essentially applied for fresh water treatment, rather than for wastewater treatment, but WWTPs including an ozonation step devoted to the removal of micropollutants can be found, now, for example in France (Sofia Antipolis) and in Switzerland (Dübendorf). Another key problem that is not completely solved is the toxicity of the by-products. Even though

most of the above-mentioned CBZ by-products do not seem to be toxic, the situation may be different for other micropollutants. In addition, the "cocktail effect" due to the presence of a mixture of reactive compounds must be accounted for, not only on toxicity, but also on reactivity, *i.e.* the possible reactions between by-products from different pollutants. On this topic, no definite answer is available at the moment.

4.2.2. UV/HYDROGEN PEROXIDE

UV/H₂O₂ is one of AOPs generally used to remove organic micropollutants from water/wastewater in which the photolysis of hydrogen peroxide leads to the formation of two hydroxyl radicals (Eq. 1). The destruction of organic species takes generally place due to two parallel actions:

1. the photolytic degradation thanks to the UV light
2. the reactions in chain with hydroxyl radicals (Rosario-Ortiz et al., 2010; Wols et al., 2013).

Most of the time, oxidation of organic compounds by hydroxyl radicals prevails in comparison with photodegradation (Rosario-Ortiz et al., 2010). It is worthwhile noting that, sometimes, some pharmaceuticals could be insensitive to one of these degrading actions or even be insensitive to both of them. Thereafter, many efforts have been made to investigate the sensitivity of pharmaceuticals, including CBZ, to photolysis and to oxidation by hydroxyl radicals, but equally to assess the efficiency of low pressure (LP) and medium pressure (MP) lamps as a source of UV light.



The photolysis of CBZ has been of paramount interest for many researchers (Donner et al., 2013; Wols et al., 2013), even though its application at industrial scale remains small. CBZ might be effectively photodegraded in the presence of a high UV dose and with a water/wastewater matrix favorable to the photolysis *i.e.* the presence of other contaminants leading to involvement of other radicals that degrade compounds. Using a high initial concentration of CBZ (6 mg/L) and a medium pressure metal-halogen UV lamp (690 W) that emits a polychromatic light of wavelength between 185 and 400 nm, Donner et al. (2013) investigated the ecotoxicity of UV photolysis products. Upon having conducted experiments following three short-term toxicity tests (inhibition of bioluminescence in the marine bacterium *Vibrio*, growth inhibition of the green algae *Pseudokirchneriella subcapitata*, immobilization of the crustacean *Daphnia magna* Straus, among others), the following conclusion was drawn: acridine and acridone, the two major products among the photolysis products of CBZ that may be formed, were found to be more toxic than the parent compound (CBZ), as the toxic response was higher to 60% for algae and bacteria after 30 min of UV exposure and for daphnia after 80 min. While the toxicity of acridone is not clearly established, acridine is well-known to be a water and air pollutant with mutagenic and carcinogenic activity. In practice, acridine has been reported to be intermediate product or a byproduct from UV/H₂O₂ of CBZ degradation in addition to many other by-products, such as 2-aminobenzoic acid, 2-hydroxybenzoic acid, 2-hydroxyphenol, hydroxyacetic, oxalic, malonic, oxaloacetic, maleic, fumaric, succinic, tartronic, malic and tartaric (Vogna et al., 2004). Although most experiments were performed in unrealistic conditions in terms of CBZ concentration compared to usual water/wastewater, acridine/acridone may be obtained in the effluents from water/wastewater treatment plants as a by-product of UV/H₂O₂

oxidation process of CBZ in trace concentrations, but with a significant toxicity level.

Wols et al. (2013) carried out a research study on the degradation of 40 pharmaceuticals including CBZ by UV/H₂O₂ in order to investigate, first, the sensitivity of each pharmaceutical to the photolysis and to the oxidation by hydroxyl radicals. Their objective was to determine the photolytic and the oxidation degradation rate constants and, then, to figure out which one of LP and MP lamp is the most efficient as a source UV light for three types of water that differ by their matrices. Some pharmaceuticals turned out to be sensitive to the photolytic degradation from UV light of LP lamp and slightly more sensitive to the photodegradation from UV-MP lamp thanks to its supply of polychromatic radiation that has a wide range of wavelength for all three water types (MilliQ

deionized water, tap water from the city of Nieuwegein, and pre-treated water from the river Meuse) However, CBZ was found to be slightly sensitive to photolysis from MP lamp only and highly sensitive to oxidation by hydroxyl radicals for both lamps. Oxidation of CBZ was higher for the water type whose matrix did not contain hydroxyl radical scavengers. Comparing the two lamps, although the MP lamp offers a high performance, its energy efficiency was not significant; thus, the degradation of pharmaceutical products was almost similar for both types of lamps. A low sensitivity of CBZ to photolysis compared with UV/H₂O₂ was mentioned in other works (Shu et al., 2013; Vogna et al., 2004). For instance, in Shu's work, the degradation efficiency of CBZ at 25 mg/L H₂O₂ concentration was increased 100 times in comparison with direct photolysis. Shu et al. (2013) also assessed the UV dose required to degrade 50% and 90 % of CBZ by direct photolysis; the UV dose was found to be 68.9 and 231.6 J.cm⁻², respectively. Once in the presence of 25 mg/L H₂O₂, the removal of 50% and 90% of CBZ were accomplished with a UV dose of 0.68 and 2.25 J.cm⁻², respectively. Thereafter, the presence of H₂O₂ dwindled significantly the UV dose and energy consumption. In UV/H₂O₂ advanced oxidation process, the degradation of CBZ is supposed to increase with a higher UV dose and an increasing concentration of H₂O₂. However, the excess of the latter could augment the scavenging capacity of water/wastewater matrix, so that some H₂O₂ molecules react with the already generated hydroxyl radicals, thereby leading to the formation of hydroperoxyl radicals the oxidation potential of which is lower than that of hydroxyl radicals.

As a conclusion, UV/H₂O₂ treatment of CBZ culminates in satisfactory results for CBZ removal yields. However, this process should be reconsidered and more profound studies should be conducted for seeking how the formation of acridine could be avoided or in which conditions this could be directly turned into less toxic compounds. In addition, this process remains costly, as ozonation, and remains subjected to light transfer limitation, which is usually a problem more difficult to handle in the scale-up methodology than gas-liquid mass transfer in ozonation process. Applications to real wastewater or micropollutants "cocktails" also lack in the literature.

4.2.3. FENTON & PHOTO-FENTON

Fenton's oxidation relies upon Fenton's reagent, composed of a solution of hydrogen peroxide and iron catalyst. This has been widely used in the literature to destroy various contaminants contained in water/wastewater. In Fenton's oxidation, iron salts are used as catalysts to decompose hydrogen peroxide for producing free hydroxyl radicals. Ferrous ions generated from iron salt dissolution react with hydrogen peroxide leading to the simultaneous production of hydroxyl radicals and ferric ions (Eq. 2). The latter, in turn,

react with H_2O_2 , which ends as an instable intermediate compound that subsequently undergoes self-decomposition to form ferrous ions and hydroperoxyl radicals (Eq. 3-4). Thereafter, ferrous ions obtained in Eq. 4 are attacked again by hydrogen peroxide following Eq. 2 (Munter, 2001).

Fenton's oxidation offers some additional advantages compared to other AOPs. Alongside oxidation of pollutants by hydroxyl radicals, coagulation of by-products or parent pollutants takes place, which contributes to an enhanced oxidation process effectiveness. In addition, iron is an abundant and a non-toxic element and hydrogen peroxide is environmentally harmless. Under UV light, alongside the formation of hydroxyl radicals by Fenton's reagent, additional hydroxyl radicals are produced thanks to the photo-reduction of Fe^{3+} to Fe^{2+} (Eq. 5) and to the hydrolysis of $Fe(OH)^{2+}$ that may be formed around pH 3 in presence of ferric ions, as shown in Eq. 6-7 (Aguinaco et al., 2014; Munter, 2001). This particular AOP, summarized by (Eq. 5-7), is called photo-Fenton process and is of great importance in the generation of hydroxyl radicals due to the production of ferrous ions produced.



Fenton and photo-Fenton processes have been used to treat effectively a wide range of micropollutants and pharmaceuticals including CBZ (Miralles-Cuevas et al., 2013, Rodríguez-Gil et al., 2010). Fenton's oxidation and photo-Fenton's oxidation being promising processes to the water/wastewater treatment (aside from their high cost), some processes deriving from these oxidative processes have been developed with the aim to boost the amount of hydroxyl radicals produced. Mohapatra et al. (2013) made a comparative study of Fenton oxidation and Fenton oxidation combined with ultrasonication (ferro-sonication) for the degradation of CBZ in wastewater. The removal yield of CBZ by Fenton's oxidation and ferro-sonication ranged from 84 to 100% and from 62 to 93%, respectively. The high removal of CBZ by Fenton's oxidation was explained by its efficiency in producing hydroxyl radicals and intermediate compounds that were found to be epoxycarbamazepine and hydroxy-carbamazepine for all these processes. CBZ removal by Fenton process combined with ultrasonication in which zero-valent iron is used as a source of ferrous ions was investigated by Ghauch et al. (2011). This ultrasonic/ Fe^0/H_2O_2 process turned out to be efficient for CBZ abatement, especially in acidic media, even in the presence of ionic species. Protons attack iron powder, leading to the formation of ferrous cations that are much needed to turn hydrogen peroxide into hydroxyl radicals. Consequently, the complete removal of CBZ (42 μ M) was achieved at pH 3 after less than 30 min. reaction, while almost 90% CBZ abatement was reached at pH 5 after 1 hour. reaction with 100 μ L H_2O_2 additives and 200 mg Fe^0 load. Sun et al. (2013) studied the CBZ degradation by Fenton-like reaction with ferric-nitrilotriacetate

complexes. Besides the fact that nitrilotriacetate is biodegradable, the addition of nitrilotriacetate to the traditional Fenton-like process with Fe(III)/H₂O₂ provided efficient degradation of CBZ by ferric-nitrilotriacetate/H₂O₂ at an initial pH range 7–9. Upon having proposed the mechanism of carbamazepine degradation, the major degradation intermediates were identified as hydroxy-CBZ, 10,11-epoxy-CBZ, quinonoid CBZ derivatives, dihydroxy-CBZs and hydroxy-CBZ-10,11-diols. The degradation of the previous intermediates can occur due to the subsequent attack by hydroxyl radicals. Ahmed and Chiron (2014) studied CBZ removal from wastewater by solar photo-Fenton by using persulphate anions as an oxidant instead of hydrogen peroxide. Under UV-Visible irradiation from solar energy or by reaction with ferrous cations, persulphate anions turned into sulphate radicals and the resulting ferric cations underwent photoreduction to produce hydroxyl radicals. As a result, CBZ was first oxidized by sulphate radicals and, subsequently, by hydroxyl radicals. In wastewater, CBZ was fully degraded in 30 min. for an initial CBZ concentration of 50 µM with an optimal persulphate/Fe(II) molar ratio of 2 and, finally, a complete CBZ mineralization was achieved with a molar ratio of persulphate over CBZ of 4.

As a conclusion, Fenton and in particular photo-Fenton process have been limited for a long time by the small range of pH, typically 3–5, in which they are efficient. They also suffer from their high cost as all the AOPs. In addition, a limited degradation of CBZ is reported in comparison to ozonation, with the advantage over UV/H₂O₂ process that highly toxic by-products have never been found in the literature. For the recent most developments, validation on real wastewater is still to develop.

4.2.4. HETEROGENEOUS PHOTOCATALYTIC PROCESSES

Heterogeneous photocatalytic processes consist of the generation of conduction band electrons and valence band holes on the surface of an appropriate semiconductor, usually titanium dioxide TiO₂ under UV irradiation. In turn, on the one hand, valence band holes can react with water and hydroxide ion to produce hydroxyl ions, and on the other hand, conduction band electrons react with adsorbed molecular oxygen, reducing it to superoxide radical anion (Munter, 2001). Thanks to hydroxyl radical and oxygen radical species, TiO₂ photocatalysis has been used to remove the micropollutants, especially pharmaceuticals including CBZ from water and wastewater (Rizzo et al., 2009, Sarkar et al., 2015). The degradation of CBZ by TiO₂ photocatalysis can be complete for wastewater/water containing that solute at lower level concentration. For instance, in the work conducted by Mohapatra et al. (2014b), the photocatalytic degradation of CBZ in wastewater was investigated using whey-stabilized TiO₂ and ZnO nanoparticles with 55 minutes of irradiation time by UV light. The photocatalytic degradation efficiency of CBZ turned out to be 100% and 92%, respectively, for an initial concentration of 295 ng/L. The main final by-products obtained were identified as epoxy-carbamazepine and hydroxy-carbamazepine (Mohapatra et al., 2014b). Even with a high CBZ concentration, considerable photocatalytic degradation efficiency could be attained; this was evidenced by photocatalytic treatments applied at high CBZ concentration (5 mg/L) on a synthetic hospital wastewater using TiO₂ nanofibers: 78% removal yield was achieved for CBZ using a photocatalytic treatment time of 4 hours (Chong and Jin, 2012).

As a conclusion, heterogeneous photocatalytic processes seem effective for CBZ removal. They suffer, however, from the same drawbacks as UV/H₂O₂, Fenton, photo-

Fenton and ozonation processes in terms of operating cost and the same limitations as UV/H₂O₂ and photo-Fenton in terms of light transfer limitation. In comparison to ozonation, the degradation of the CBZ molecule is also more limited, for example to epoxycarbamazepine, with the advantage in comparison to the UV/H₂O₂ process that more toxic by-products, such as acridine, have never been reported in the literature. The biodegradability of these by-products must, however, still be better assessed before concluding definitively on the applicability of heterogeneous photocatalytic processes for CBZ elimination. The same stands for applications involving real water/wastewater.

4.3 ADSORPTION OF CBZ

Adsorption is a low-cost separation process widely used in water treatment. The most common application involves the removal of organic pollutants using activated carbon as the adsorbent, but adsorbents can be found for almost all the existing pollutants, including inorganic anions and cations. A limitation of adsorption is that it only displaces pollution, especially if a costly adsorbent is used, which implies that regeneration is compulsory. However, regeneration, especially thermal regeneration may be an expensive process. In practice, many solid phases can be used as low-cost adsorbents. First, various organic waste can be a resource to prepare activated carbons, but some of them can also be used directly as adsorbents. Similarly, clays are usually good candidates. Nevertheless, CBZ is a particular organic compound that is known to be neither purely hydrophobic, nor hydrophilic, which explains its low, but non-negligible solubility in water. Consequently, CBZ usually adsorbs weakly on conventional adsorbents, including activated carbons (Rivera-Utrilla, 2013).

Recently, two commercial activated carbons, coconut shell- and wood-based, were chosen to evaluate the mechanisms of CBZ and sulfamethoxazole (SMX) adsorption from a low (ppm level) concentration of these pharmaceuticals. It was found that not only porosity, but also surface chemistry plays an important role in the adsorption process. The results show that extensive surface reactions take place during adsorption and adsorbates undergo significant transformations in the pore system. The ability of carbon surfaces to form superoxide ions results in the oxidation of CBZ and SMX and in their partial decomposition. Surface chemistry also promotes dimerization of the latter species. Moreover, functional groups of CBZ and SMX, mainly amines, react with oxygen groups of the carbon surface. Thus, not only microporous carbons with sizes of pores similar to those of adsorbate molecules, but the carbons with large pores, rich in oxygen groups, can efficiently remove these pharmaceuticals following the reactive adsorption mechanism (Nielsen et al., 2014). Alternative activated carbons include the carbonization of sewage sludge and fish waste, which is a promising technology leading to a feasible way of waste elimination/recycling. The produced adsorbents were shown to be successful in removing CBZ from an aqueous solution, most likely as a result of the favorable surface chemistry. Trials with varied carbonization temperatures indicated that higher temperatures yielded more effective adsorbents of CBZ. Varying the proportion of fish waste to sewage sludge content did not show a clear trend; however, the addition of a small amount of fish waste led to an improved performance of the adsorbent with a clearly visible synergistic effect. The high adsorption capacity of this particular material was linked to a favorable combination of a highly dispersed polar inorganic phase being able to interact with CBZ through polar, acid-based interactions and complexation, and to the presence of the carbon phase which provided hydrophobicity in micropores (Nielsen et al., 2015).

As an alternative to activated carbons, fixed-beds of transition metal (Co^{2+} , Ni^{2+} or Cu^{2+}) inorganic–organic pillared clays were tested to study single- and multi-component non-equilibrium adsorption of a set of pharmaceutical and personal care products (PPCPs: salicylic acid, clofibric acid, CBZ and caffeine) from water (Cabrera-Lafaurie et al., 2015). Multi-component adsorption tests showed a considerable decrease in adsorption capacity for the acids and an unusual selectivity toward CBZ depending on the transition metal. This was attributed to a combination of competition between PPCPs for adsorption sites, adsorbate–adsorbate interactions, and plausible pore blocking caused by CBZ. It was also found that the adsorption of pharmaceutical and personal care products, such as salicylic acid and CBZ from water, using Na^+ -Y zeolites modified with extra-framework transition metal cations and a surfactant was significantly influenced by the pH of the solution, the type of modification (transition metal with and without surfactant), and the physical-chemical properties of the adsorbate. Simultaneous incorporation of both a transition metal (Cu^{2+} , Co^{2+} or Ni^{2+}) and a cetylpyridinium (CPY+) cation increased the equilibrium salicylic acid adsorption capacities considerably, especially in the pH range 6–11. A better selectivity toward salicylic acid over CBZ was clear in all cases, but the uptake capacity for the latter also varied depending on the modification of the zeolite. In addition to that, fixed bed adsorption studies performed for single- and multi-component PPCP feeds on (Cu^{2+} , CPY+)-Y at pH about 6 and ambient temperature indicated that the adsorption process is not limited by the diffusion of the salicylic acid and that most of the bed depth is used efficiently (*i.e.*, with negligible dispersion problems). The multi-component adsorbate tests also revealed a considerable increase in adsorption capacity toward salicylic acid, highlighting once more the weak adsorption of CBZ in comparison to other PPCPs (Cabrera-Lafaurie et al., 2014).

As a conclusion, adsorption process is a possible way to remove CBZ. Despite its weak adsorption properties, several solid phases including zeolites and activated carbons able to adsorb CBZ have been reported in the literature. However, competitive adsorption tests are still necessary, as CBZ adsorption may be impaired at low concentration by the presence of other compounds for which adsorption is more favorable.

5. ANALYTICAL TECHNIQUES FOR CBZ IDENTIFICATION AND QUANTIFICATION

In this section, the techniques devoted to the analysis of pharmaceutical micropollutants in general and CBZ in particular will be described. Even though this is not the objective of this Ph.D., analysis is a necessary step to investigate the occurrence of CBZ and the efficiency of water treatments. The presence of CBZ at trace concentrations in the different environmental sites makes its quantification very complex and complicated. The advanced analytical techniques based on the coupling of chromatography to mass spectrometry have been used and turned out to be efficiently able to analyze CBZ qualitatively and quantitatively down to very low concentration of ng/L (Fatta et al., 2007). Gas/liquid chromatography are separation techniques that are composed of ionisation source, detection system and analyser; mass spectrometry is an analytical method whereby the ratios mass/charge (noted m/z) of ionised molecules and products from fragmentation are measured, thereby allowing the structural study, identification and quantification of the analytes of interest. Mass spectroscopy combines the advantages to provide a universal and highly sensitive detection system for chromatography owing to which the detection and the identification of organic micropollutants has become

feasible. For CBZ for example, the interesting methods can be made up of gas chromatography coupled either to mass spectrometry (GC-MS) or to tandem mass spectrometry (GC-MS/MS) and liquid chromatography coupled either to mass spectrometry (LC-MS) or to tandem mass spectrometry (LC-MS/MS).

5.1. SAMPLE PREPARATION, EXTRACTION AND CLEAN-UP OF CBZ

The efficiency of the aforementioned diverse analytical methods depends on the samples collection, irreproachable sample preparation to the extraction and clean-up or purification of a target compound, as could be delineated in the Figure 1 (Kostopoulou and Nikolaou, 2008; Fatta et al., 2007). The clean-up step consists of preconcentrating CBZ and separating the other compounds that may interfere with it throughout its trace level quantification, which leads to an enhancement of the accuracy and the reproducibility of results. For water and wastewater samples, after filtration ensued by acidification (Bayen et al., 2013; Yuan et al., 2014) or by the addition of complexing agents, such as Na₂EDTA (Yuan et al., 2014), the enrichment of samples in CBZ can be carried out by several methods, such as liquid-liquid extraction LLE (Queiroz et al., 2008), (automated) solid phase extraction SPE (Lacina et al., 2013) and solid-phase micro-extraction SPME (Moeder et al., 2000). For the sewage sludge, wastewater sludge, biosolid, sediment and soil, after the samples have undergone freeze drying, CBZ can be and highly sensitive extracted by soxhlet extraction SE (Noeon et al., 2009), microwave-assisted extraction MAE (Mohapatra et al., 2012), ultrasonic solvent extraction USE (Ternes et al., 2005; Mohapatra et al., 2012), the QuEChERS method (acronymic name for quick, easy, cheap, effective, rugged and safe) (Cerqueira et al., 2014) or by pressured liquid extraction PLE (Ferreira da Silva et al., 2011; Chen et al., 2013; Miao et al., 2005) also called accelerated solvent extraction ASE (Mohapatra et al., 2012). Upon the extraction of CBZ, the resulting aqueous samples may be subjected to the clean-up and preconcentration by one of the several methods mentioned above, but most of the time by SPE (Ferreira da Silva et al., 2011; Mohapatra et al., 2012; Chen et al., 2013). Upon extraction of analytes, the extract obtained may also undergo centrifugation or evaporation prior to the clean-up step (Mohapatra et al., 2012). PLE or ASE (Mohapatra et al., 2012) and SPE (Fatta et al., 2007; Kostopoulou and Nikolaou, 2008) as extraction method and enrichment methods, respectively, have gained more popularity thanks to various advantages they offer in comparison with other methods. PLE is performed at high temperature and pressure in the range of 40–200°C and 65–170 bars, respectively (Mohapatra et al., 2014b), but mostly often at 100 bars (Miao et al., 2005; Ferreira da Silva et al., 2011; Chen et al., 2013). Higher temperatures permit to solubilise considerably the analyte, to enhance the speed of elution and to shorten the extraction time. However, higher temperatures may as well allow the extraction of unwanted interfering matrices compounds and could lead to the degradation of a thermally labile analyte as CBZ (Miao et al., 2005). The efficiency of concentration of CBZ depends on the stationary phase and the elution solvent. The samples are passed through the stationary phase and the latter adsorbs the analyte while the impurities are washed. Finally, CBZ is eluted in the solvent passing through the stationary phase (Kostopoulou and Nikolaou, 2008). Hydrophilic–lipophilic balance cartridges are the stationary phase the most widely used for the preconcentration and purification of CBZ thanks to its high efficiency (Azzouz et al., 2010). Besides being a rapid and sensitive method, SPE has an additional advantage of its practical automation (Azzouz et al., 2010). The purification of CBZ by automated SPE offers diverse advantages summed up below (Trenholm et al., 2009; Azzouz et al., 2010):

- The use of a continuous system avoids the error linked to manipulation;
- The volume miniaturization of the samples (1-2.5 mL instead of 25 mL) can be achieved, so that requirements in elution solvent and other reagents, such as the derivatizing reagents, are minimized;
- An increase in reliability and sensitivity results in an increase of detection limit in the low ng/L range.

5.2. GC-MS, GC-MS/MS, LC-MS AND LC-MS/MS

GC-MS and GC-MS/MS constitute one of the analytical methods to analyse the organic pollutants at trace level. However, trace compounds about a few ng/L could not be detected without the above-mentioned sample preparation procedures. GC which is the separation technique based on the volatility of the organic components thermally stable, is performed using capillary columns types and helium as carrier gas for the separation of CBZ prior MS (Gómez et al., 2007; Azzouz et al., 2010). To optimise the efficiency of detection, two-dimensional gas chromatography, so-called tandem mass spectrometry MS/MS, is often used after both GC and LC. The derivatization of CBZ takes place to enhance its volatility prior to GC/MS in order to improve chromatographic behaviour/separation and the mass spectrometric selectivity (Moeder et al., 2000; Azzouz et al., 2010; Lacina et al., 2013). The derivatization is generally recommended for the acid compounds (Öllers et al., 2001; Sacher et al., 2001) and may have some drawbacks, such as the incomplete derivatization that leads to underestimation of target analyte and the use of more toxic or carcinogenic reagents (Öllers et al., 2001). CBZ being a neutral pharmaceutical, many researchers preferred to perform the quantification of CBZ without the derivatization step (Öllers et al., 2001; Sacher et al., 2001, Ternes et al., 2001). It has been shown that CBZ could be prone to thermal degradation after injection in GC-MS that results in iminostilbene formation (Ternes et al., 2001). However, in the subsequent studies, the thermal degradation turned out to be minor and the reproducible results were obtained with a lower variability (Gómez et al., 2007; Togola et al., 2008).

To date, the most highly-convincing results have been obtained for the trace-level concentration of CBZ and other pharmaceuticals by LC-MS and LC-MS/MS. Those advanced analytical methods have been performed using preferably the C18 columns type in which the organic mobile phases based on methanol or acetonitrile with the formic acid or ammonium acetate as the additive agents are used to separate CBZ for conventional LC (Chen et al., 2013; Bahlmann et al., 2014; Cerqueira et al., 2014), LC-MS and LC-MS/MS. Diverse ionisation sources such as electrospray ionisation (Chen et al., 2013) atmospheric-pressure ionization, atmospheric pressure chemical ionization and different analysers, such as quadrupole time of flight MS (Li et al., 2014), quadrupole linear ion trap MS (López-Serna et al., 2010) and triple quadrupole MS/MS (Loos et al., 2010, Yuan et al., 2013) have been used. The quantification of CBZ by LC coupled to MS or MS/MS is becoming an emerging analytical method due to the thermal instability and the low volatility of CBZ, which makes this latter an ideal candidate for LC separation, in addition to drawbacks of derivatization mentioned earlier prior to GC separation. Ternes et al. (2001) concluded that LC-ESI-MS/MS was the most efficient alternative method to GC-MS for the quantification of CBZ for the aqueous samples. Even though the trace-level determination of CBZ could be performed with GC-MS and GC-MS/MS generally without or with derivatization, LC-MS or LC-MS/MS is being preferred especially for CBZ and generally for other polar pharmaceuticals. This is confirmed in several works related to environment monitoring of the emerging organic

contaminants where both GC-MS/MS and LC-MS/MS were used and in which the quantification of CBZ was finally carried out using LC-MS/MS (Trenholm et al., 2006; Robles-Molina et al., 2014;).

As a conclusion, the recent progress in chromatographic techniques, in particular LC-MS/MS and sample preconcentration methods has considerably improved the detection and the quantification of organic micropollutants in water/wastewater, especially CBZ which is considered as a witness micropollutant. Even though the accumulation of organic micropollutants deriving from home care products or pharmaceuticals is clearly proved, their number in the environment will go on increasing also because of the continuous progress of the analytical tools able to detect them.

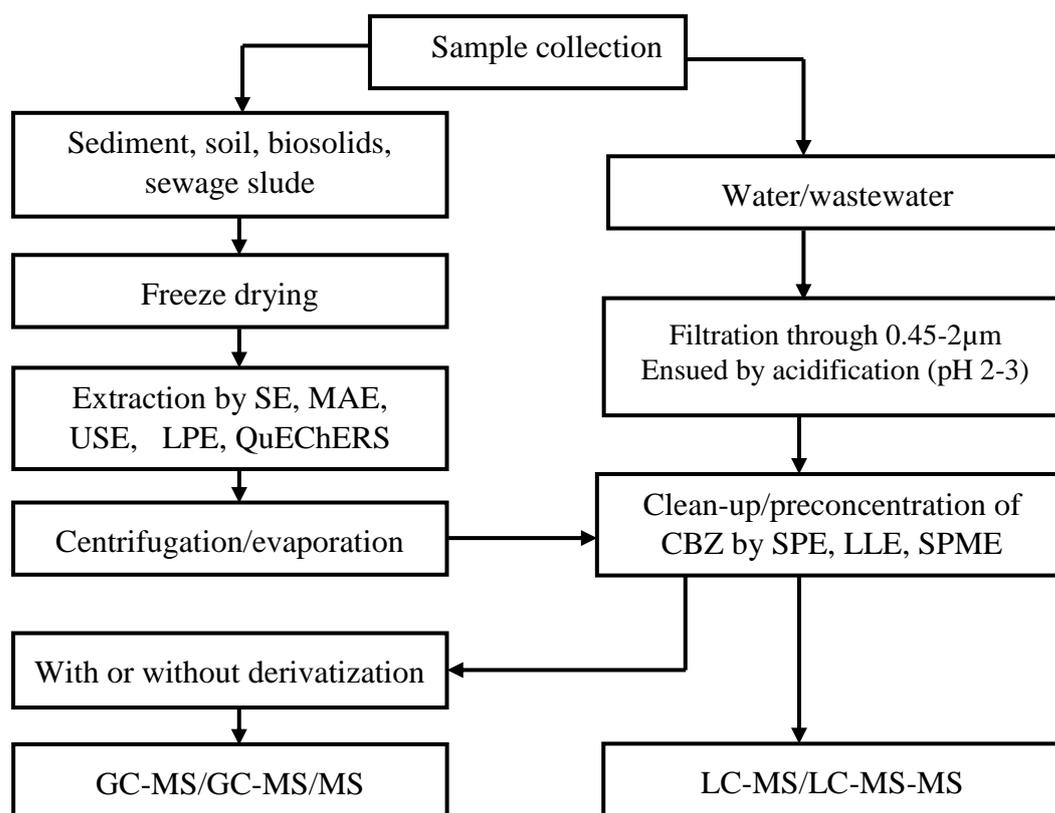


Figure 1: Diagram displaying the main steps involved for the trace-level determination of CBZ by GC-MS, GC-MS/MS, LC-MS and LC-MS/MS.

6. CONCLUSION

In this chapter, we have shown that carbamazepine excessive usage renders it a potential hazardous pollutant in the aquatic environment. After administration, CBZ and its metabolites are found in the urine and faeces which are finally destined to the water bodies. Moreover, the direct disposal of CBZ in the hospital wastewater further augments its content and its metabolites concentrations in aquatic ecosystem. CBZ is also a highly biorefractory molecule, which means that it is persistent in the environment and that only 10% CBZ removal is achieved in conventional WWTPs. When larger values are observed, biosorption on sludge and, therefore, a direct access to soil pollution may sometimes be suspected. In addition, the analytical tools able to detect CBZ even at trace concentrations in water and soils are now clearly established and available. As a result,

WWTPs necessitate improved or innovative treatment methods to address CBZ removal and, more generally, that of all the persistent micropollutants. The aim is, therefore, to find a cost-effective and efficient process capable of completely eliminating the CBZ from water without producing non-biodegradable metabolites and, in the worst case, toxic by-products. In this chapter, alternative biological treatments, adsorption and Advanced Oxidation Processes (AOPs) have been described. AOPs correspond to various technologies among which ozonation is, at the moment, the only one applied to treat real wastewater effluents at the industrial scale. Adsorption stays limited due to pollution displacement. Alternative bioprocesses mainly address the bioaugmentation of the activated sludge or the use of an alternative biological step in WWTPs.

In this work, we will study, in the next chapters, the second pathway using phycoremediation, *i.e.* a biological treatment involving algae/microalgae as the microorganism. This is an original approach that has been studied extensively in the literature for CBZ removal. However, before the analysis of this biological process, we will investigate first a physicochemical process an electrochemical process that has not been described in this chapter: electrocoagulation (EC). EC presents the advantage to combine an oxidoreduction process with an adsorption process. This will be the aim of the next chapter. The advantages expected over the above-mentioned AOPs are a reduced-cost in comparison to all the AOPs and the absence of toxic by-products.

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CHAPTER V: REMOVAL OF CARBAMAZEPINE BY ELECTROCOAGULATION: INVESTIGATION OF SOME KEY OPERATIONAL PARAMETERS

This article is published online on March 2015 in The Environmental Engineering and Management Journal. Consequently, it follows the guidelines of this journal.

Tania Yehya, Lidia Favier, Yassine Kadmi, Fabrice Audonnet, Nidal Fayad, Maria Gavrilescu, Christophe Vial, 2015. The Environmental Engineering and Management Journal, Vol.14, No. 3, 639-645.

ABSTRACT

The performance of electrocoagulation (EC) process, a non-specific electrochemical technology, was investigated for the removal of carbamazepine (CBZ), an antiepileptic drug, from water. Experiments were carried out in synthetic wastewater in a batch cell. The respective influences of some key process parameters were studied, such as mixing conditions, initial pH, and current on aluminium electrodes. Experimental results showed that a CBZ removal efficiency of 62% was observed under slightly acidic initial conditions (pH 4) with a current density as high as 44 mA cm^{-2} ($I=4.5 \text{ A}$) using Al electrode. This clearly indicates that CBZ removal proceeds through an electrochemical mechanism, while the adsorption of CBZ onto the aluminum hydroxide flocs was shown to be negligible. Furthermore, the increase of initial pH to alkaline values was shown to decrease the drug elimination efficiency. Conversely, as expected, an increase of current intensity improved the removal of CBZ. As a result, low initial pH 4 coupled with high current elevates the electrochemical elimination of CBZ: in this case, one metabolite could also be detected.

1. INTRODUCTION

Water resources are contaminated when pollutants are directly or indirectly discharged into wastewater without adequate treatment to remove harmful compounds. This provides a serious threat to human health on one side, and to plants and organisms living in these bodies of water on the other side (Aziz et al., 2010; Caliman et al., 2002). Water contamination has been caused over decades by a number of natural and anthropogenic pollutions, such as the spillage of pesticides and herbicides in agriculture, hospital discharges, industrial discharges, for example, industries involving fuels, wood preserving operations and textile production. This leads to the presence of rather different types of pollutions, such as organic products which derive for example from agro-food waste, home and personal care products, textile dyes, pharmaceuticals, but may also correspond to heavy metals cations or oxianions, or to inorganic anions, in particular sulfide, fluoride, and nitrate.

Pharmaceuticals and their bioactive metabolites are continuously introduced in the aquatic environment, where they are detected at trace concentrations (*i.e.* found in the ng L^{-1} or $\mu\text{g L}^{-1}$ range, so that they are often referred to as “micropollutants”), and become pseudo-persistent (Caliman and Gavrilescu, 2009; Gavrilescu et al., 2015; Semrany et al., 2012; Sirés et al., 2012). First, most pharmaceuticals are not completely degraded after ingestion and they may be excreted directly or also produce secondary pollutants, *i.e.* metabolites and subsequently enter and harm the aquatic ecosystem. This results in the detection of pharmaceutically active compounds, such as lipid regulating drugs, analgesics, antibiotics, antiseptics, antidiabetics, barbiturates, beta-agonists, psychiatrics, receptor antagonists,

hormones, and chemotherapy and beta-blocking heart drugs in wastewaters, streams, and ground-water resources. Their occurrence in the environment is mainly due to:

1. the excretion of the fraction of pharmaceuticals that are not metabolized by human or animal bodies into wastewater, or their metabolites;
2. the discharge of unused or expired medications;
3. the discharge of hospital wastewater;
4. the residues from pharmaceutical manufacturing.

Carbamazepine (CBZ), commercialized as *Tegretol* (Zhang et al., 2008), is a pharmaceutical imminostilbene derivative, and a lipophilic, neutral tricyclic compound (Atkins et al., 2013; Bahlmann et al., 2014). It is mainly used as an anticonvulsant drug, and also as a specific analgesic for trigeminal neuralgia (Popa et al., 2014; Rao et al., 2010). Its efficacy and safety profiles have made it first choice for adults. It is administered chronically in high dosages of 100-2000 mg daily and, hence, its annual production is high (Kosjek et al., 2009). Approximately 72% of orally administered carbamazepine is absorbed, while 28% is unchanged and subsequently discharged through the faeces (Zhang et al., 2008). Environmental studies confirm the presence of CBZ as one of the most frequently detected pharmaceuticals in the effluents of sewage treatment plants, in river and sea water (Miao et al., 2005), in comparison to the other pharmaceutical micropollutants in Europe, America and Asia. For instance, the presence of CBZ has been reported at concentrations about $6.3 \mu\text{g L}^{-1}$ in wastewater, $1.1 \mu\text{g L}^{-1}$ in surface water, and 30 ng L^{-1} in drinking water (Mohapatra et al., 2014) in Canada. It has also been detected about 2300 ng L^{-1} in Canada in a wastewater effluent and about 258 ng L^{-1} in the USA, but below 10 ng L^{-1} in Germany (Metcalf et al., 2003). As a consequence, health-based guidance values have been established for CBZ upon fishery products consumption in both marine and freshwater, such as $2000 \mu\text{g/kg}$ biota or $130 \mu\text{g.L}^{-1}$.

Due to the persistence and toxic effects of this molecule, various water remediation technologies have been investigated to remove CBZ from wastewater and drinking water, including conventional biological and physicochemical treatments, but also advanced oxidation and biological processes. Several studies showed that, the abatement yield of CBZ by the conventional activated sludge process is limited (typically below 10%) due to its high resistance to biodegradation, independent from hydraulic retention times (Hata et al., 2010). Other studies have investigated the removal efficiency of this molecule by white-rot fungi. A CBZ elimination yield of about 60% was obtained with fungal laccase, an enzyme from *Trametes versicolor* after 48h of treatment (Hata et al., 2010), while less than 10% CBZ elimination were achieved after treatment with membrane bioreactors using *Pseudomonas sp.* (Li et al., 2013). The efficiency of physicochemical treatments, such as coagulation and flocculation/flotation, was also investigated and these did not operate rather better than biological treatments, with typical yields of CBZ elimination from 20% to 35% (Carballa et al., 2005; Suarez et al., 2008). Conversely, many contributions from the literature showed that ozonation and advanced oxidation processes (AOPs) including Fenton, photo-Fenton and heterogeneous photocatalysis could be more efficient for the removal of this molecule from wastewater. For example, ozonation was found to remove up to 99% CBZ (Hua et al., 2006); UV/hydrogen peroxide in the presence of 25 mg.L^{-1} of H_2O_2 promoted the elimination of 90% CBZ at 2.25 J.cm^{-2} UV dose (Shu et al., 2013); Fenton and photo-Fenton process could possibly achieve a complete elimination of CBZ by Fenton oxidation (Mohapatra et al., 2013), and heterogeneous photocatalytic processes with more than 90% elimination (Doll and Frimmel, 2005; Martínez et al., 2011). However, AOPs are highly expensive for wastewater treatment (Betianu et al., 2008; Sirés et al., 2012). Other cheaper treatments such as electrodeposition, electrocoagulation, electroflotation, electrodisinfection, electrooxidation,

and electroreduction are important alternatives for wastewater treatment, due to their high efficiency in pollution abatement, easy operation, and compact facilities (Al-Shannag et al., 2014; Behbahani et al., 2013).

The objective of this paper is to investigate the potential applicability of electrocoagulation process (EC), an electrochemical treatment, as a possible way to remove CBZ from water and wastewater. Up to now, electrochemical methods have been disregarded in the literature for the removal of CBZ. This paper will also analyze how CBZ removal is affected by mixing conditions, pH, and current intensity which is the major process parameter of EC.

2. EXPERIMENTAL

In this study, EC was applied to investigate CBZ removal from synthetic water in which the initial concentration C_0 of CBZ is 12.5 mg L^{-1} . All solutions were prepared with carbamazepine of analytical purity (99%) supplied by Sigma-Aldrich (France). The composition of the synthetic water includes also KCl (6.33 g L^{-1}) as a supporting electrolyte. The initial conductivity of water is 2.8 mS cm^{-1} and pH is 8.2. Initial pH is then adjusted between 4 and 9 by the minute addition of either 0.1 M hydrochloric acid or sodium hydroxide solutions.

For EC process, two rectangular aluminum electrodes were used as the anode and the cathode, of surface area $S=102 \text{ cm}^2$ each, with an inter-electrode distance of 1 cm. EC consists of the controlled electrodisolution of the anodic material, as shown by Eq. (1).



At the cathode, hydrogen gas is released through the electroreduction of water (Eq. 2).



When pH is between 4 and 10, aluminium cations rapidly form insoluble oxyhydroxides and hydroxides, which readily precipitate and form flocs. This is usually summarized as given by Eq. (3).



As a result, several mechanisms can promote pollution removal, among which:

1. the coagulation of colloids or slightly soluble species;
2. the adsorption of pollutants onto the flocs;
3. the electrooxidation or electroreduction of the pollutants onto the electrodes.

To enhance these mechanisms, the EC cell consisted of a batch reactor of volume $V=4.0 \text{ L}$, mechanically stirred using a standard Rushton turbine. Tests were carried out in an intensiostatic mode by means of a BK-Precision (USA) generator with a current (I) ranging between 1.5A and 4.5A. The electrolysis time of each run ranged between 30 and 120 minutes. The respective effects of mixing speed (from 100 to 400 rpm), current, and initial pH (pH_i) were investigated. Experiments were done at room temperature under atmospheric pressure. Analytical tools can be summarized as follows. The conductivity and the pH of the solution were recorded online. The concentration of soluble CBZ at time t (C_t) was obtained using a sampling procedure, followed by HPLC analysis (*Waters 2410, UV*, France) under isocratic mode using a C18 column (*Waters SAS, Symmetry*, France). The mobile phase

consisted of a solution of acetonitrile (*Sigma-Aldrich*, France) and ultra-pure water at 30:70 (v/v). The flow rate was $0.5 \text{ mL}\cdot\text{min}^{-1}$, leading to a retention time of 20 min for CBZ when detected at a wavelength of 230 nm. Total organic carbon in the liquid phase was measured using a total organic carbon analyzer (TOC-V CSN, Shimadzu, Japan). At the end of EC experiment, the flocs recovered by decantation or flotation were filtered, washed, and dried at 105°C overnight before being weighted. BET surface area of the flocs was then estimated using nitrogen adsorption (*Tristar II, Micromeritics Instr.*, USA). To detect the presence of adsorbed species on the dried solid, the solid phase was dissolved using a 0.1 N HCl solution and, then, subjected to chemical analysis using the total organic carbon analyzer and the HPLC described above. Fig. 1 summarizes the experimental setup coupled with analytical tools.

3. RESULTS AND DISCUSSION

3.1. INFLUENCE OF MIXING AND INITIAL PH USING AL ELECTRODES

Preliminary results were devoted to the analysis of the influence of mixing conditions. The rotation speed of the Rushton turbine was varied between 100 and 400 rpm and its potential influence on CBZ removal was investigated. Results showed that this parameter had a limited effect in the studied range on the CBZ removal. This means that regardless of the mechanism of depollution (oxidoreduction at the electrodes or adsorption onto the flocs), there is no apparent limitation due to mass transfer in the EC process which is in accordance with other EC studies done on wastewater containing nitrate (Yehya et al., 2014) or for the removal of trivalent chromium (Golder, 2006).

This is of utmost importance because both oxidoreduction and adsorption may be controlled by mass transfer. For this work, a rotation speed of 100 rpm has been finally retained for the Rushton turbine: this presents not only the advantage to prevent swirl, but also reduces the power input for mixing purpose. Unlike the effect of the stirring rate which can be easily overcome, pH is always the key parameter affecting the elimination of pollutants by EC both in terms of effectiveness and operating cost (Chafi et al., 2011). In a batch cell, pH varies with time and only the initial pH, pH_i , can be controlled.

Accordingly, experimental data highlighted a strong influence of pH_i on the abatement of CBZ over time during EC.

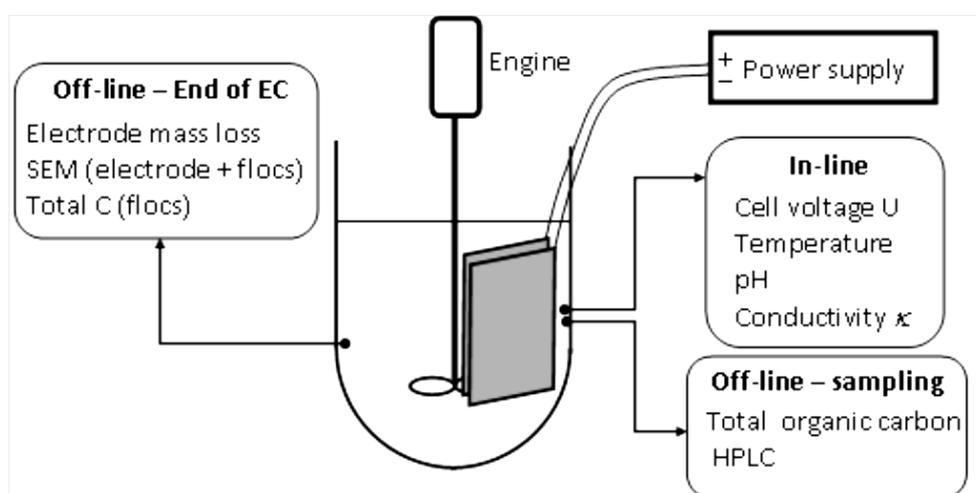


Figure 1 : Experimental setup.

Concerning CBZ, it is known to have two pKa values of 13.9 and -0.49; this means that it is out of our pH range (4 to 9), and that it would be always chemically stable. However, the results obtained have shown a different behavior than expected. Fig. 2 highlighted a poor removal yield of CBZ when pH_i was 6 or 9, with values lower than 10% after 120 min of electrolysis, with a slightly higher yield when pH_i was 6. Conversely, CBZ was shown to be removed more efficiently when pH_i corresponded to acidic pH values, *i.e.* when pH_i was 4. Under these conditions the eliminated CBZ amount was up to 62%.

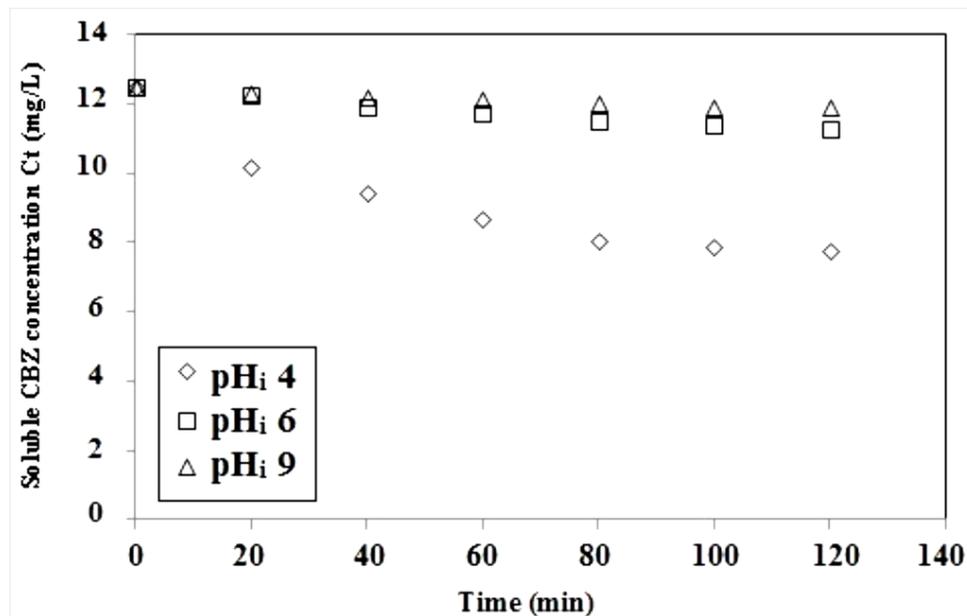


Figure 2: Effect of initial pH during EC on the abatement of CBZ over time at $I=3A$.

As pH increases during the EC process, the elimination rate of CBZ decreases, this means that CBZ removal mainly occurs when pH is between 4 and 6. This is clearly confirmed by Fig. 3: a sharp decrease of CBZ content is observed within the first five minutes, when pH varies rapidly from 4 to 6. Then, CBZ concentration passes through a plateau region when pH varies from 6 to 8.6, and then decreases again, but far slower when pH is equal or higher than 9.

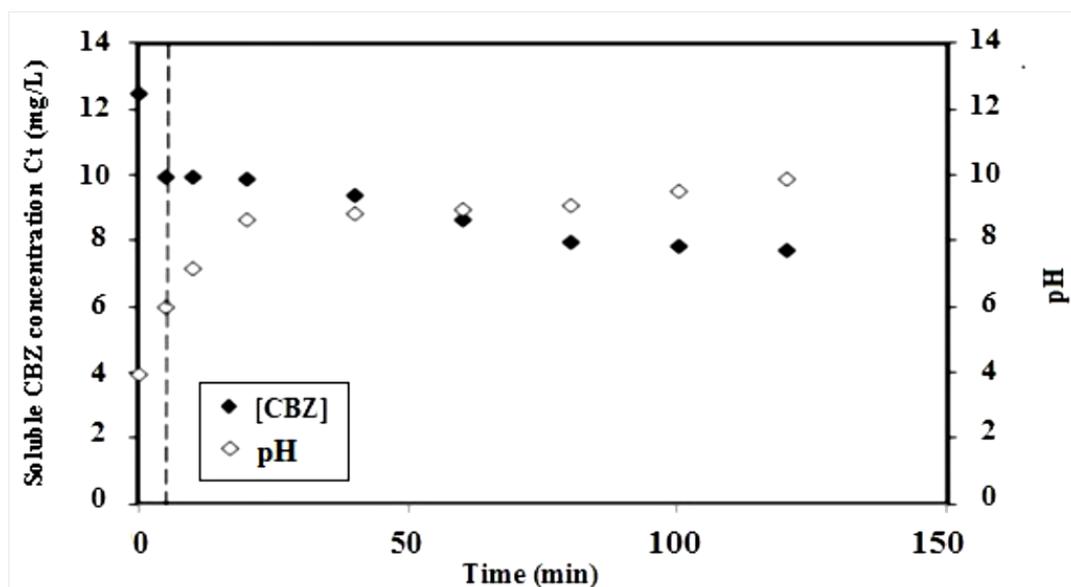


Figure 3: Effect of the change of pH during EC on the abatement of CBZ at $I=3A$ and pH_i 4, as a function of time.

Similar changes of pH can be observed at different current values, as shown in Fig. 4 at $I=4.5A$. This figure also shows that final pH values are close, regardless of pH_i after 120 min, which means that final pH cannot be correlated to CBZ removal yield.

Moreover, it was observed that, initial pH also affected the amount of flocs formed at the end of EC. This varied from 7.3, 9.8 and 6.1 g in the conditions of Fig. 4 when the initial pH increased from 4, 6 and 9 after 120 min of electrolysis, respectively.

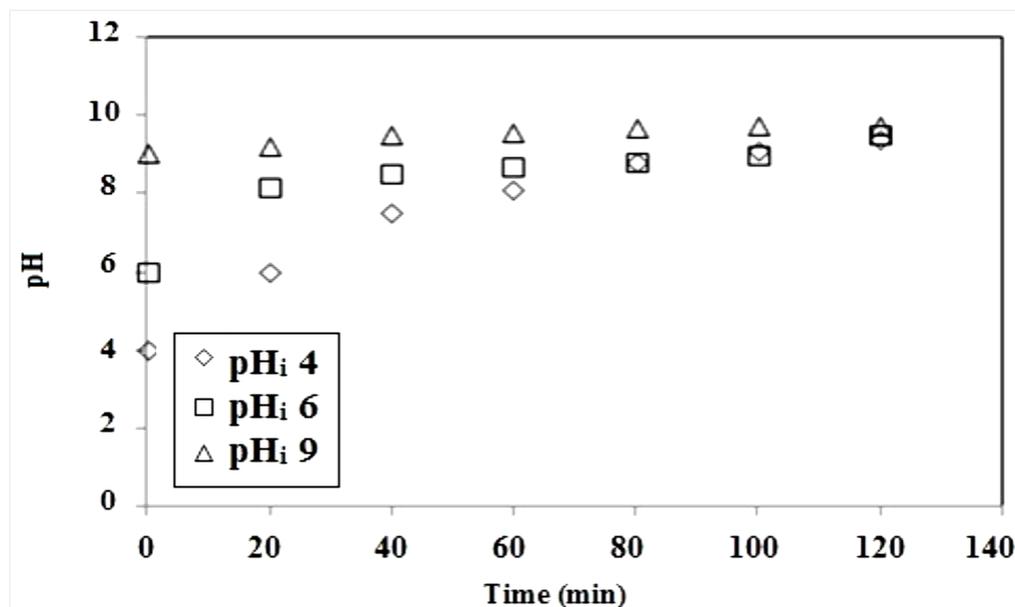


Figure 4: Effect of initial pH on the change of pH over time during EC at $I=4.5A$

These results agree with the speciation of aluminium: soluble Al^{3+} cations dominate at low pH, soluble aluminate anions $Al(OH)_4^-$ prevail at a pH higher than 10 and the insoluble $Al(OH)_3$ hydroxides reign at intermediate pH. As a result, initial pH equal to 6 maximizes the mass of flocs because their formation is impaired only at the end of EC, while it is reduced at the beginning and the end when pH_i is 4 and during a large part of the electrolysis time when pH_i is 9. However, as for the final pH, no correlation could be found between the mass of flocs and CBZ removal yield.

This indicates that adsorption is unlikely to be the mechanism governing the CBZ elimination. On the contrary, HPLC highlighted the presence of a metabolite that was detected at the same wavelength as CBZ, but with a far smaller retention time (3 min).

As there was no other organic compound in the synthetic water, this could only derive from CBZ. In addition, the increase in concentration of this new compound was always observed in parallel with a decrease of the CBZ concentration. As a result, its content was maximized at the end of electrolysis and was observed mainly when pH_i was 4.

A typical evolution of the metabolite production with time can be seen in Fig. 5 in which the areas of the detected peaks are compared because this compound has not been identified yet. This confirms the idea that an oxidoreduction mechanism at the electrode surface is responsible for CBZ removal.

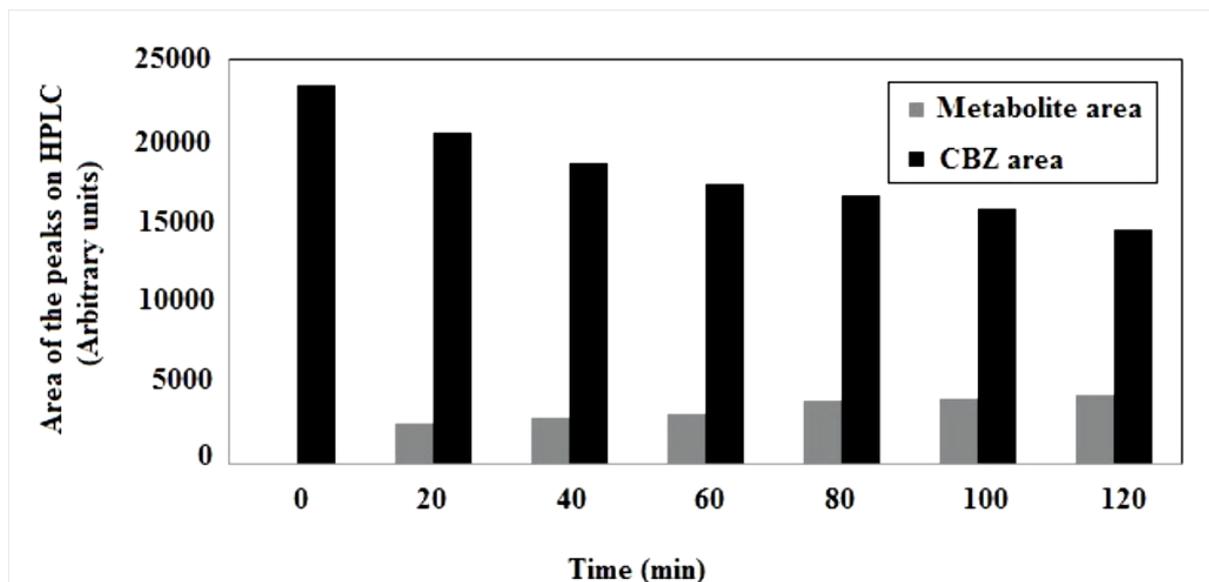
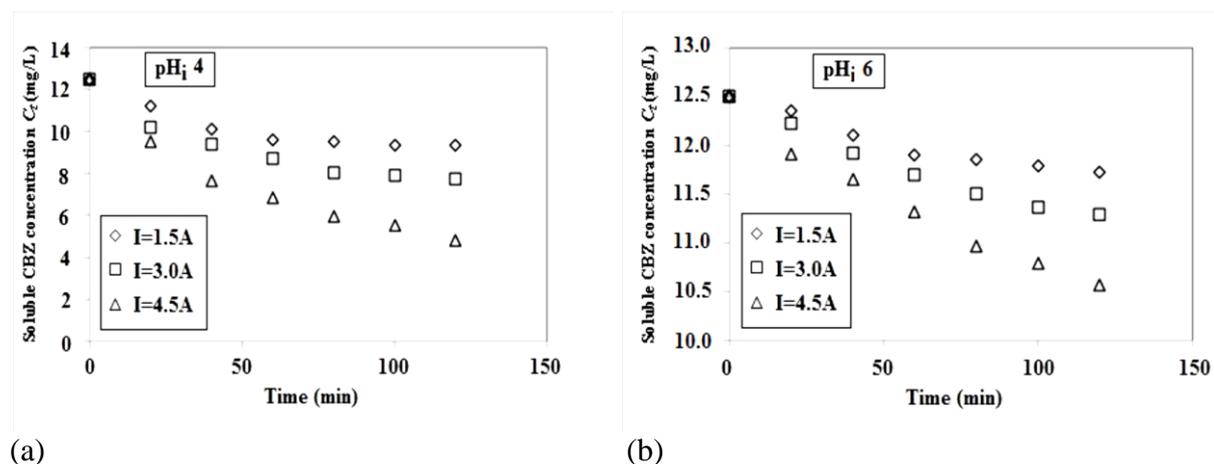
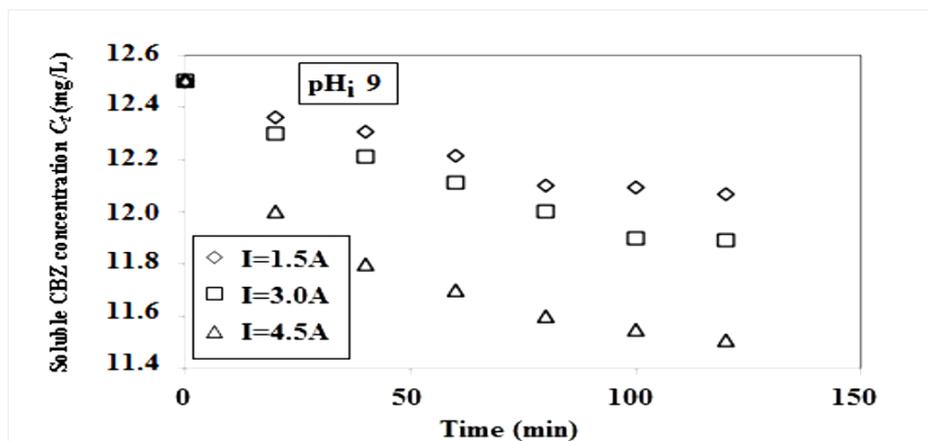


Figure 5: Bar graph showing the peak area of a CBZ metabolite and of CBZ detected by HPLC during EC at pH_i 4 and $I=4.5\text{A}$ as a function of time

3.2. INFLUENCE OF CURRENT

The new set of experimental runs was dedicated to the study of the influence of the current intensity I on CBZ elimination using EC with Al electrodes. The results showed that an increase of current results in an acceleration of the CBZ removal at all pH_i values (Fig. 6) in particularly at pH_i 4 (Fig. 6a). It was shown that the elimination was rapid at early times during EC and slowed down during the EC process.





(c)

Figure 6: Effect of current intensity on CBZ elimination: (a) at pH_i 4, (b) at pH_i 6, (c) at pH_i 9

The reason is the elevation of pH and the consequent decrease in the probable oxidoreduction rate of CBZ. Current was also found to have an effect on the amount of Al³⁺ released and hence on the amount of flocs formed. Results obtained from the HPLC analysis showed the effect of the current intensity on the metabolite concentration that increased with the increase of current and with the decrease of CBZ concentration. It was also found that the pH change rate depended strongly on the current applied to the EC unit. The highest current employed, led to the fastest rate of pH change during EC (Fig. 7) for all values of initial pH leading to the consequent decrease in the probable oxidoreduction rate of CBZ. Current was also found to have an effect on the amount of Al³⁺ released and hence on the amount of flocs formed.

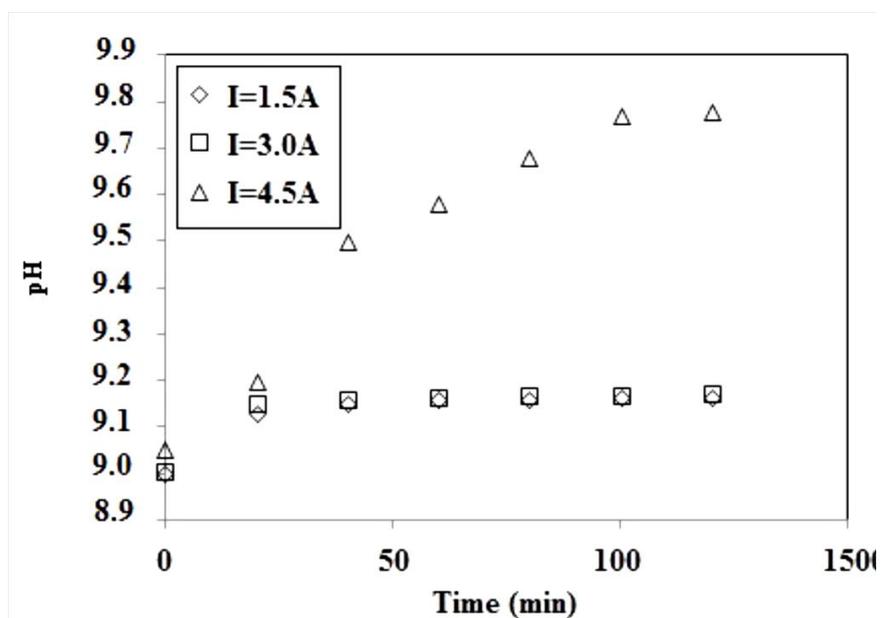


Figure 7: Effect of current on the change of pH with pH_i 9

3.3. SPECIATION OF THE LIQUID AND THE SOLID PHASES

The analyses of the liquid sample at $t=120$ min during EC at $I=4.5A$ and pH 4 on the HPLC found that almost 62% of CBZ has disappeared. The HPLC analysis showed that the

disappearance of CBZ, was accompanied by the appearance of a new molecule that would possibly be a metabolite of CBZ having it appearing at the same wavelength of CBZ.

If there was no adsorption on the solid phase or no gas release, 100% of the initial carbon should be found in the liquid samples when tested on the total organic carbon analyzer at nearly all times. However, the amount of carbon found in the liquid samples when tested on the total organic carbon analyzer was about 79-82% of the initial amount of carbon found in CBZ particularly after $t=20$ min where the corresponding pH is around 7. So, this means that the rest (around 21%) was either been adsorbed on the solids throughout the experiment or released as CO_2 . Analysis of the solid phase by nitrogen adsorption isotherm showed that it exhibited a high specific surface area as the BET method provided values that varied between 200 and $320 \text{ m}^2\text{g}^{-1}$ floc as a function of current.

The dissolution of the flocs of EC with 0.1M HCl showed, by the analysis done on the total organic carbon analyzer, the presence of carbon entities on the solid phase that increase with the increase of current and the decrease of pH_i . The amount of these carbon entities comprises almost 20% of the total amount of carbon found as the form of CBZ at the beginning of EC. The same samples were passed on the HPLC to test for the adsorbed species and revealed that the metabolite was found on the flocs with no minimal presence of CBZ. The inability of CBZ to adsorb on the flocs was confirmed at all the pH used in this study: the same solid phase was produced with the same composition, however, with no CBZ being added. Then, the solids were added to CBZ solutions of different concentrations. The solids were set in contact with CBZ for 24 hrs to attain equilibrium in order to test for adsorption. Analyzing the solutions on the total carbon analyzer showed no decrease of the CBZ concentration, hence no CBZ adsorption on the solid phase.

The total amount of carbon (on the solid phase and in the solution) depending on what is obtained from the total organic carbon is almost 98%, accounting for experimental error, from which we can conclude that there was no release of gaseous CO_2 , or no formation of HCO_3^- or CO_3^{2-} anions in water.

Thus, we conclude that the elimination of CBZ by EC at $I=4.5\text{A}$ and pH_i 4 is primarily an oxidoreduction mechanism comprising the change of 62% of initial CBZ concentration into a metabolite which in turn adsorbs onto the flocs at a relatively neutral pH comprising 20% of the initial carbon amount found in CBZ.

4. CONCLUSIONS

The electrochemical treatment of a biorefractory pharmaceutical molecule, CBZ, has been tested in this work using EC process. Collected data demonstrate that the CBZ is apt to electrochemical oxidoreduction reactions. CBZ was found to be eliminated mostly at pH 4 and at the highest current density of 44 mA cm^{-2} (4.5A) on Al electrodes.

The CBZ was shown to exhibit the highest elimination at pH between 4 and 6. The solid phase was found to capture a new molecule, a probable metabolite of CBZ comprising 20% of its initial carbon content. The increase of the concentration of the soluble and the adsorbed metabolite is in harmony with the decrease of CBZ. Compared to other biological and physicochemical treatments, EC was proven to be more effective in the treatment of CBZ from water than many other conventional techniques. Moreover, by optimizing the parameters of EC, this latter can be used rather than the expensive AOP treatments.

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CHAPTER VI: TOWARDS A BETTER UNDERSTANDING OF THE REMOVAL OF CARBAMAZEPINE BY ANKISTRODESMUS BRAUNII: INVESTIGATION OF SOME KEY PARAMETERS

This article is submitted online in Water Research. Consequently, this chapter follows the guidelines of this journal.

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ABSTRACT

Nowadays, water pollution by pharmaceuticals is a major problem that needs an urgent solution, as these compounds, even when found at trace or ultra-trace levels, could cause harmful effects to organisms. Carbamazepine (CBZ) is a pharmaceutical product that is detected as a micropollutant in many water resources. Different treatment methods were lately employed for the treatment of CBZ, which are often cheap but inefficient, or efficient but expensive. Yet, there are no available studies on the elimination of this molecule by algae despite their well-known highly adaptive abilities. In this study, the biological treatment of CBZ is carried out using green microalgae, *Ankistrodesmus braunii* (*A. braunii*). The respective effects of the culture medium, the initial inoculum and CBZ concentrations were studied on CBZ removal. Lastly, the mechanism of CBZ elimination by *A. braunii* was investigated. The presented data clearly demonstrates that the presence of this molecule did not completely repress the *A. braunii* growth and the ability of these algae to remove CBZ. After 60 days of incubation, the highest percentage of CBZ elimination achieved was 87.6%. Elimination was more successful in bold's basal medium than in proteose peptone medium. Finally, the removal mechanism was also investigated to provide a better understanding about the transformation mechanism of this molecule. It was showed that, the main removal mechanism was the bioaccumulation of CBZ inside the *A. braunii* cells, but the biotransformation of about 20% of the initial CBZ into metabolites inside the cells was also observed.

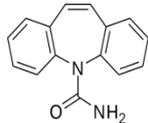
1. INTRODUCTION

Water is more and more polluted by various types of anthropic contaminants or pollutants. Ranging from macro- to micro-pollutants, these are caused by different human activities, such as the urban and industrial development, the use of chemical fertilizers and pesticides in agriculture, the mining activities and the disposal of pharmaceuticals and personal care products (see, e.g., Schwazzenbach et al., 2006; Semrany et al., 2012; Yehya et al., 2014).

Water pharmaceuticals contamination has been an environmental issue of concern since the late 1980s (Doerr-McEwen et al., 2006). During the 1990s, pharmaceutically active compounds, such as lipid-regulating drugs, analgesics, antibiotics, antiseptics, hormones, and chemotherapy and beta-blocking heart drugs, were detected in wastewaters, streams, and ground-water resources across Europe (Stackelber et al., 2004). These molecules can have diverse and adverse effects on organisms and the aquatic life. For this reason, the pharmaceutical products discharges of human and veterinary usage are the object of an intensive research since several years (Mudgal et al., 2013). Numerous studies in Europe and North America have shown that carbamazepine (CBZ) is among the pharmaceutical products

the most frequently detected in the effluents of the wastewater treatment plants and in the river waters (Mohapatra *et al.*, 2014). Indeed, this molecule has a very low biodegradability and is considered as environmentally persistent and refractory (see detailed information in Table 1).

CBZ is a human pharmaceutical approved by the United States (US) Food and Drug Administration for treating epileptic seizures and trigeminal neuralgia. It is also used “off-label” to treat bipolar depression, excited psychosis, and mania (Thacker, 2005). CBZ is composed of two benzene rings fused to an azepine group, which in turn is connected to an amide group (Hai *et al.*, 2011). Its doses commonly range from 100 to 2000 mg per day (Kosjek *et al.*, 2009; Yehya *et al.*, 2015), which is reflected by an annual consumed quantity of CBZ in the world about 1014 tons (Miao *et al.*, 2005). Post-administration, approximately 72% of oral CBZ can be absorbed, metabolized, and excreted with urine, whereas 28% is unchanged and discharged into waters through feces (Li *et al.*, 2013). Consequently, CBZ and its metabolites: trans-10,11-dihydro-10,11-dihydroxycarbamazepine (CBZ-diol) and 10,11-dihydro-10,11-epoxycarbamazepine (CBZ-epoxide) were found in water. Several monitoring studies carried out in Europe, America and Asia have detected CBZ in the aquatic environment at the highest frequency compared to the other pharmaceutical micropollutants. It is most frequently detected in sewage treatment plants effluents, in river water and in seawater (Miao *et al.*, 2003). It was found at the highest concentration of 1.075 µg/L in surface waters in Germany (Heberer *et al.*, 2002), but also at 2.3 µg/L in Canada in a wastewater effluent (Metcalf *et al.*, 2003).

Table 1: Therapeutic use and physicochemical properties of carbamazepine	
<i>Carbamazepine</i>	
Therapeutic class	Antiepileptic
Formula	C ₁₅ H ₁₂ N ₂ O
Structure	
Molar mass (g/mol)	236.27
pK _a at 25°C	14
Boiling point (°C)	411
Solubility in water at 25°C (mg/L)	17.66 ^{a,b}
Log K _{ow} – Octanol Water Partition Coefficient	2.25 ^c
K _{oc} – Adsorption Coefficient (mL/g)	510
Henry's constant (atm·m ³ /mol) at 25°C	1.08·10 ⁻¹⁰ ^b
^a Vieno <i>et al.</i> (2007)	
^b Favier <i>et al.</i> (2015)	
^c Alder <i>et al.</i> (2006)	

The wastewater treatment plants (WWTPs) has been identified as the most relevant sources of pollution with this molecule. Indeed, the average removal rate of CBZ by the conventional activated sludge process is below 10%, and that it is not significantly improved by the use of modern configurations such as membrane bioreactors, though these are frequently effective at removing other pharmaceuticals (Sipma *et al.*, 2010; Popa *et al.*, 2015). The same is reported

for sequencing batch reactors in which CBZ appears to be resistant to microbial biodegradation (Zhang et al., 2008). However, other alternatives are more efficient: biological treatments with *Trametes versicolor*, for example with laccase, eliminated about 60% of CBZ after 48h of contact time (Hata et al., 2010). The first drawback of the biological processes, in general, is the possible biodegradation of CBZ into more toxic compounds. The second is that removal may be driven by a biosorption mechanism, which only displaces the pollutant. Many studies have tested the toxicity of CBZ by various microorganisms. For instance, CBZ toxicity was tested on the green algae *Ankistrodesmus braunii* (*A. braunii*) and *Selenastrum capricornutum* (Andreozzi et al., 2002). They found out that no toxic effect on *A. braunii* could be observed by working at very low concentrations of CBZ and that over 50% of CBZ had been removed in 60 days.

In practice, physicochemical treatments of CBZ were often found more efficient than biological treatments. For example, membrane processes with reverse osmosis showed more than 85% elimination of CBZ and of other pharmaceutical compounds (Deegan et al., 2011). Moreover, ozonation (Hua et al., 2006) removed up to 99% even when present in trace concentrations. Additionally, Fenton and photo-Fenton processes showed a complete elimination of CBZ by Fenton oxidation (Mohapatra et al., 2013), and heterogeneous photocatalytic processes achieved more than 90% elimination (Doll et al., 2005). Hybrid processes that consist of the combination of several conventional processes were also investigated and found efficient for CBZ elimination. For instance, in the treatment based on UV/H₂O₂, the presence of 25 mg/L of H₂O₂ eliminated 90% of CBZ at 2.25 J/cm² UV dose (Shu et al., 2013). Ozonation, as Fenton and photocatalytic processes belong to the class of advanced oxidation processes (AOPs). Despite their ability to eliminate rapidly highly biorefractory chemicals, such as CBZ, they present, however, two major drawbacks:

- their cost is too high;
- mineralization is not complete, and they may also produce secondary pollutants.

This is the reason why research still focuses on the development of innovative biological treatments, for example involving the bioaugmentation of activated sludge.

In this work, the objective is to investigate the potential of a green microalgae *A. braunii* for CBZ removal. Andreozzi et al. (2002) have investigated the toxicity of this molecule on *A. braunii* and noted that about 50% of the initial CBZ had been removed after 60 days. At the moment, it is still unknown whether these algae are able to degrade or transform this refractory pharmaceutical molecule and even less about the degradation mechanism. The aim is, now, to confirm this result at various CBZ initial concentrations using two culture media, the Bold's Basal (BB) and Proteose Peptone (PP) media, for different inoculum concentrations as a function of cultivation time. First, the influence of CBZ on the growth of the algae will be studied. Then, the elimination of CBZ will be investigated quantitatively over time. Finally, the mechanism of CBZ removal will be analyzed.

2. METHODS AND EXPERIMENTAL PROCEDURES

2.1 CHEMICALS

Carbamazepine (99.8 % pure) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile of HPLC grade was supplied by Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used for analytical purposes or for culture media formulation were of analytical grade and were obtained from standard manufacturers. Ultrapure water was produced by an Elga Option-Q DV-25 system (Antony, France).

2.2 ANKISTRODESMUS BRAUNII AND CULTURE MEDIA

The algae tested for the elimination of CBZ was *Ankistrodesmus braunii*, strain CCAP 202/7a, from the Culture Collection of Algae and Protozoa (CCAP, Ambleside, UK). *A. braunii* was cultivated in two liquid culture media having different elemental composition: namely, the Bold's basal medium (BB) and the Proteose Peptone medium (PP) to compare the relative growth of the algae and its ability to remove CBZ in these media.

The components of these liquid culture media and the used protocols are described below. For the BB media, different salt stock solutions at a given concentration have been prepared as follows: NaNO₃ (5.0 g/200 mL), MgSO₄ (1.5 g/200 mL), NaCl (0.5 g/200 mL), K₂HPO₄ (1.5 g/200 mL), KH₂PO₄ (3.5 g/200 mL) and CaCl₂·2H₂O (0.5 g/200 mL). Stock solutions were stored unsterilized at 4°C. Culture media was prepared by combining 10 mL of each of the six stock solutions with 1 mL of each of the following solutions: alkaline EDTA (5.0 g/100 mL of EDTA-KOH), acidified iron (4.98 g/L of FeSO₄ and 1 mL of H₂SO₄), boron (1.14 g/L of H₃BO₃) and trace metals solution. The volume was finally filled up to 1 L with ultrapure water. The metals solution used was composed of: 8.82 g of ZnSO₄, 1.44 g MnCl₂·4H₂O, 0.71 g of MoO₃, 1.57 g of CuSO₄ and 0.49 g of Co(NO₃)₂·6H₂O dissolved in 1 L ultra-pure water. The Proteose Peptone medium contained per liter: 20 mL of each of the following stock solutions (MgSO₄ (1.0 g/L); K₂HPO₄ (1.0 g/L); KNO₃ (10.0 g/L)) and 1 g of proteose peptone powder. The pH of both culture media was adjusted to 7 with NaOH (1 mM) and then sterilized in an autoclave for 15 min. at 120°C.

2.3 GROWTH EXPERIMENTS

The precultures were conducted in Erlenmeyer flasks containing 100 mL of the considered medium aseptically inoculated with 10 mL of CCAP algae suspension. These cultures were kept to grow reasonably dense under controlled light and temperature conditions (23±1)°C, irradiance 100 µEs⁻¹.m⁻², the day and night pattern 16 hrs. day – 8 hrs. night, using Philips Master (TL-D 18W/827 lamps, Poland) in a stove (INFROS, Switzerland). The obtained precultures were then used to inoculate the cultures for the degradation experiments. These tests were carried out in batch mode, in conical flasks containing 1 L of culture medium at two different pollutant concentrations 2.5 and 10 mg/L. CBZ was directly dissolved in the culture media (BB or PP) to give the desired initial concentration. Erlenmeyer flasks were autoclaved at 121°C for 15 minutes prior to inoculation to avoid contamination and provide rapid dissolution of CBZ. No degradation of CBZ was observed during autoclaving.

For these cultures two different initial biomass concentrations (10⁴ and 10⁵ cells/mL) were used in order to investigate the influence of this parameter on algae growth and CBZ biodegradation. Flasks were then, incubated as a reference at 30°C during 60 days under agitation at 135 rpm. The agitation rate was chosen from the range 100-150 rpm usually used to inoculate algae (Truhaut et al., 1980; Iqbal et al., 1993). Appropriate controls without the target compound were conducted for each of the tested cultures conditions to investigate the algal growth, but also for analytical purposes. Indeed, the controls allow to distinguish metabolites resulting from the breakdown of CBZ from those arising from the base metabolism of algae. Thus, twelve different experimental conditions were investigated in duplicate to evaluate the growth of *A. braunii* as well as CBZ degradation. Samples were taken at different time intervals and evaluated for cells concentration and CBZ concentration as described below. Culture transfer and sampling were done aseptically to minimize contamination.

2.3 MICROSCOPY

The growth of algae during all the experiments was followed by cell counting using a Malassez hemocytometer (Tiefe Depth, 0.200 mm) and a microscope (Olympus BX41TF, Japan) at a magnification $\times 100$ (oil immersion lens) in the absence and in the presence of CBZ at regular time intervals over a period of 60 days, on each of the twelve culture conditions described above. Microscopy was also employed for the morphological observation of algal culture during different tests and to ensure the complete bursting under high pressure conditions of the algal cells.

2.4 ANALYTICAL PROCEDURE

HPLC-DAD ANALYSIS

Samples taken from each of the cultures were filtered using adequate polyester filters (Chromafil Xtra PET, 0.45 μm , 25 mm diameter) and transferred to HPLC vials for subsequent HPLC determination. The quantification of CBZ in the different samples was carried out on HPLC (Waters 2410, France) equipped with a diode array detector (DAD) using a reverse-phase C18 column (Waters, Symmetry: 5 μm , 4.6 mm \times 250 mm). The mobile phase was acetonitrile in ultra-pure water (30:70 v/v) and detection was carried out at the wavelength $\lambda=230$ nm. The flow rate was 0.5 mL/min. Analysis were conducted in isocratic mode and the retention time of CBZ was about 20 min.

IDENTIFICATION OF CBZ METABOLITES

Organic reaction intermediates obtained under optimum process conditions were identified using an ultra performance liquid chromatography tandem mass spectrometry (UHPLC/MS/MS). The analyses were performed with an Acquity™ UHPLC H-Class system (Waters, Saint-Quentin en Yvelines, France) coupled with a Waters Acquity™ triple quadrupole mass spectrometer (MS/MS) equipped with an electrospray ionization source. Separation was achieved with a reversed phase column BEH C18 (50 mm \times 2.1 mm, 1.7 μm) placed in an oven at 45°C. Elution was carried out with a mixture of acetonitrile/ultrapure water (30/70 ratio) containing 0.1% (v/v) of formic acid and in isocratic mode with a flow rate of 0.5 mL.min⁻¹. For the analysis, a sample volume of 5 μL was used. The MS analyses were carried out in positive mode electrospray ionization.

2.5 IDENTIFICATION OF THE MECHANISM OF CBZ ELIMINATION

Samples taken from the reference solutions of CBZ in BB and PP in the absence of algae were analyzed first using HPLC to identify the effect, if any, of the high temperature used to sterilize and dissolve the CBZ on the CBZ elimination. These preliminary results confirmed that in the absence of algae and at high temperature, no possible chemical degradation of CBZ could occur. To check for the fate and mechanisms of CBZ elimination by *A. braunii*, cells were burst at 239 MPa using a high pressure cell disruption equipment (Constant systems, UK) and the CBZ concentration was, then, measured by HPLC using the procedure described above. Cells lysis was confirmed by microscopic examination employing an electronic microscope (Olympus CX41, Japan) coupled to a camera (QImaging, Canada).

3. RESULTS AND DISCUSSION

3.1 EFFECT OF CBZ ON THE *A. BRAUNII* GROWTH

The growth of the *A. braunii* was followed in both culture media in the absence and in the presence of CBZ to detect whether there would be any effect after the addition of CBZ at both initial inoculum concentrations of, namely 10^4 and 10^5 cells/mL. The growth curves (data from the measurements using a Malassez cell) are reported in Fig. 1 at the two initial inoculum concentrations. First, all the curves in the figure showed a short latent phase for both media without CBZ of about 5 days. This latent phase was, however, longer when the pollutant was added to both media, about 8 days for both tested inoculum concentrations. This is probably due to the time necessary for the algae to adapt to the presence of pollutant in the culture media.

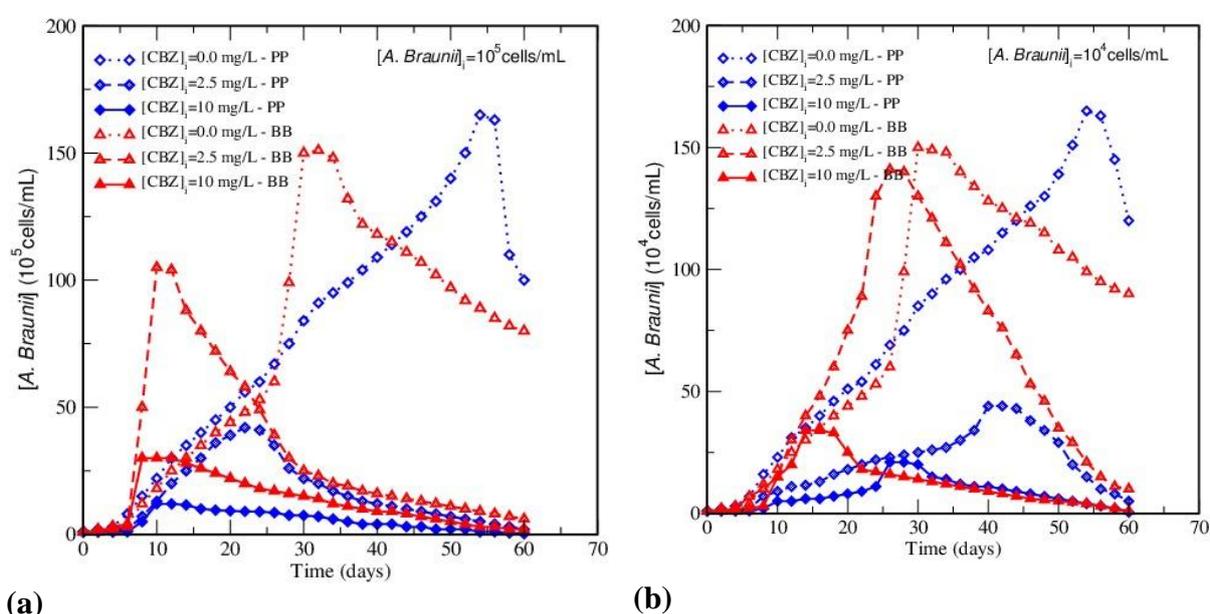


Figure 1: Growth curves of *A. braunii* cultured in BB and PP media at different initial CBZ concentrations (0.0, 2.5, and 10 mg/L, respectively) for an initial inoculum concentration (a) 10^5 cells/mL, (b) 10^4 cells/mL, during 60 days of incubation.

When comparing the growth rates, it was found that the growth of algae in the absence of CBZ followed, first, approximately the same rate in both media. Discrepancies emerged after 25 days, as growth rate suddenly rose steeply in BB, whereas it followed the same trend with a constant slope in PP. This might be due to the difference in nutritive content, particularly the nitrogen source: proteose peptone of bovine source in PP and EDTA in BB, respectively. As a result, growth was faster in BB than in PP in the absence of CBZ, but the exponential growth phase was shorter and the death phase started earlier in BB. The consequence is that the maximum amount of algae was higher in PP, but was reached far later than in BB. It must, however, be pointed out that the amount of algae present was still considerable after 60 days of incubation: due to the delayed maximum, the algae concentration was higher in PP, but the death rate was also higher, while it followed a smooth decreasing shape in BB. The comparison between Fig. 1a and Fig. 1b also revealed that the algae concentrations exhibited exactly the same shape when inoculum concentration was changed and that, at any time t , the algae concentration was roughlyly proportional to the inoculum concentration in the absence of

CBZ, which means that maximum algae concentrations were observed at the same time, independently from the inoculum concentration without CBZ.

Conversely, this pattern changed greatly when CBZ was present in both media in Fig. 1. The addition of CBZ shortened the growth phase in both cases. Consequently, the onset of the death phase started earlier, a lower maximum biomass concentration was achieved than without CBZ, and an almost complete death was observed at the end of the 60 days of cultivation. However, differences emerging from the presence of CBZ were media-dependent. For example, the maximum algae concentration was observed in BB, while it was reported in PP without CBZ. The comparison of the results obtained for the cultures with and without CBZ highlighted that the maximum algae concentrations fell due to CBZ in PP, while they were only slightly decreased in BB. A striking result is also that the rapid increase of the algae concentration was forwarded in BB in Fig. 1, but with growth rates nearly as high as without CBZ. This differed strongly in PP: although the maximum algae concentration was also forwarded, the values and the growth rate were always smaller than without CBZ. The comparison between Fig. 1a and Fig. 1b also highlighted that if the maximum algae concentration was nearly divided by a factor 10 when the inoculum concentration was reduced from 10^5 and 10^4 cells/mL (as without CBZ) the time at which this maximum was achieved depended strongly on the inoculum concentration when CBZ was present in both media, which was not observed without CBZ. This showed clearly that the ratio between inoculum and CBZ concentrations played a key role in the *A. braunii* growth.

The differences between BB and PP could be related to a possible interaction of CBZ with the proteose peptone in PP, as CBZ is known to interact with proteins (Fortuna et al., 2013). This would lead to a decrease in the amount of the proteins available for the algae to grow, and consequently to a weaker growth rate. However, no interaction between proteose peptone and CBZ was found using HPLC, although this could vanish in the acetonitrile/water eluent. Another explanation is that CBZ is toxic for *A. braunii*, but that CBZ assimilation is less rapid in the presence of EDTA (nutritive source of C and N in BB), which also accelerated algae growth under these conditions. Conversely, the rapid algae death that followed could be attributed to a toxic effect of CBZ under EDTA limitation. This contradicts the results of Andreozzi et al. (2002) who reported no toxicity of CBZ on *A. braunii*, but used a far lower initial concentration of CBZ, about 2.1×10^{-6} g/L. Finally, for an efficient and rapid growth of *A. braunii* microalgae, experimental data shows that PP should be preferred, but in the presence of CBZ, BB should be retained because of the evidenced strong interaction between CBZ and algae growth.

Consequently, the influence of the initial CBZ concentration was investigated for both media and initial inoculum concentrations. Two initial CBZ contents (2.5 mg/L and, 10 mg/L, respectively) were investigated in this work. Experimental results showed that, for all the experiments, a higher concentration of pollutant further shortened the exponential growth phase and lowered the obtained maximum algae concentration. Indeed, a high initial CBZ content forwarded the onset of the death phase and led to an earlier *A. braunii* death when compared to working at 2.5 mg/L of CBZ. Roughly, when the initial CBZ concentration was multiplied by a factor 4, the maximum concentration of algae was divided by the same factor. In addition, the growth profiles of the microalgae presented the same shape for both of tested inoculum concentrations, but kinetics showed an interaction between CBZ and algae contents. Thus, when the initial inoculum concentration was multiplied by a factor 10, the culture time necessary to reach the maximum algae concentration was divided approximately

by a factor 2 at constant initial CBZ concentration, whatever the considered initial pollutant concentration.

As a conclusion, the obtained data clearly showed that the presence of the target compound did not repress the *A. braunii* growth despite its continuous exposure. For the all tested conditions, the algae successfully grew in the presence of CBZ. This result is in agreement with previous studies which reported that the toxicity of organic compounds on cells can be attenuated by the uptake of non-toxic nutriment, such as the alternative carbon sources (Saéz and Rittmann, 1991; Gauthier et al., 2010; Popa et al., 2014). However, the initial CBZ concentration impairs the duration of the exponential phase of growth phase and the maximum cell number was reduced for all of investigated conditions when the initial CBZ content was increased. Thus, the presence of pollutant negatively affects the metabolic reactions associated to the algae main metabolism (growth media BB or PP without CBZ). These trends corroborate the probable toxicity of CBZ on *A. braunii* which increased with the initial xenobiotic concentration. Similar results were reported by (Ziagova et al., 2009) for the growth of *S. xylosus* in the presence of 2,4 dichlorophenol. In addition, the above results also indicate that an additional carbon source is needed to support the cells growth in the presence of toxic compounds such as CBZ.

3.2 EFFECT OF THE CULTURE CONDITIONS ON THE POLLUTANT ELIMINATION

3.2.1 EFFECT OF CULTURE MEDIUM

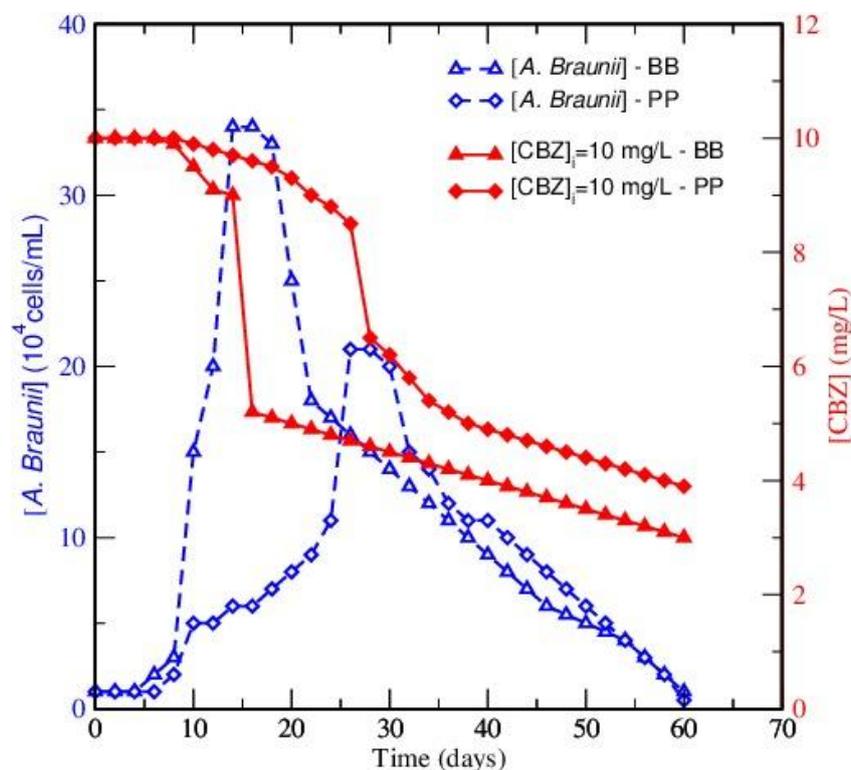


Figure 2: Effect of the culture media on CBZ removal for an initial pollutant concentration of 10 mg/L and an initial inoculum concentration of 10^4 cells/mL.

The effect of the composition of culture media used for the *A. braunii* growth on the removal of CBZ was investigated. Figure 2 plots the time profiles for the residual CBZ concentration for both of the investigated culture media and an initial inoculum and pollutant concentration of 10^4 cells/mL and 10 mg/L respectively. As shown in this figure, the evolution of CBZ concentration over time exhibited the same profile in both media. Three phases are really discriminated for the both investigated media: first, it was constant or decreased slightly and then, a steep decrease was reported, followed by a slow decrease of CBZ content. The key difference between BB and PP media was the period at which the steep decrease was reported: it occurred 12 days before in BB than in PP. However, BB was shown to be more favorable than PP for the CBZ elimination after 60 days of incubation, as a higher removal yield was achieved. For example, with an inoculum concentration of 10^4 cells/mL and an initial CBZ content of 10 mg/L, this yield was $70\% \pm 4$ in BB medium, but only $66\% \pm 3$ in PP. However, BB medium also leads to a faster removal. This is in accordance with the results presented previously (Section 3.1).

A. braunii cells concentration and CBZ elimination increased simultaneously, suggesting that CBZ could be used as a nutritive source by the microalgae, but other explanations can be found. For example, these trends could be also related to a biosorptive or metabolic role of *A. braunii*, as the kinetics of these mechanisms are also proportional to the amount of algae. The results presented here are not unexpected, as algae are known to be able of degrading recalcitrant organic compounds. Indeed, Todd et al. (2002) demonstrated the biotransformation of naphthalene and diaryl ethers, complex aromatic pollutants, by *Chlorella*, *Ankistrodesmus* and *Scenedesmus* strains. Other studies reported that numerous algal strains induce biotransformation of exogenous steroids or low-molecular weight phenols (Pollio et al., 1994; Pinto et al., 2003; Della Greca et al., 2003). However, to the best of our knowledge, no previous study has demonstrated the removal of CBZ with algae pure cultures.

For a better understanding of experimental results, the growth curves and the residual CBZ concentration were plotted together in Fig. 2 for each tested culture medium. The obtained data highlights the interaction between these two parameters: the delayed exponential growth phase in PP led to a delayed death phase and to a delayed onset of CBZ elimination. The role of *A. braunii* in CBZ elimination is, therefore, assured by comparing growth kinetics and the consequent CBZ amount in both culture media. Figure 2 showed a synchronized behavior between the *A. braunii* cell growth and the residual amount of CBZ. For example, when working at an initial inoculum concentration of 10^4 cells/mL and an initial CBZ concentration of 10 mg/L, the amount of CBZ at the very beginning of the culture was almost stable during the first 5 to 8 days corresponding to the latent phase of the algae, but this was followed by a rapid decrease of CBZ concentration that took place at the time where the highest cell concentration is attained in both BB and PP. Thus, during the first phase, cells seem to prepare themselves to better assimilate the pollutant and no appreciable changes in the biomass and CBZ concentration were observed. The improved performance of *A. braunii* in the removal of the xenobiotic compound becomes effective during the second phase, when a rapid increase in the growth rate and pollutant consumption was observed. Finally, the removal of the target pollutant continues during the negative cell-growth period. A similar trend was observed by Saéz and Rittmann (1993) for the elimination of 4-chlorophenol by *Pseudomonas putida*.

As a conclusion, it can be noticed that the presented data assess the role of *A. braunii* on CBZ removal in both media and that CBZ removal is enhanced in BB in comparison to PP, noting

that the growth of the algae is also favored in BB in the presence of CBZ. Moreover, it should be pointed out that the addition of conventional carbon sources in the culture medium can substantially modify the cell density, especially the extracellular enzymes under the considered conditions, and as consequence, the removal of the target compound. Other studies also reported for bacteria the positive role of additional carbon sources via a co-metabolism in the removal of toxic organic compounds (Larcher and Yargeau, 2013). Similarly, the literature reports that a primary electron-donor substrate is required to grow and sustain the biomass capable of degrading any cometabolite which is an obligate secondary substrate (Saéz and Rittmann, 1993), which may also explain our experimental results.

3.2.1.1 EFFECT OF INOCULUM CONCENTRATION ON THE REMOVAL OF CBZ

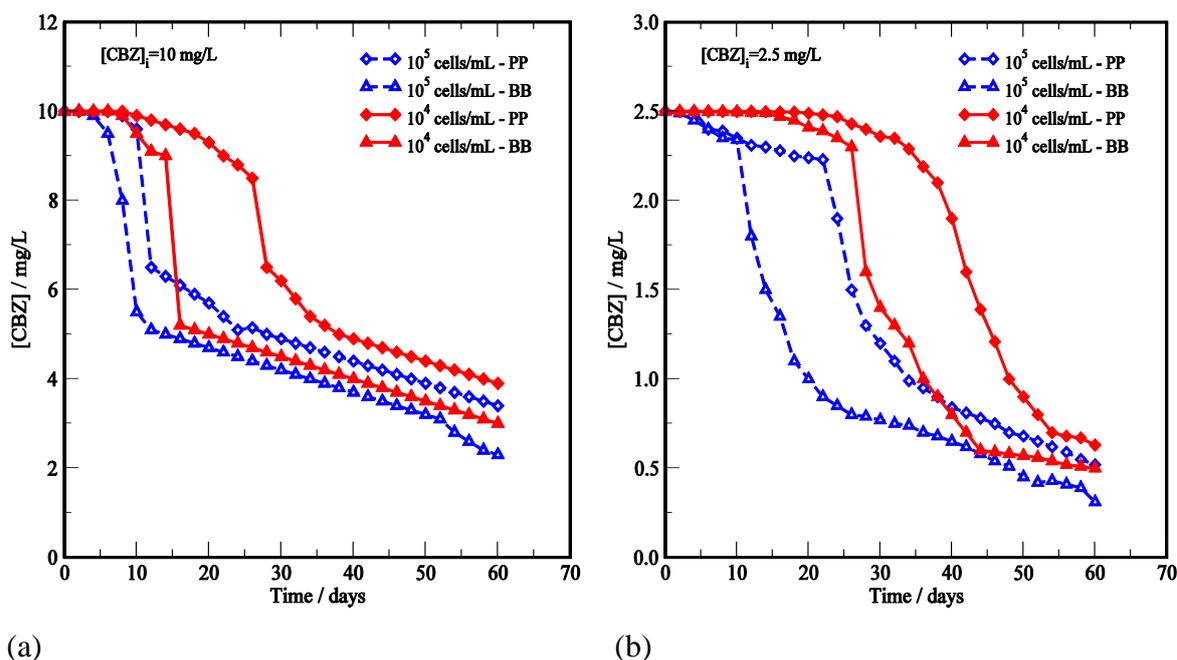


Figure 3: Effect of inoculum concentration on the removal of CBZ in both media at: (a) 10 mg/L, and (b) 2.5 mg/L of CBZ.

To further clarify the effect of inoculum concentration, a new set of experiments was carried out in order to follow the effect of the initial inoculum concentrations (10^4 and 10^5 cells/mL) on the elimination of CBZ. The tests were carried out at both initial concentrations of CBZ (2.5 and 10 mg/L) and in both media (BB and PP). By comparing the HPLC data, it was shown that the removal yield of CBZ was enhanced and that the onset of CBZ elimination was forwarded by the highest initial *A. braunii* inoculum concentration (10^5 cells/mL) in both culture media and for both CBZ initial concentrations (Figure 3a and 3b). For example, it arises from this figure that in BB medium for the same initial CBZ concentration of 2.5 mg/L, an increase in the removal of CBZ from 80% to the highest elimination percentage attained of 87% in this work is observed after 60 days of incubation when the concentration of *A. braunii* increases from 10^4 to 10^5 cells/mL. It must, however, be pointed out that the increase in removal yield due to higher inoculum content from 10^4 to 10^5 cells/mL never overpassed 8%, whatever the removal yield, and that it never approached complete removal. This may be explained as follows: while the additional amount of the eliminating agent, the algae, leads to an improved elimination of CBZ, the medium is exhausted more rapidly due to a stronger competition on nutritive sources. As a result, the algae starts more rapidly to consume CBZ as the only possible nutritional source and a higher quantity of algae is

removed from water, but toxic effects of CBZ also grow simultaneously and prevent a complete elimination of this molecule.

As a conclusion, CBZ removal can be enhanced by increasing the initial inoculum concentration and, more generally, by increasing the concentration of algae in both media, even though a complete elimination cannot be achieved. It is important to notice that the concentrations tested in this work are really higher than those presented in the influents of the wastewater treatment plants (in the range of $\mu\text{g/L}$). However, it is also important to take into account that pharmaceuticals are not always detected at trace levels. Thus, in their work, Larsson *et al.* (2007) reported high concentrations of pharmaceutical compounds for effluents from health and care industry: some of the mentioned molecules exhibited concentrations of the same order of magnitude as in this study (concentrations levels of 28, 2.4 and 1.3 mg/L were detected for ciprofloxacin, losartan and cetirizine, respectively). The main objective of our work at this point was to evaluate if the considered algae are able to degrade this refractory pharmaceutical compound. The next step will be focused on the design of degradation tests in culture conditions closer to the real ones (micropollutant concentration, pH, temperature, real wastewater...).

3.2.2 EFFECT OF CBZ INITIAL CONCENTRATION ON ITS ELIMINATION

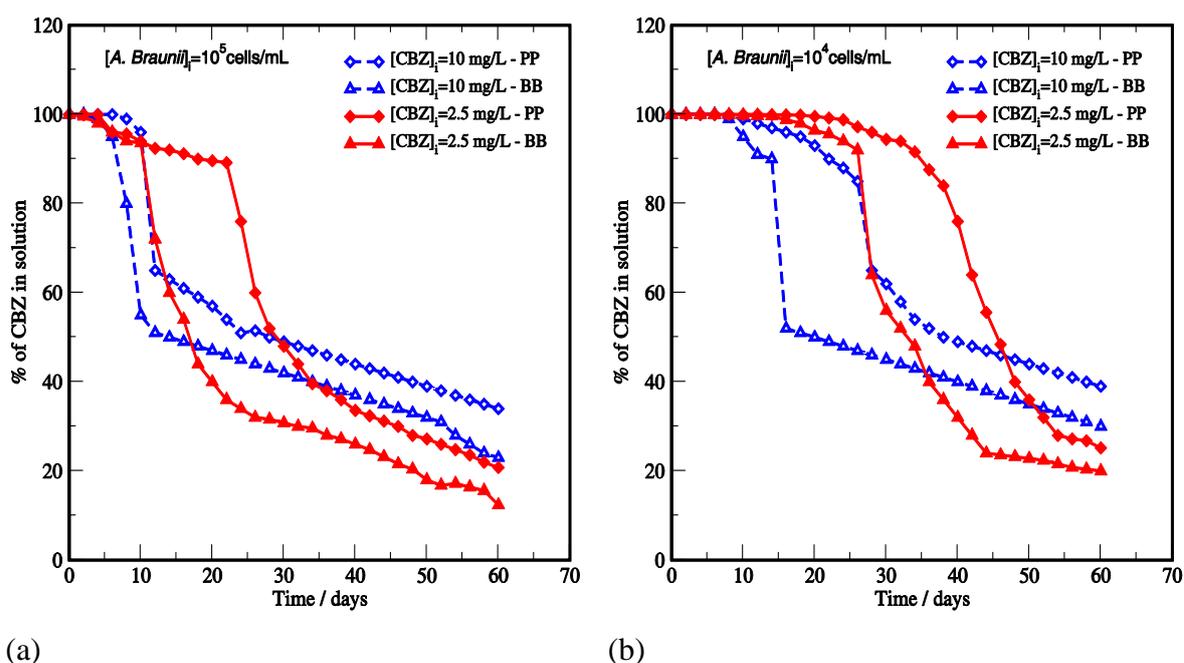


Figure 4: Effect of CBZ initial concentration on the elimination of CBZ at initial *A. braunii* content: (a) 10^5 cells/mL, and (b) 10^4 cells/mL.

The effect of the initial concentration of CBZ was analyzed using experimental data at two initial CBZ concentrations of 2.5 and 10 mg/L, in both media, starting with both initial inoculum concentrations. Experimental results in Fig. 4 showed that the increase of the initial CBZ concentration led to a decrease in the percentage of its elimination in both media. For instance, for the same *A. braunii* inoculum concentration 10^5 cells/mL, increasing the initial CBZ concentration from 2.5 to 10 mg/L in PP medium induced a decrease of the percentage of elimination from 79.2% to 66.0% after 60 days. This increase also forwarded the onset of the elimination of CBZ, worth noting, that corresponds to the relative growth of *A. braunii* at that time. This results in a more rapid elimination, but with a lower final yield when starting

with 10 mg/L of CBZ than when starting with 2.5 mg/L. In fact, this is due, on the one side, to the more rapid consumption of the higher concentration of CBZ (10 mg/L), that turns to be more toxic on the *A. braunii*, leading to forwarded death, and on the other side, to the smaller amount of CBZ needed to be harvested by the same inoculum concentration when the initial CBZ content is 2.5 mg/L, leading to higher elimination yield and a reduced toxicity. It must, indeed, be pointed out that even though the yield decreased, the amount of CBZ removed strongly increased when its initial content was increased by a factor 4. This highlights the dual role of CBZ, at the same time a substrate and a toxic compound for microalgae.

As a conclusion, the elimination yield of CBZ is enhanced when the initial CBZ concentration decreases, as this causes less toxic effect on the algae and hence, and enhanced capability for approaching a “complete” CBZ elimination. This is an advantage, as CBZ is usually present as a micropollutant in water, but the drawback is that a lower CBZ content induces a slower elimination kinetics. Moreover, the obtained data indicate that *A. braunii* is able to utilize the target compound either as carbon or nitrogen source, whatever these are made available by simpler substrates in the culture media.

3.3 SUMMARY OF THE REMOVAL YIELD OF CBZ AFTER 60 DAYS

The removal yield of CBZ as a function of culture media, initial inoculum concentrations, and initial concentration of CBZ after 60 days have been summarized in Table 2.

Initial Concentration of CBZ	Initial Concentration of <i>A. Braunii</i> C_i	CBZ % of elimination in PP Medium	CBZ % of elimination in BB Medium
2.5 mg/L	10^4 cells/mL	74.8%	80.0%
2.5 mg/L	10^5 cells/mL	79.2%	87.6%
10 mg/L	10^4 cells/mL	61.0%	70.0%
10 mg/L	10^5 cells/mL	66.0%	77.0%

This table shows that the values range between 60% and 90%, which is quite high for a biological treatment, accounting for the biorefractory character of CBZ. This assesses the ability of *A. braunii* to remove CBZ. The results achieved in this work were quite similar to those reported for the degradation of carbamazepine or clofibric acid by white rot fungi including *Trametes versicolor* (Marco-Urrea et al., 2009). It is also important to note that, the removal efficiencies obtained with *A. braunii* were much higher than the ones reported previously based on the activated sludge systems (Oppeheimer et al., 2007; Stackelberg et al., 2007).

As discussed before, Table 2 emphasizes that the lowest yields in both media correspond to a high initial concentration of 10 mg/L of CBZ. It also shows that for any value of the initial concentration of *A. braunii* in both media, the removal yield decreases by about 10% for a factor 4 increase in the initial concentration of CBZ. In addition to this, for any value of the initial concentrations of CBZ, a factor 10 increase in the initial inoculum concentration leads to around a 7% increase in CBZ elimination. In other words, this shows that a factor 4 of the

initial concentrations of CBZ has more influence than a factor 10 of the inoculum concentration on the elimination of CBZ. This demonstrates again the toxic effect of CBZ on *A. braunii* in the range of concentrations studied, but also that this toxicity does not significantly impair the ability of the algae to remove CBZ.

3.4 FATE OF CBZ

Besides studying the removal of the considered pharmaceutical compound, it is very important to investigate and determine the mechanism involved in elimination of this molecule. Generally, different mechanisms such as biosorption, accumulation and biotransformation are involved in the removal of organic pollutants. However, they cannot be clearly distinguished, since for example, live cells can also degrade adsorbed pollutants by intracellular mechanisms (Blázquez *et al.*, 2004). In an effort to understand the mechanisms involved in the removal of CBZ by *A. braunii*, it is important to assess transformation products. A first insight emerged in the HPLC chromatograms (obtained by successive analysis) which showed the presence of a new peak corresponding to an extracellular compound produced by the algae, the concentration of which increased in parallel to the decrease of CBZ. Being identified at the same wavelength, this exhibited a shorter retention time (3 min., while the retention time of CBZ is 18 min.). As a consequence, this seemed to indicate the possible biotransformation of CBZ into metabolites and can be considered as indicator of the biodegradation process.

To confirm this result and better analyze the fate and the mechanisms of elimination of CBZ by *A. braunii*, cells were burst under high pressure and then imaged to ensure the complete bursting of the cells, so that the CBZ accumulated in the cells, if any, was released in the medium and easy to quantify by HPLC analysis. Figure 5 shows the magnified images of the cells with the elongated shaped *A. braunii* before pressure treatment, and the randomly shaped destroyed cells after the cell burst. This makes also clear, as shown in Figure 5a, the asexual mode, binary fission, of *A. braunii* reproduction in the presence of the target compound.

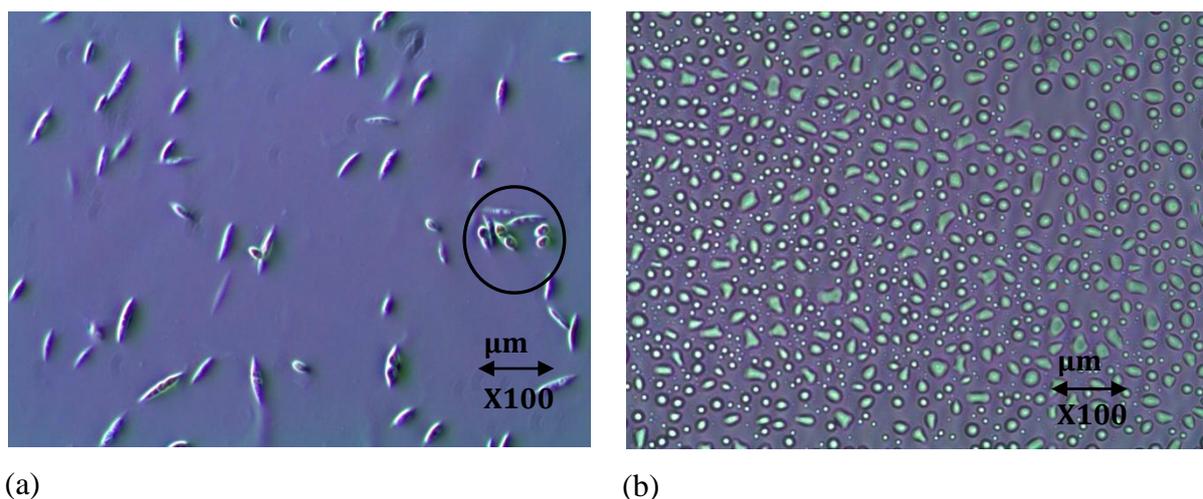


Figure 5: Magnified images of *A. braunii*, (a) non-burst, where the asexual reproduction of binary fission is shown in the circle, and (b) burst cells.

HLPC analysis of the liquid phase after *A. braunii* bursting showed that a high quantity of eliminated CBZ was recovered into the medium. Figure 6 gives a comparison of the CBZ

concentrations determined in burst and non-burst cells cultured in BB and PP media. It can clearly be concluded from this figure that the bioaccumulation of CBZ in the cells plays a key role in the removal of this molecule by *A. braunii*. However, it can also be observed that the total amount of CBZ found in the solution even after the algal bursting does only correspond to 80% of the initial CBZ concentration which is 2.5 mg/L. Surprisingly, the lowest amount of CBZ recovered in the medium corresponds to the case of the highest CBZ elimination, *i.e.* in the BB medium starting with 10^5 cells/mL of *A. braunii* and 2.5 mg/L of CBZ.

Moreover, the HPLC data analysis also highlighted that the surface area of both the CBZ and the other peak increased simultaneously in both media after bursting *A. braunii* cells and that the area of the second peak was maximized in the BB medium starting with 10^5 cells/mL of *A. braunii* and 2.5 mg/L of CBZ (data not shown).

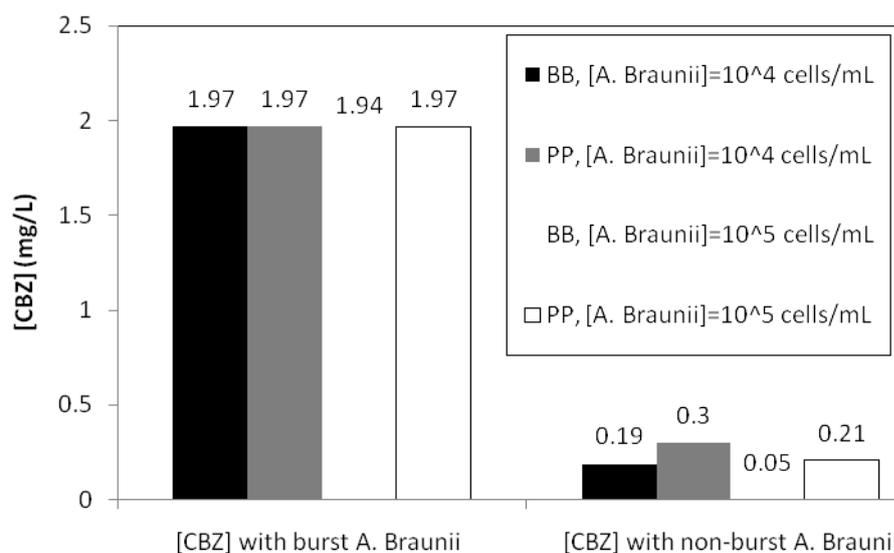
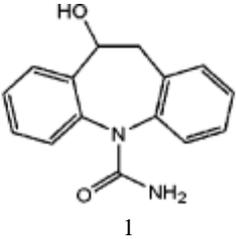
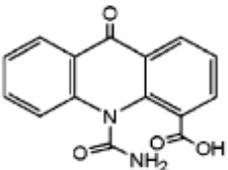
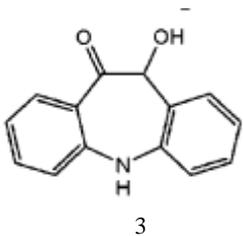


Figure 6: CBZ concentration in BB and PP media with burst and non-burst cells for an initial CBZ concentration of 2.5 mg/L and an inoculum concentration of *A. braunii* between 10^4 and 10^5 cells/mL.

These results confirm that these algae may take up this substrate into the cell and another mechanism, namely a biodegradation occurs intracellularly in parallel to the bioaccumulation of CBZ, even though a possible biosorption of this molecule on the *A. braunii* cells cannot be neglected.

In addition, to confirm the presence of the transformation products generated by the by the CBZ transformation by *A. braunii*, MS analyses were carried out in positive mode electrospray ionization. UHPLC/MS/MS data have showed the qualitative presence of three different metabolites in the cell-free supernatants (Table 3), confirming the biotransformation role of *A. braunii* cells on CBZ. The most abundant compound detected was 10,11-dihydro-10-hydroxycarbamazepine (10-OH CBZ). From this results 10-OH CBZ is the one mostly resembling CBZ, we could assure that this metabolite is the one that appears on the HPLC at earlier times at the same wavelength. The other detected metabolites did not appear in the HPLC chromatograms, despite the sensitivity of this analytical method. This suggests that these are unstable and/or are present in concentrations which are below the instrumental limit of detection (LOD) of the used HPLC method.

Table 3: CBZ metabolites detected on UHPLC/MS/MS.	
Metabolite	Experimental m/z
 <p>1</p>	255
 <p>2</p>	283
 <p>3</p>	226

Based on the chemical structure of the organic intermediates (degradation products), a possible mechanism of the formation of 10-OH CBZ consisting in two-step reaction that could be explained by the epoxidation of carbamazepine, followed by a direct ring-opening of the resulting epoxide and the formation of the resulting alcohol is proposed in Figure 7. From this, the major bioaccumulative and biosorptive role of *A. braunii* cells in the elimination of CBZ at about 80%, and the other 20% biometabolic oxidative transformation of CBZ mainly into 10-OH CBZ could be concluded.

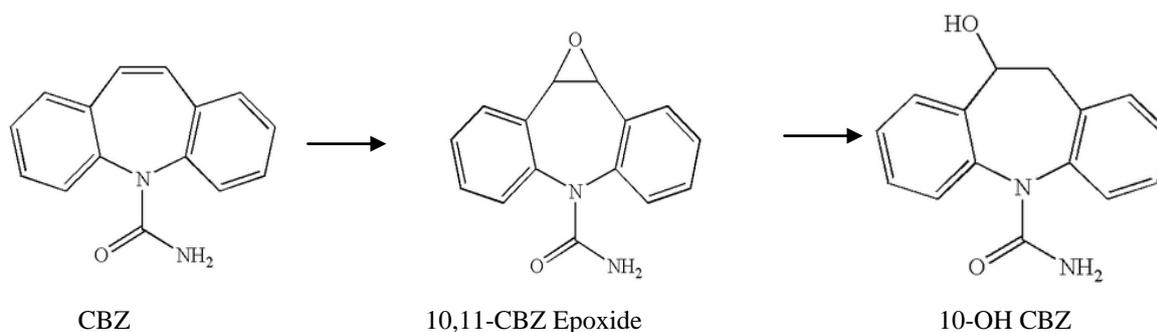


Figure 7: Proposed oxidation reaction of CBZ by *A. braunii* and the formation of 10-OH CBZ.

To the authors' knowledge, this is the first study reporting on the detection CBZ intermediates formed during the CBZ biodegradation by *A. braunii*. However, more detailed analysis is needed in the future in order to clarify the mechanism of biotransformation step and to extend the knowledge on the role of *A. braunii* in the elimination of this refractory pharmaceutical compound.

4. CONCLUSION

An original biological treatment for the removal of carbamazepine (CBZ), a highly persistent molecule in wastewater treatment plants, has been investigated in this work using the algae *Ankistrodesmus braunii* (*A. braunii*). Experimental data demonstrated first that CBZ is efficiently removed: up to 87.6% CBZ was found to be eliminated at high *A. braunii* concentrations of 10^5 cells/mL, starting with an initial CBZ concentration of 2.5 mg/L. The removal of CBZ was enhanced by increasing the concentration of algae and impaired by initial higher concentrations of CBZ after 60 days. On the contrary, from a kinetic point of view, high CBZ contents and lower inoculum concentrations promoted a more rapid elimination of CBZ, despite the lower final values of the removal yield. This highlights the dual role of CBZ that is a nutritive source for the algae, but seems to be toxic at high dose. An analysis of the fate of CBZ confirmed that about 80% of the CBZ removed was accumulated in the *A. braunii*, but the presence of several metabolites found in the cell free supernatant and in the cells indicated that a biotransformation of CBZ also occurred in parallel: three main metabolites of CBZ have been identified and the surface area of secondary peaks in HPLC chromatograms were clearly negatively correlated with the peak of CBZ.

Finally, all these results prove that a biological treatment using *A. braunii* could be more efficient for the removal CBZ from water than many other conventional techniques, either biological or physicochemical. However, further work is still needed, first to optimize the parameters of the *A. braunii* growth, and then to investigate the ability of *A. braunii* to remove CBZ in real water in relation to the influence of the culture media used in this work.

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CHAPTER VII: ELIMINATION OF ORANGE II, CARBAMAZEPINE, AND DICLOFENAC BY SACCHAROMYCES CEREVISIAE IMMOBILIZED ON ALGINATE IN WASTEWATER

1. INTRODUCTION

The treatment of industrial wastewater effluents is a major challenge in modern research on water technology. In particular, biorefractory compounds constitute a key problem resulting from human activities that cannot be treated using the conventional activated sludge process. Two typical examples of this class of compounds can be found in pharmaceuticals and dyes. To limit their accumulation in the resource, a specific treatment is necessary. In this work, the objective is to test the efficiency of depollution of a biological treatment by *Saccharomyces cerevisiae* (*S. cerevisiae*) immobilized on alginate beads on two pharmaceuticals, Diclofenac (DCF) and Carbamazepine (CBZ), and on an azo dye, orange II (OII). The experimental study focuses on the influence of the presence or the absence of *S. cerevisiae*, the initial concentration for all three pollutants (CBZ: 2.5 mg/L, 5 mg/L, 10 mg/L; DCF: 0.5 mg/L, 1 mg/L, 2 mg/L; OII: 6 mg/L, 12.5 mg/L, 25 mg/L), the effect of the amount of alginate beads (2.5 g, 5.0 g, 7 g, 10.0 g), and of the pH (1, 5.5, 7). The work was conducted in batch with time over 24 hrs. The best results in terms of pollutant removal were obtained after 24 hrs. in acidic conditions with the maximum concentration of alginate and the highest concentrations of pollutants. This was obviously reported in the case of both CBZ and DCF, but did not perfectly describe the results with OII. However, the removal yield remained low (around 55%), except for the OII (up to 80%). As a conclusion, this work shows that OII could be effectively removed by *S. cerevisiae* immobilized on alginate beads at low and medium pH. On the contrary, CBZ and DCF exhibited lower elimination yields at low pH and low elimination yields at high pH values.

2. MATERIALS AND METHODS

S. cerevisiae was immobilized on sodium alginate beads and used as a biosorbent for the elimination of OII, CBZ and DCF. OII dye ($C_{16}H_{11}N_2NaO_4S$) of molecular weight 350.33 g.mol⁻¹ was provided by Dystar Colours Deutschland GmbH. This molecule is a sulphonate sodium salt with pKa about 11. CBZ ($C_{15}H_{12}N_2O$) and DCF ($C_{14}H_{11}Cl_2NO_2$) of molecular weight of 236.27 g.mol⁻¹ and 296.148 g mol⁻¹, respectively were provided by Sigma Aldrich. The three pollutants structural formulae and their respective pKa values are shown in Table 1. To analyze the role of *S. cerevisiae* in the elimination of OII, CBZ and DCF, this study was performed on two groups of samples: the first contained only sodium alginate and the second comprised the *S. cerevisiae* immobilized on the alginate beads. The respective influences of pH (between 1 and 7), the contact time (up to 24 hrs.), the dry mass of alginate (between 2.5 and 10 g/100 mL of water) and the initial concentration of OII dye (between 6 and 25 mg/L), CBZ (2.5, 5 and 10 mg/L), DCF (0.5, 1 and 2 mg/L) were studied. The elimination yields of OII, CBZ and DCF were followed by spectrophotometric analysis (JENWAY 6405, UV/Vis, France) at wavelengths 483, 230, 220 nm, respectively. Using the respective wavelengths of

3. EXPERIMENTAL RESULTS

3.1 EFFECT OF PH, PRESENCE OF *S. CEREVISIAE*, AND INITIAL CONCENTRATION OF OII, CBZ, AND DCF.

Experimental results (Figure 1) showed that the removal yield of OII was maximized about 80% at pH 1 for an initial concentration of 25 mg/L and an alginate quantity of 10 mg/100 mL. These were achieved in the presence of *S. cerevisiae*, whereas in its absence, the elimination was only 59%. It was also observed that the removal yield of OII decreased when the pH increased and that at higher pH, the highest removal yields were obtained in the absence of *S. cerevisiae*. The percentage of elimination, in the absence of *S. cerevisiae*, decreased slightly when the pH passed from 1 to 5.5, and strongly from 55.5% to 25% when the pH was changed from 5.5 to 7. With *S. cerevisiae*, however, only 15% of OII was eliminated at pH 7. The same trends were perfectly followed in the case of CBZ and DCF but with lower maximal elimination yields of 55% and 40%, respectively at pH 1 and at the highest pollutants' concentrations and masses of alginate beads used in the presence and in the absence of *S. cerevisiae* as well.

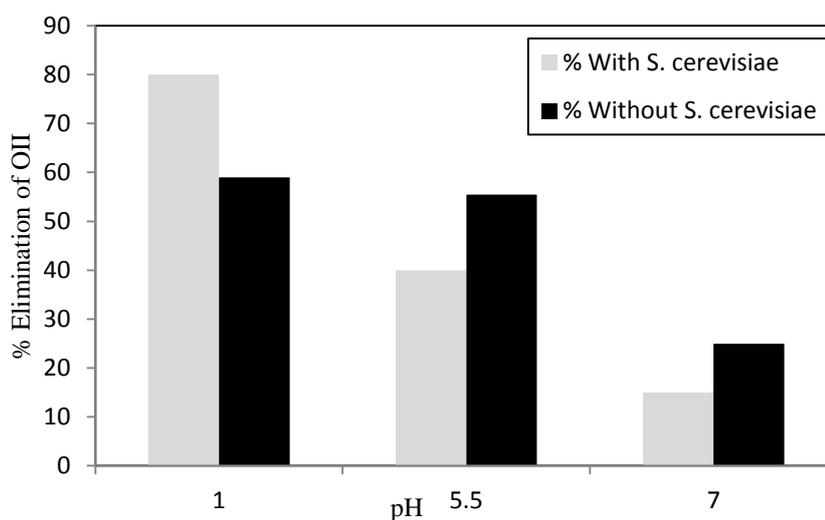


Figure 1: Removal yield of OII with or without *S. cerevisiae* as a function of pH for a mass m 10 g/100 mL of alginate beads and an initial concentration of OII of 25 mg/L.

Moreover, concerning the effect of the initial concentration of the OII dye in the treated solution, the results showed that the percentage of elimination of OII increased when increasing the initial concentration of OII. Figure 2a illustrates the results at pH 1 for a mass of 7g/100 mL of alginate in the absence of *S. cerevisiae*. As this figure shows, the elimination yield decreases from 50% to 23% when decreasing the initial concentration of OII from 25 to 6.25 mg/L. The adsorption experiments of OII under the same conditions and using the three different initial concentrations (6.25, 12.5, and 25 mg OII/L) on the alginate beads and in the absence of *S. cerevisiae* (Figure 2b) showed that OII had high adsorption affinities on alginate. After equilibrium was achieved, OII concentration did not vary any more: one could consider that an equilibrium was reached and an adsorption isotherm could be deduced, as

displayed in Figure 2b. This highlights an "unfavrouable" isotherm trend that could be fitted by a Freundlich isotherm $q=kc^{1/n}$ with $n<1$.

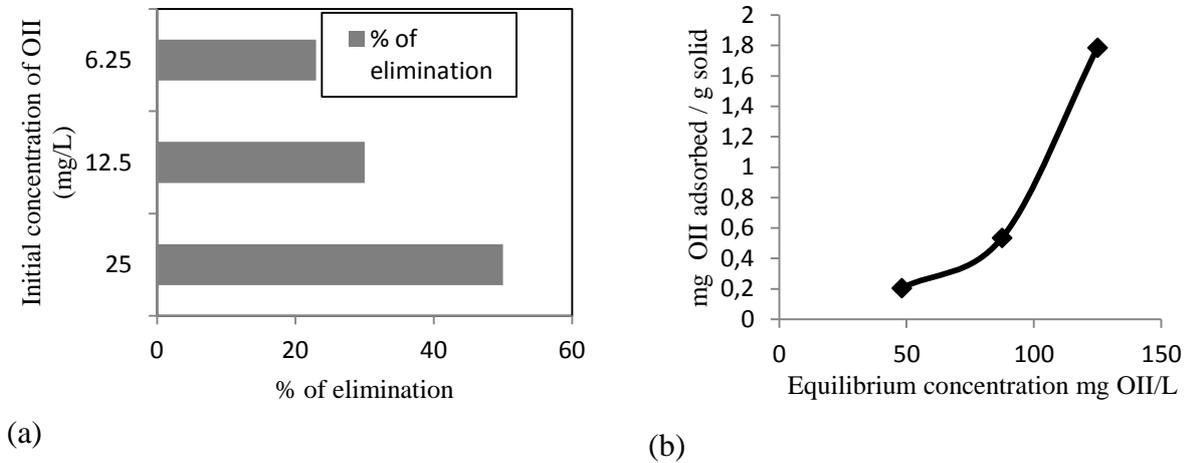


Figure 2 : (a) Removal yield of OII as a function of its initial concentration; (b) isotherm of OII adsorption on the alginate beads at pH 1 and a dry mass of alginate of 7 g/ 100 mL in the absence of *S. cerevisiae*.

In the case of CBZ and DCF, the same behaviour as for OII was followed under the effect of changing the initial concentration on elimination, *i.e.* for all values of pH, mass of alginate beads, in both the absence and the presence of *S. cerevisiae* at pH 1 and for a dry mass of alginate of 7 g/100 mL in the absence of *S. cerevisiae*. For example, on the one hand, the elimination yields of CBZ increased from 3 to 10% when increasing the initial concentration of CBZ from 2.5 to 10 mg/L at pH 7 in the absence of *S. cerevisiae* using 2.5 g/100 mL of alginate (Figure 3a). On the other hand, the elimination yields of DCF increased from 19 to 43% when the initial concentration of DCF increased from 0.5 to 2 mg/L (Figure 3b).

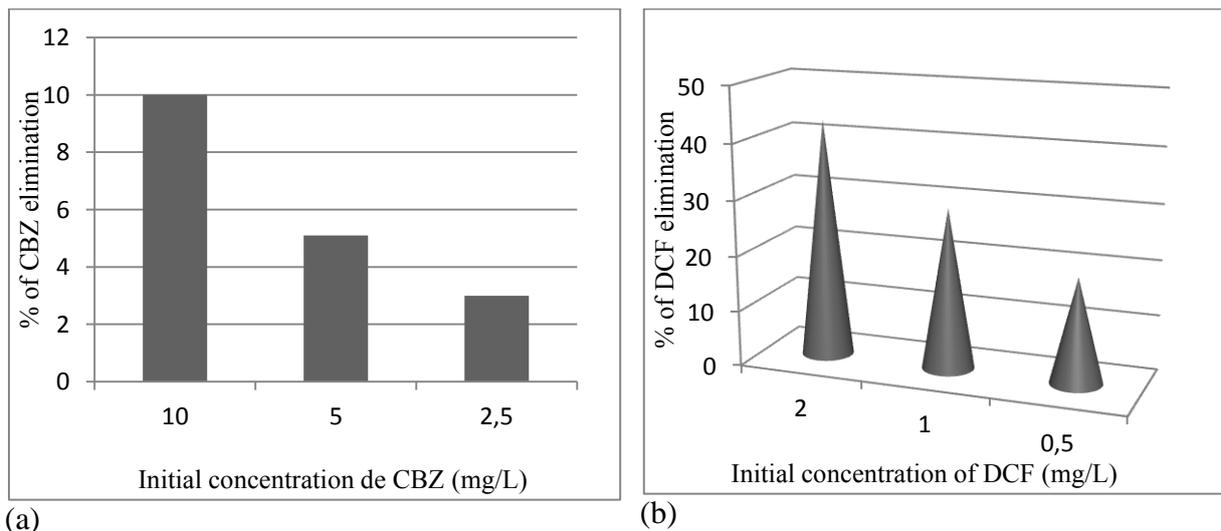


Figure 3: Elimination yield of: (a) CBZ at pH 1 for dry mass of alginate of 7 g/ 100 mL in the absence of *S. cerevisiae*; (b) DCF at pH 1 and alginate dry mass 10 g/100mL; as a function of the initial concentration of pollutant.

3.2 EFFECT OF THE DRY MASS OF ALGINATE BEADS AND TREATMENT DURATION OF OII, CBZ AND DCF

The increase in the mass of alginate beads used in the experiments induced an increase in the OII, CBZ and DCF elimination yield for all the studied conditions (for all pH values, with or without *S. cerevisiae*). Figure 4 highlights this effect on the removal yield at pH 7 without *S. cerevisiae* for an initial concentration of 6.25 mg/L OII. This figure displays a slight, but significant, increase in the elimination percentages from 2% to 12% when the dry mass of alginate beads increases from 2.5 to 10 g/100 mL. Moreover, Figure 5 shows the reponse of CBZ elimination when the mass of alginate is increased at an initial concentration 5 mg/L using 10 g/100 ml alginate. It proves that elimination yields increased from 3% at 2.5 mg/100 mL to 9% in the presence of *S. cerevisiae*. The same trend was seen with DCF for which the elimination yield increased from 5% to 14% at pH 5 when the initial concentration of DCF was 1 mg/L in the absence of *S. cerevisiae* (Figure 6).

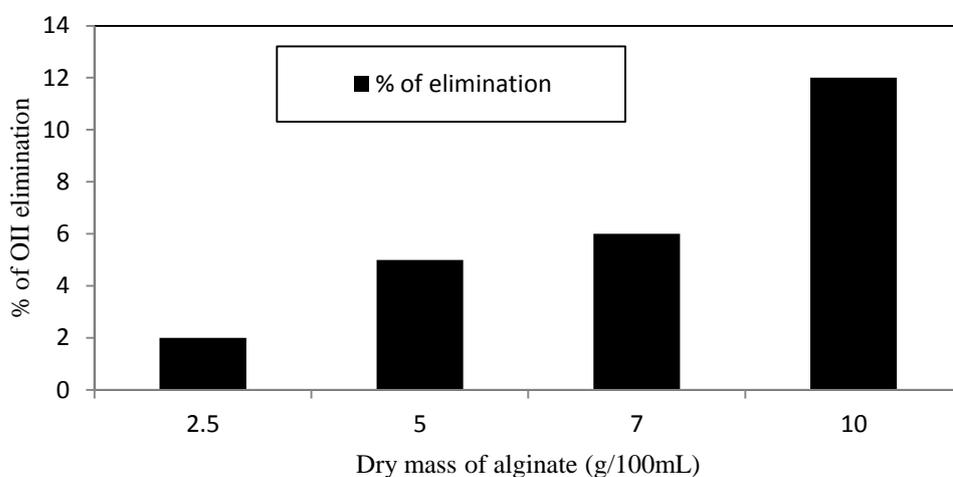


Figure 4: Elimination yield of OII as a function of the dry mass of alginate at 6.25 mg/L OII concentration and pH 7 in the absence of *S. cerevisiae*

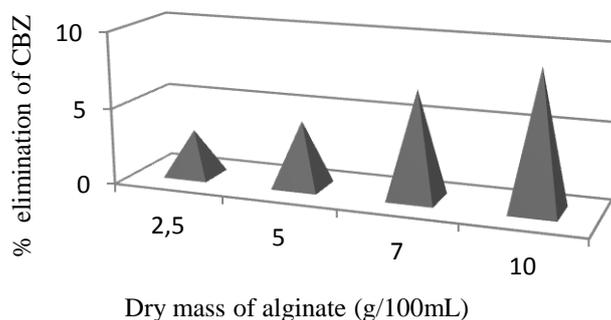


Figure 5: Elimination yield of CBZ as a function of alginate dry mass at pH 5, initial concentration of CBZ of 5 mg/L in the presence of *S. cerevisiae*

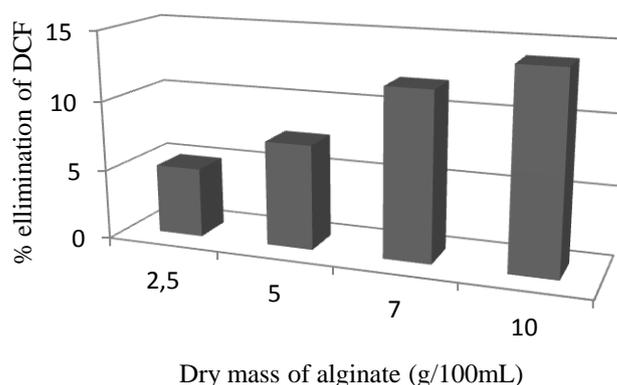


Figure 6: Elimination yields of DCF as a function of dry mass of alginate at pH 5 and initial DCF concentration of 1 mg/L in the absence of *S. cerevisiae*

The spectrophotometric data after 24 hrs. of the onset of the treatments were compared to those obtained after 8 hours. The elimination yield of OII increased after 24 hrs. in all the conditions studied. For pH 1, for instance, a dry mass of alginate of 10 g/100 mL with an initial concentration of 25 mg/L, without *S. cerevisiae*, the elimination percentage increased by 5% from 25% to 30%. However, in the case of CBZ and DCF, the HPLC analyses showed that, generally, there was almost no variation in the eliminated amount of CBZ and DCF where the same tests on the spectrophotometry showed the increase of these values between 8 and 24 hours of contact.

4. DISCUSSION

For the three pollutants, OII, CBZ and DCF, the results highlighted that at pH between 5 and 7, the highest removal efficiency is achieved in the absence of *S. cerevisiae*. This shows that it is mainly due to the alginate beads that entrap the pollutants. Indeed, the addition of *S. cerevisiae* in the beads results in a decrease in mass of the alginate in the beads, consequently lowering the percentages of elimination. Knowing that calcium alginate has no metabolic activity, an adsorption mechanism is probably responsible for capturing the molecules in the gel porosity. This analysis is fortified by the fact that the removal efficiency increases with both the mass of alginate beads and the initial concentration of pollutant.

In all cases, in the presence of *S. cerevisiae*, the results showed that the highest removal efficiencies are achieved at pH 1, for the three pollutants. For diclofenac and OII, there is a possible change in the chemical structure of the molecules at pH 1. Indeed, the DCF is an acid with a pKa about 4, which means that it is dissociated at both high pH studied (5 and 7), but not at pH 1. This could be further justified by the fact that the anionic form is known to adsorb less than the neutral form. However, the trend observed at pH 1 cannot be only explained by the protonation of OII, as pKa of OII is about 11. Several interpretations are possible: a degradation of OII by the yeast, adsorption on *S. cerevisiae* or bioaccumulation in the yeast... In the case of CBZ, this pollutant stays in its neutral form at all pH values studied (1, 5 and 7). However, its highest elimination at pH 1 could be correlated to how alginate carboxyl group behave by changing from COO^- to COOH , which renders it more hydrophobic.

Alginate is, indeed, a carboxylated polysaccharide wherein the carboxyl groups have a pKa about 3.5; although anionic carboxylates are stabilized by calcium ions into the beads, it is possible that some of them are protonated at pH 1. The fact that the protonated forms adsorb more suggests that either the interaction between alginate and pollutants are mainly of hydrophobic type, or of hydrogen bonds. For *Saccharomyces cerevisiae*, due to the poor contribution of the yeast in the conventional pH range, we will not investigate further to distinguish biosorption from bioaccumulation on *S. cerevisiae*, but we will conclude that both the parallel mechanisms of adsorption on alginate and on the yeast increase when the pH decreases.

At pH 1, we see that for all the pollutants, the removal efficiency is higher in the presence of *S. cerevisiae*. In any case, it shows that an additional mechanism is added, but not necessarily the main one, to the adsorption on the alginate. Several interpretations are possible: a degradation of molecules by yeast or adsorption on *S. cerevisiae*. The results obtained on HPLC validate the first proposition since CBZ was observed as a peak at 18 minutes and another peak at 3 min, which tends to be a metabolite.

For CBZ and the DCF, after 24 hours, there is an increase of the values obtained by spectrophotometry, even in the absence of additional pollutant disposal. This can be either explained by the little dissociation of the CBZ and the DCF adsorbed after 24 hours - as the analyses done on the HPLC did not give huge variations from the concentrations obtained at 8 hours, or there is a possible dissolution of alginate at low pH value, herein pH 1. In conclusion for both molecules, it does not seem necessary to extend the treatment beyond 8 hours.

In practice, adsorption is rather slow. For OII, however, the removal percentage increases after 24 hours; this shows that there is still elimination of the dye between 8 and 24 hours. It may be the extra time needed by the dye to diffuse into the beads. Thus, two solutions could rise for the treatment of OII with alginate; the first might be the elongation of the contact time between the alginate and the OII dye, and the second might be the employment of beads of smaller diameter to facilitate the OII access into these beads. Finally, when the OII concentration increases, the removal efficiency increases, which corresponds to an unfavorable isotherm and means that this approach is not suitable for very low concentrations, *i.e.* when OII is a micropollutant.

In general, it was found that when the solution concentration increases, the removal efficiency increases. This can be related to the high possibility that the alginate beads were not saturated. When the mass of alginate increases, the removal efficiency increases, as expected. This is justified by the increase in the beads number available for adsorption and consequently the contact surface area between the alginate and the pollutant which could also enhance the removal kinetics.

5. CONCLUSION AND PERSPECTIVES

This study aimed to verify the efficiency of the use of *Saccharomyces cerevisiae* immobilized on alginate of two pharmaceuticals, namely Carbamazepine and Diclofenac and the dye Orange II in water, three biorefractory molecules with different chemical structures and properties. We showed that OII was the molecule the most highly eliminated among the others. Our results explained that the alginate beads were able to adsorb OII, CBZ, and DCF in the presence and in the absence of the yeast confirming the adsorptive role of alginate.

Moreover, protonation of the alginate at low pH (pH 1) was perhaps a secondary mechanism explaining the high elimination percentages at this pH. It was found that the elimination yield increases with the increase of initial concentration of OII, CBZ and DCF, with the increase of the dry mass of alginate beads and with treatment time. The highest removal attained of 80% was achieved for OII, this value was reported at pH 1 and the yield collapsed at pH between 5 and 7. Accordingly, *Saccharomyces cerevisiae* supported on alginate beads does not allow to achieve sufficient removal efficiencies under conditions of interest of pH values. However, this does not hinder the possibility of the use of other microorganisms supported (other than *Saccharomyces cerevisiae*) on alginate beads in order to benefit from the adsorbent alginate capacity and thus facilitating their elimination by these microorganisms.

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CONCLUSIONS AND PERSPECTIVES

This thesis comprises two submitted literature review articles and four articles three of which are published/ and one is submitted. The main objective of this work was to develop a technically and economically reliable non-conventional processes for the quantitative removal of two water pollutants, namely nitrates and the biorefractory pharmaceutical Carbamazepine (CBZ). In this context, two different processes have been studied. Nitrate was first treated electrochemically using electrocoagulation (EC), and the success of this process for denitrification (95% yield) led to manage its implementation for CBZ removal. The treatment of CBZ with EC, however, was not as efficient as for nitrates (only 62% yield). An alternative based on phycoremediation was, therefore, developed. This biological treatment is based on the green algae called *Ankistrodesmus braunii* (*A. braunii*) and an enhanced removal of CBZ was reached, about 87%.

In more details, the first study was subjected to remove nitrates when found at relatively higher concentrations of those found in nature using EC process. EC, a non-specific electrochemical process for water depollution, has been shown to be able to remove efficiently nitrate anions, whatever the initial concentration, and to reach nitrate concentrations far below the guideline value. This agrees with literature data, but no general conclusion could be found on the mechanism of nitrate removal in the literature in which electro-reduction into ammonium or into nitrogen gas, or even adsorption were proposed as the main mechanism. This work first proves that nitrates are first electro-reduced into ammonium cations, with nitrites as intermediates, and then, that nitrogen gas released was found negligible. Conversely, the elimination of nitrogenous species was found to be mainly due to the adsorption of ammonium on the oxyhydroxide flocs produced by electrocoagulation process. Both phenomena, namely the electro-reduction of NO_3^- into NH_4^+ and the NH_4^+ adsorption on the flocs, are consecutive mechanisms that proceed at the same time, following first-order and zero-order kinetics, respectively. It was also found during this work that the use of EC, other than being an efficient depollution process of nitrates, could be at the same time cost effective when used to pretreat wastewater comprising multiple contaminants that cannot be removed by the conventional biological denitrification process. This work was finalized by defining a simple model that can predict the nitrogen speciation and nitrogen removal during EC.

The second part of this work was the implementation of EC process for the removal of CBZ, compound for which most biological processes fail. The collected experimental results have proved that the electrochemical oxido-reduction of CBZ during the EC process mostly takes only place at a pH lower than 4 and under high current. Further investigations were conducted on the fate of CBZ; it was found that the removal of a fraction of the initial carbon content was due to the adsorption of a possible metabolite of CBZ on the aluminium hydroxide flocs produced during the EC process. The comparison of the cost and of the efficiency of electrocoagulation to those of other depollution treatments on CBZ, it could be concluded that the 62% elimination yield was high enough using EC, but only in a narrow range of pH. A more robust treatment is, therefore, necessary to avoid the cost of pH adjustment.

As a continuation of the second part of this work, a third study consisted of the treatment of CBZ using a biological process. Phycoremediation as an original process was studied the use of green algae, *A. brauni* for CBZ elimination. Experimental data proved that this microalgae

can be effectively used to remove CBZ from water, especially when high inoculum concentrations are used. It is also efficient when CBZ is found at low concentrations which renders this process more applicable to the current concentrations in wastewater (except hospital wastewater in which concentrations can be close to those of this work) and thus more feasible. Moreover, the further analysis of the fate of CBZ confirmed that about 80% of the CBZ removed was accumulated in *A. braunii*, but the presence of several metabolites found in the cell free supernatant and in the cells indicated that a biotransformation of CBZ also occurred in parallel. This constitutes a highly interesting result because the biosorption of CBZ usually remains weak. By a comparison with diclofenac and an azo textile dye, Orange II, that are also biorefractory, Orange II was shown to be easily removed by *S. cerevisiae* immobilized on alginate beads, while CBZ as in the case of DCF, was not removed, except at very low pH. In this case, both biosorption and metabolization were not observed at neutral pH, which confirms the interest of phycoremediation.

Therefore, it emerges that further work is still needed because none of the methods studied to remove CBZ are fully satisfactory up to now. EC is able to remove various pollutants at the same time, but acts on CBZ only in a narrow range of pH, while phycoremediation is efficient, but very slow and the algae must be recovered after treatment because they contain CBZ. Even for nitrates, EC cannot compete economically with the conventional denitrification treatment, except when biorefractory compounds have to be removed in parallel to nitrates. So, the perspectives of this work can be summarized as follows:

- For nitrates, an obvious but challenging continuation of this work would be to implement the influence of the pH in the model and to better estimate the dependence on pH of NH_4^+ formation and adsorption. This cannot be found in the literature and would constitute a significant improvement for the design and scale-up of EC process;
- For nitrates, still using EC, the perspective is also to apply a continuous process using the continuous EC cell available in the laboratory. The aim is to apply alternating polarity on the electrodes so that they behave periodically as anodes and cathodes, in an automated process, as in the industry;
- For nitrates using EC, finally, the process can be assessed by treating a “cocktail effect” involving multiple classes of contaminants, and co-anions (e.g. by the addition of phosphates, heavy metals...), and study the effect of their presence on the denitrification process. It would then be a reasonable perspective to upgrade the use of EC to the treatment of these compounds found in real river or ground water;
- For phycoremediation, the first perspective is to assess the ability of the algae to remove CBZ in the presence of other substrates. The objective is to check whether the CBZ will continue to be metabolized and consumed by *A. braunii* when carbon and nitrogen compounds easy to assimilate are present;
- The second perspective is to enhance the growth kinetics of microalgae, using photobioreactors, so as to enhance productivity, even though high productivity photobioreactors are probably too expensive for the objective of wastewater treatment;
- Finally, EC could also be used as a separation process for phycoremediation. EC is known to be a very efficient technique to harvest microalgae for 3rd generation biofuels. Results obtained in master program and an ongoing Ph.D. at Institut Pascal have shown that this result is not general: *Chlorella vulgaris* can be easily harvested, but this is not the case of *Spirulina platensis* that sticks on the electrodes. As a result, EC as a recovery process for

the algae that have accumulated CBZ should be studied, which could open the possibility to define a hybrid physicochemical/biological process for water treatment.

Abstract

Water is vital to the existence of all living organisms, but this valued resource is increasingly being threatened and polluted as human populations and activities grow and demand more water of high quality for domestic purposes and economic activities. Wastewater treatment for resource preservation is nowadays one of the first concerns of research in this field of science. In this work, two typical pollutants from agriculture and domestic activity, Nitrates and Carbamazepine, are quantitatively addressed by non-conventional electrochemical and biological treatment methods. The study focuses, on the one side, on electrocoagulation (EC) that exhibits the advantages to be non-specific and to combine various depollution mechanisms (adsorption, electro-oxidation...) that act simultaneously; on the other side, innovative and low-cost biological treatments using green algae, *Ankistrodesmus braunii*, are developed. Finally, the respective advantages, limitations and perspectives of these processes are compared to the literature and discussed.

Key words:

Electrocoagulation, Phycoremediation, Carbamazepine, Nitrates, Ankistrodesmus braunii

Résumé

L'eau est vitale pour l'existence de tous les organismes vivants, mais cette ressource précieuse est de plus en plus menacée et polluée à cause de l'augmentation de la demande en eau potable qui résulte à la fois de l'accroissement de la population mondiale mais aussi de l'activité économique tant au niveau de l'agriculture que de l'industrie. La préservation de cette ressource est aujourd'hui l'une des premières préoccupations de la recherche dans le domaine du traitement des eaux. Dans ce travail, l'élimination de deux polluants typiques des activités humaines, les nitrates et la carbamazépine, est étudiée au moyen de méthodes de traitements électrochimiques et biologiques non-conventionnelles. Le travail se concentre d'une part sur l'électrocoagulation (EC) qui associe les avantages d'être non-spécifique et de combiner plusieurs mécanismes de dépollution simultanés (adsorption, électro-oxydation ...); d'autre part, un traitement biologique innovant de faible coût utilisant une algue verte, *Ankistrodesmus braunii*, a été développé. Enfin, les avantages, limitations et perspectives de ces deux procédés sont comparés à ce qui existe dans la littérature et sont discutés.

Mots-clés :

Electrocoagulation, phycoremediation, carbamazépine, nitrates, Ankistrodesmus braunii