Year: 2020 **Thesis N°: 184 /ST2I**

جامعة محمد الخامس بالرباط
Université Mohammed V de Rabat

École Nationale Supérieure d'Informatique et d'Analyse des Systèmes
Centre d'Études Doctorales en Sciences des Technologies de l'Information et de l'Ingénieur

THÈSE DE DOCTORAT

INDUSTRIAL WASTEWATER TREATMENT AND ENERGY PRODUCTION BY MICROBIAL FUEL CELLS: NOVEL MEMBRANES, CONFIGURATIONS FOR CONTINUOUS FLOW AND MICROALGAE APPLICATION

Presented by:

Zakarya BAICHA

October 17th, 2020

Doctoral program: Engineering Sciences- Environmental Engineering
Research team: Materials and Environmental Engineering

JURY MEMBERS

Professor El Mostapha LOTFI PES, ENSAM, Mohammed V University in Rabat	**Chairman**
Professor Mohammed El MAHI PES, ENSAM, Mohammed V University in Rabat	**Thesis director**
Professor Moulay Abderrahim El MHAMMEDI PES, FPK, Sultan Moulay Slimane University, Khouribga	**Reporter**
Professor Souad EL HAJJAJI PES, FSR, Mohammed V University in Rabat	**Reporter**
Professor Malika SERGHINI IDRISSI PES, FSR, Mohammed V University in Rabat	**Reporter**
Professor Abdelmajid SKALLI PES, ENSET, Mohammed V University in Rabat	**Examiner**
Professor Najoua LABJAR PH, ENSET, Mohammed V University in Rabat	**Examiner**

Abstract

Climate change is a complex and cross-cutting problem that needs an integrated and transformative systems approach to respond to the challenge. The complex interdependencies between climate change, water, energy and other social and environmental factors present a challenge for researchers and policy makers.

MFCs (microbial fuel cells) are an emerging biotechnology that could contribute to overcome the current energy crisis and meet water needs in developing world countries. Thus, this technology has become the focus of many research studies trying to improve its performance by investigating alternative materials and determining optimal operating conditions. In this work, a new single-chamber air-cathode microbial fuel cell configuration has been used to operate in continuous mode with vertical and horizontal up-flow. This design incorporates a novel embedded ionic liquid-based membrane-cathode assembly working as separator. The ionic liquid selected for the present work is triisobutyl (methyl) phosphoniumtosylate, $[P_{I4;I4;I4;1}^{+}][TOS^{-}]$. MFC performance is investigated in terms of electricity production and wastewater treatment for various feed flow rates. It has been concluded, that the effectiveness of treatment is directly dependent to the HRT, pH, T° operated in continuous mode, and the highest performance of wastewater treatment was reached for the flow of 0.25 $ml.min^{-1}$.

Nutrients transport through polymer inclusion membranes based on different concentrations of methyltrioctylammonium chloride, was also investigated in this work, in order to broaden the application range of these kinds of membranes. Calcium chloride ($CaCl_2$) and sodium hydrogen phosphate (Na_2HPO_4) nutrients were used at the concentration of 1 $g.L^{-1}$ in the feeding phase. The evolution of the concentration in the receiving phase over time (168 h) was monitored and the experimental data fitted to a diffusion-solution transport model. The results show very low permeation values for $CaCl_2$.

By contrast, in the case of Na_2HPO_4 the permeation values were higher and increase as the amount of ionic liquid in the membrane also increases. The surface of the membranes was characterized before and after being used in the separation process by scanning electron microscopy coupled to energy dispersive X-Ray spectroscopy (SEM–EDX) and elemental mapping analysis. The SEM–EDX images show that the polymer inclusion membranes studied

are stable to aqueous solution contacting phases and therefore, they might be used for the selective transport of nutrients in separation processes.

Microalgae have been identified as one of the newest bio-material due to its use in microbial fuel cells (MFCs). The resulting microalgae-MFC systems can produce electricity using the electrons released to the anode during microalgae degradation. Furthermore, microalgae can be grown in the cathode chamber, capturing the CO_2 therein and using light as power source.

Keywords:

Bioenergy, Continuous microbial fuel cell, Energy production, Industrial wastewater, Ionic liquids; Membrane stability, Microbial fuel cells, Microalgae, Microalgae-microbial fuel cells, Nutrients, Polymer inclusion membrane, Permeation, Wastewater treatment

Acknowledgement

Over the course of conducting this research, I was extremely fortunate to have been surrounded by many great people who have challenged me and provided opportunities to learn and grow.

Firstly, I much appreciated the most valuable support, advice and comments from my supervisor, Professor Mohammed El MAHI from ENSET- Mohammed V University in Rabat-ENSET, I would like to express my gratitude to him for giving me the possibility to learn more by having responsibilities beyond my PhD thesis and for his invaluable and warm assistance and endless support during this long doctoral experience.

I warmly thank Pr. Francisco Jose Hernández-Fernández, from Technical University of Cartagena, Spain, who gave me the opportunity to work in his research group in the Department of Chemical and Environmental Engineering. His guidance during this period has been extremely valuable. Thank you for your insight, and feedback throughout this process, your wisdom, support and optimism have made this difficult endeavor possible.

I would like to express my sincere thanks to the supervisory committee members; Pr. El Mostapha LOTFI, from ENSET, Mohammed V University in Rabat, Pr. Moulay Abderrahim El MHAMMEDI, from FPK, Sultan Moulay Slimane University. Pr. Souad EL HAJJAJI and Malika SERGHINI IDRISSI from FSR, Mohammed V University in Rabat, Pr. Abdelmajid SKALLI and Najoua LABJAR from ENSET, Mohammed V University in Rabat who generously committed themselves to the review of this work, for valuable advice and insights on my studies.

Secondly, many thanks are due to co-authors and colleagues, María José Salar-García, Víctor Manuel Ortiz-Martínez, F.J. Hernández-Fernández, Antonia Perez de los Ríos, Diego P. Maqueda Marín, J.A. Collado and F. Tomás-Alonso, Salma and Lahbib. To my friends in Universidad Politécnica de Cartagena who were always sources of joy and moral support, I would never forget the positive energy and true friendship that I've had with Khaled Touati, Professor Carlos Godinez, Laura, Teresa Canales, Masood, Iacob, Denis, Tereza, Denisa, Oxana, Andrej, and Yassine.

Finally, my whole heartfelt gratitude to my family. Words cannot express how grateful I am to them: my lovely Mother for her unconditional love and motivation, dear Father for all his constant support and encouragement, my brothers, sisters for their support, love and

encouragement. A special thanks to my lovely wife for her patience and support during all the period of my PhD. All the people who supported loved and encouraged me and told me that everything is possible as long as you decide so. I'm very proud and fortunate to have them as my family.

Thank you! Without your love and support, I don't think I can persist to the end of my PhD track.

Funding: Erasmus+ program (Ref: 2015-1-ES01-KA107-014539) en collaboration with the Technical University of Cartagena. the Project EU Mare Nostrum, Erasmus Mundus Action 2, Strand 1, Lot 1 (Ref: 2011 4050/001-001 EMA2).

Table of contents

Chapter IV: Use of microalgae as alternative in microbial fuel cells

Chapter V: General Conclusions and Recommendation for Future Research

List of Tables

Chapter II:

Chapter III:

Chapter IV:

List of Figures and Illustrations

Chapter I:

Chapter II:

Chapter III:

Chapter IV:

List of Symbols, Abbreviations and Nomenclature

AEMEDSA	Aceites Especiales del MEDiterraneo S.A
BOD	Biochemical Oxygen Demand
$CaCl_2$	Calcium chloride
CE	Coulombic efficiencies
CO_2	Carbon dioxide
COD	Chemical Oxygen Demand
COD_R	Chemical Oxygen Demand consumed
Cl^-	Chloride
EC	Electrical Conductivity
EDX	Energy dispersive X-ray
Eh	Redox Potential
EIS	Electrochemical impedancespectroscopy
ETC	Electron transport chain
FAMEs	Fatty acid methyl esters
FAO	Food and Agriculture Organization
Fe^{2+}	Ferrous ion
FRA	Frequency response analysis
GHG	Greenhouse gas
H_2	Hydrogen
HPO_4^{2-}	Hydrogen phosphate

HRT	Hydraulic retention time
I	Current
IC	Ionic chromatography
IEA	International Energy Agency
IL	Ionic Liquid
MCA	Membrane cathode assembly
MEA	Membrane electrode assembly
MFC	Microbial Fuel Cell
N	Nitrogen
N_2	Dinitrogen
Na^+	Sodium
Na_2HPO_4	Sodium hydrogen phosphate
NO_3^-	Nitrate
OCV	Open Circuit Voltage
[OMIM][NTf_2]	1-Octyl-3-Methylimidazolium Bis(trifluoromethanesulfonyl)imide
ORR	Oxygen reduction reaction
P	Phosphorus
PBRs	Photobioreactors
PEM	Proton exchange membrane
PEMFC	Proton exchange membrane fuel cell
pH	Potential Hydrogen
[$P_{I4;I4;I4;1}^+$][TOS^-]	Tri-iso-butyl(methyl)phosphonium tosylate

PILIMs	Polymer ionic liquid inclusion membranes
PIM	Polymer inclusion membrane
PMFCs	Phosynthetic microbial fuel cells
PVC	Polyvinylchloride
R_{ext}	External load resistance
rpm	Rotation per minute
SEM	Scanning electron microscopie
SO_4^{2-}	Sulfate
SILMs	Supported ionic liquids membranes
THF	Tetrahydrofuran
TP	Total Phosphorus
TSS	Total suspended solids
UFM	Ultrafiltration membrane
V	Voltage
WWTP	Wastewater Treatment Plant
Ω	Ohm

General Introduction

Global situation:

Climate change is a complex and cross-cutting problem that needs an integrated and transformative systems approach to respond to the challenge. The complex interdependencies between climate change, water, energy and other social and environmental factors present a challenge for researchers and policy makers.

Furthermore, governments have identified water as a key to climate change adaptation and most efforts to reduce greenhouse gas emissions depend on reliable access to water resources.

Nowadays worldwide, access to safe water of about 3.5 billion people are not fulfilled, nearly 2.5 billion people do not have admittance to improved sanitation and around 768 million people do not have admittance to improved source of water, and water contamination and lack of sanitation and hygiene cause approximately 1.7 million deaths every year.

According to some estimation, through 2050 more than 40% of global population will live in conditions of severe water stress. The population growth, economic development and urban expansion will increase demand of freshwater in the future, which in turn will affect water-intensive industries.

The United Nations has completed recently a report that calls for a radical rethink of policies to manage fresh drinking water. It has been mentioned that freshwater is not being used sustainably, and water management is fragmented, concluding that the future of the humanity is increasingly uncertain, and risks are set to deepen. Efficient management of water resources is therefore of vital importance, especially in areas that are heavily urbanized or industrialized.

Morocco was listed among the 27 countries facing a high level of water stress according to a report issued recently by the World Resources Institute. Morocco was ranked 22nd in a list where Qatar, Lebanon were described as most countries facing an extremely high level of water shortage threat.

By 2020 Morocco is predicted to have a water crisis. The South of Morocco, for instance, is already under considerable water stress. Food, chemicals, electronics, construction materials, mechanical, metal, leather, and textiles are the main industries in Morocco. The north west of the country knows the major concentration of industrial activity. The majority of domestic and industrial wastewater of the urban and rural centers is released in the nature, without

preliminary treatment. "Rivers receive directly approximately 30% of total water pollution and 27% is absorbed by soil and groundwater. Industrial effluents convey heavy organic and toxic pollution. 98% is poured into the sea (944.7 million of m^3) and the remainder into the water network or directly onto the ground".

From another side and according to the latest World Energy Council's Energy, Morocco ranks 68^{th} in energy dependency. High external energy dependency is the main reason behind a very poor performance in the area of energy security – and responsible for this low overall rank. The other two dimensions of energy equity and environmental sustainability are similarly below ideal levels. Morocco spent 69 billion MAD on imported energy in 2017. However, energy demand has been continuously growing over the last ten years at an annual rate of 4%.

The world is dependent on energy both for human wellbeing and society's continued development. Energy use is however also one of the human systems that is most directly influenced by changes in climate, which makes it crucial to gain insight into the impacts of climate change on energy demand.

Indeed, the most widely used energy sources are based on fossil fuels even though it is well know that the reserves of oil, gas, coal or uranium will be exhausted in 55–75 years. The increase in the world population, estimated at around 9.7 billion people in 2050, along with a sharp economic growth of emerging countries as China and India, will increase the energy needs of the world.

Energy demand worldwide grew by 2.3% last year, its fastest pace this decade, an exceptional performance driven by a robust global economy and stronger heating and cooling needs in some regions. The continued use of fossil fuels has several negative environmental impacts due to greenhouse gas (GHG) emissions to the atmosphere, such as CO_2, which contributes to global warming and the acidification of the oceans.

The long-term solution to such environmental problems is the development of renewable technologies to produce energy that will reduce CO_2 emissions to almost zero in 2100. To stimulate and promote research in the bioenergy field new policies have been adopted around the world. Thus, in July 2015, Denmark generated 140% of its electricity needs from wind energy and shared its excess with other countries.

This demonstrates that renewable energies are feasible for satisfying the world's energy needs. A recent report from the International Energy Agency (IEA) reveals that the energy produced from biofuels and waste have the highest potential. The energy obtained from biofuels and waste represented 10.0% of the world's total primary energy supply, compared with 2.4% from water, and 1.1% from other sources such as the wind or the sun. Therefore, it is expected that biofuels will play an important role in the future as sustainable energy source.

MFCs (microbial fuel cells) are an emerging biotechnology that could contribute to overcome the current energy crisis and meet water needs in developing world countries. MFC is a device that transforms the chemical energy present in a substrate into electrical current. Simple substrates such as acetate or lactate can be used as fuel, but the real potential of this technology lies in employing different types of wastes as fuel, usually domestic or industrial wastewater.

In this way, microbial fuel cells would allow these effluents to be treated while simultaneously generating electricity, offering a two-fold benefit. However, in order to facilitate the commercialization of this technology, it is necessary to reduce the overall cost of the devices as well as improving the energy harvesting. Thus far, different kinds of membranes have been explored, for instance, Ion-Liquid-based membranes have shown promising results in gas separation and metal extraction. The use of IL-based membranes addresses both challenges by replacing the expensive and sometimes low-efficiency commercial membranes.

Another available option to produce bioenergy and one of the most promising is the use of microorganisms. Microalgae are photo synthetic microorganisms that have the ability to accumulate lipids, hydrocarbons, etc.

Many studies demonstrate that the development of this biotechnology could supply 30% of the global fuel demand in an environmentally friendly way, without having a negative impact on food production. Some research works confirm that the production of biodiesel using microalgae is more sustainable than its production from agricultural crops since the process does not affect the supply of agricultural products.

Recent research on microalgae have recognized this new bio-material as an auspicious technology for wastewater treatment, bioenergy production, high value added products development and CO_2 capture. Microalgae can be used to produce bioethanol, biodiesel, hydrogen or methane.

In recent years, MFCs technology was combined with algae as the oxygen supplier. In this case, bacteria degrade the organic matter in the anodic chamber while algae grow in the cathodic compartment, providing the oxygen necessary for completing the redox reaction on the cathode. Furthermore, algae are able to capture carbon dioxide and the algal biomass produced could be used for the production of added-value compounds.

This novel approach could improve the wastewater treatment efficiency and the power performance of MFCs.

Moreover, the transport of specific compounds from the anode to the cathode might also help algae growth, reducing the nutrients added to the cathode chamber. The selective transport of nutrients through the membrane will allow us to design a more efficient system for both wastewater treatment and bioenergy production.

Consequently, our work will focus on the following aspects:

- Wastewater treatment and energy production by continuous MFC
- Nutrients transport throught membrane for microalgae culture
- Microalgae as alternative in MFC

Overview, objectives and focus of the work

Nowadays, society is facing various problems, among which are finds the wastewater treatment and the high amount of energy linked to those equipments, including the problem of energy dependence on fossil fuels and environmental problems caused by this kind of fuels.

Fossil fuels are an ideal source of energy, as they provide a high-density, transportable energy. But the current energy model based on fossil fuels has three major drawbacks. On the one hand, the inevitable lower rate of return and its non-renewable character. On the other hand, the problem of environmental pollution, both globally and locally perceived which derives from its intense use. Finally, the geographical distribution of the reserves, only a few countries control the world production of these hydrocarbons, with the consequent economic consequences and shortages that a political conflict between these countries and the rest could be generated. Therefore, the development and implementation of alternative renewable energy is the subject of many studies.

As for the problem of wastewater, all activities carried out by humans involve the generation of contaminated effluents. In the case of domestic and industrial wastewater, the main pollutant is organic matter. The best-known treatment for eliminating organic matter from wastewater is the biological activated sludge process. This treatment has some drawbacks, such as the high energy consumption involved in supplying oxygen and generating a large amount of sludge. Furthermore, this treatment does not make use of the energy content of the organic matter in the wastewater. If this energy could be recovered, wastewater treatment plants could become self-sufficient. Currently, a large number of research groups are studying the energy recovery of wastewater. One of the newest technologies that are receiving more attention is microbial fuel cells.

The main objective of this Doctoral Thesis was the wastewater treatment of effluent whose main pollutant is biodegradable organic matter, such as wastewater from the oil industry, and energy recovery by means of microbiological fuel cells. The aim was not only to remove organic matter from the wastewater but also to produce electricity taking advantage of the energy content of the organic matter removed. The main objective has been divided in three specific research works:

- Study the treatment of industrial oil wastewater and the simultaneous production of electricity in a self-sustaining and environmentally friendly Microbial Fuel Cell that performs in two different reactors with two different flows, with a focus on factors influencing wastewater treatment in microbial fuel cell.
- Study the nutrients transports through polymer inclusion membranes based on ionic liquids, in the aim of their potential application in double-chambers microbial fuel cells, giving a new way to feed microalgae in double compartments MFCs in the aim of their culture.
- Present methods that allow taking advantage from microalgae benefits by their use in MFCs systems, either in a double chamber MFC or in a single countinous flow MFC, It can be used in the anode as an electron donor or in the cathode as an electron acceptor.

This PhD thesis consists of five chapters; it includes a background discussion of the most relevant literature regarding wastewater treatment and energy production by microbial fuel cells using new configurations and substrate.

This dissertation is divided into five chapters:

Chapter 1 is a general introduction to separation processes, reactor design, single & double chamber microbial fuel cell configuration.
The basis of this first chapter is also to provide a general background of operational factors affecting MFC performance, electron transfer mechanisms, investigation of IL based membrane in transport, practical applications, scale-up, and finally resuming the opportunities and challenges for MFCs in wastewater treatment.

Chapter 2 describes Ionic-liquid based PIMs, tested mainly as separator in new horizontal and vertical configurations of MFCs fed with wastewater. the efficiency of wastewater treatment through the MFC system, the effluent from the reactor is examined with regard to, COD (Chemical Oxygen Demand), total P (Phosphorus), TSS (Total Suspended Solids), SO_4^{2-} (sulfate), Cl^- (Chloride), NO_3^- (Nitrate), HPO_4^{2-} (Hydrogen phosphate), Fe^{2+} (Iron) and pH, Total suspended solids, hardness, alkalinity and ion analysis.

Chapter 3 aim is to analyze the transport of nutrients such as $CaCl_2$ and Na_2HPO_4 through polymer inclusion membranes based on ammonium-based IL. The concentration evolution in

the receiving phase over time is monitored and the experimental data fitted to a diffusion-solution transport model.

The surface of the membranes is characterized before and after being used in the separation process by scanning electron microscopy coupled to energy dispersive X-Ray spectroscopy (SEM–EDX) and elemental mapping analysis.

Chapter 4 is a Review describing the current technologies based on microalgae used to produce bioenergy and discusses their advantages, limitations and future prospects. Microalgae appear to be a solution for many environmental problems. The resulting microalgae-MFC systems can produce electricity using the electrons released to the anode during microalgae degradation. Moreover, microalgae can be cultivated in the cathode chamber, using light as power source and capturing the CO_2.

Chapter 5 presents the major conclusions drawn from this dissertation. Recommendations for potential future research are also addressed in this chapter.

Chapter I: Bibliography

1. Electricity production

The energy model that is currently spreading throughout the world, dominated by hydrocarbons, is confronted with two major constraints: (i) the scarcity of stock energy (coal, natural gas, oil, uranium) on a human scale, a constraint often associated with another concern - the volatility of energy prices -, and (ii) the phenomenon of anthropogenic global warming. It is based on the massive exploitation of stock energy stock, non-renewable, high-yield energy. These limits justify the need to make a transition towards a more sustainable energy system, giving a more important place to renewable energies. Consequently, it is important to work on other green technology before the natural resources completely ran out. Morocco's energy sector is almost entirely dependent on imported fossil fuels. Oil only accounts for about 62% of these imports. Morocco's ambition is to strongly develop renewable energies in the electricity production sector: in 2009, it was set as a target to increase the share of these sectors to 42% of the total installed capacity of the Moroccan electricity fleet by 2020. Morocco has since specified that renewable energies should account for more than 52% of the country's electricity capacity by 2030. To achieve this, the country is banking on the various renewable energy sources: wind power (9.5% of electricity production in 2017), hydroelectricity (4%), solar power (1.3%), including in particular thermodynamics plants, etc. Another concern in Morocco is the water scarcity and the focus on new wastewater treatment technologies which can reuse treated water in agriculture and other industries. Further research still needed in the fields of renewable energy and wastewater treatment, in particular, Microbial Fuel Cells (MFC) which is an emerging technology that could treat wastewater and simultaneously generating electricity.

2. Wastewater treatment

The total amount of wastewater (sewage, industrial and agricultural) globally discharged to water bodies is tens of millions of cubic meters per day [18]. According to some estimation, about 80-90 % of all wastewater in developing countries is not treated [19]. For instance, an estimated treatment capacity for sewage generated in major cities in India is only about 30 % [4]. Whereas in EU about 82 % of all generated urban wastewaters have received secondary treatment in 2009-2010 [20].

Amount of industrial wastewater varies significantly from country to country. In general, almost all water utilized for industrial purposes ends up as wastewater. In developing countries, quantities of wastewater generated by the same type of industry are generally higher [5,21].

There are several types of pollutants in wastewater. They have been identified progressively and thanks to improvements in detection techniques. The first elements to attract attention were bacteria, which cause health problems such as cholera. Domestic wastewater is also a source of organic matter, dissolved or in particulate form or in particulate form which contains carbon, nitrogen and phosphorus; these nutrients in too high concentrations enrich the environment where they are discharged, soils and surface waters.

Then new problems arrived. The industrial revolution brought many metallic pollutants as well as toxic chemicals such as nonylphenols found in the paper industry. The pollutants that we are discovering today are active compounds such as detergents, medicines, hydrocarbons and endocrine disruptors. Their presence is directly related to their daily use by the population. These new pollutants are as many challenges to be taken up and as many new technologies to be created to meet them.

The conventional aerobic treatment of low-strength wastewaters such as domestic wastewater faces not only high capital expenditure but also considerable operational and energy consumption costs. The aeration of sewage represents an energy demand of about 0.5 kWh/m3, amounting to an energy use of the order of 30 kWh per capita per year.

Furthermore, large amounts of excess sludge are produced, requiring an appropriate treatment and disposal [118].

2.1. Conventional Methods of Wastewater Treatment

Wastewater treatment methods are diverse and can be classified into three categories: primary, secondary and tertiary treatments. A physical and biological classification can also be attempted, which roughly amounts to distinguishing primary treatments on the one hand and secondary and tertiary treatments on the other hand.

2.1.1. Physico-chemical treatments: Primary treatments

Pre-treatment is a coarse purification phase. All the large and coarse solid elements are eliminated (sand, fats) which could damage the installations later. It should be noted that about 35% of the pollutants are removed. [22]

The primary treatment in the strict sense is a physical-chemical treatment. It is possible to add coagulants and flocculants to the water. A large number of suspended particles can then be recovered by decantation or flotation (physico-chemical sludge).

This step allows to eliminate 90% of the particles and objects in suspension. [30, 32, 57] It is common to most wastewater treatment plants.

2.1.2. Biological treatments: Secondary treatments

These treatments are biological and allow to eliminate dissolved pollutants. For this purpose, populations of micro-organisms capable of consuming them are used. The general principle is to promote the growth of communities of aerobic bacteria, i.e. which take O_2 for their metabolism. Among the wide range of existing processes, we quote below the main technologies:

- Lagooning: This is an extensive system in which wastewater flows through one or more shallow basins. While the suspended solids settle at the bottom of the basins, self-purification is carried out naturally, and therefore quite slowly, which requires long residence times and therefore large surface structures. No energy source is necessary because oxygenation is natural and water circulation is by gravity.

- Activated sludge: a mainly aerobic heterotrophic biomass is kept in suspension by an aeration system maintained in suspension by an aeration system which also ensures the oxygenation of the of the basins. These aeration systems can be air diffusers (fine, medium or large bubbles) air diffusers (fine, medium or large bubbles) located on the inverts of the tanks or surface agitators mixing water and air by mechanical agitation.

- The bacterial bed: the water flows by gravity over a lined reactor on which the biomass develops. The substances contained in the wastewater flowing over the packing are trapped by the biofilm and then degraded by the attached biomass. Periodically, plates of biofilm are

detached from the lining and are carried away with the water. At the exit of the bacterial bed, the water goes through a clarifier where it is separated from the sludge, consisting of the detached biofilm fractions, by decantation. The oxygenation of the biomass is done by natural air circulation through the packing and by diffusion of oxygen through the biofilm.

2.1.3. Tertiary treatments

These treatments are both physical-chemical and biological. They are carried out after the primary and secondary treatments in order to eliminate residual nutrients, resistant organic pollutants, metals and pigments. The most common processes are microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), reverse osmosis (RO) and filtration on granular media (sand, anthracite,...). Infiltration-percolation can also be used as a finishing treatment.

3. Microbial Fuel Cells

Microbial fuel cells (MFCs) are devices that generate electricity from the degradation of organic compounds by bacteria. They operate on the principle of fuel cells with an anodic part, producing electrons and a cathodic part consuming electrons. These electron transfers are carried out by living microorganisms in the form of biofilms. These biofilms which develop on the two electrodesallow the multiplication ofbacterial communities so-called electroactive. Bacterial electroactivity is defined for a biofilm as its ability to exchange electrons with a conductive surface without the use of external mediators [23].

The concept of the microbial fuel cell (MFC) was discovered by the professor of botany at the University of Durham, Michael Cresse Potter in 1911, when he published that animmersed platinum electrode in yeast cultures (Saccharomyces cerevisiae) or bacteria (Escherichia coli) is able to generate an electrical current or an E.M.F. In 1931, B. Cohen confirmed these results after serially connecting microbial fuel cells that generate a voltage of about 35 V for a current of 2 mA.Until the 1960s, interest in MFCs decreased because of the low power densities produced and the lack of understanding of the physical phenomena involved to improve them. In 1963, MFCs gained popularity when NASA proposed to feed space applications with MFCs created from human waste [119]. This renewed interest is also due to the advances made in the world of biochemistry and microbiology. Over the last decade, understanding the different

mechanisms governing the operation of MFCs has made it possible to optimize their design and thus increase power output [24-25].

Research has led to the development of MFCs that recover energy from various naturally occurring substrates, such as terrestrial soils, wastewater or urine. Since the early 2000s, there has been a significant increase in the number of publications devoted to MFCs.

3.1. Principle of MFC Electricity Generation

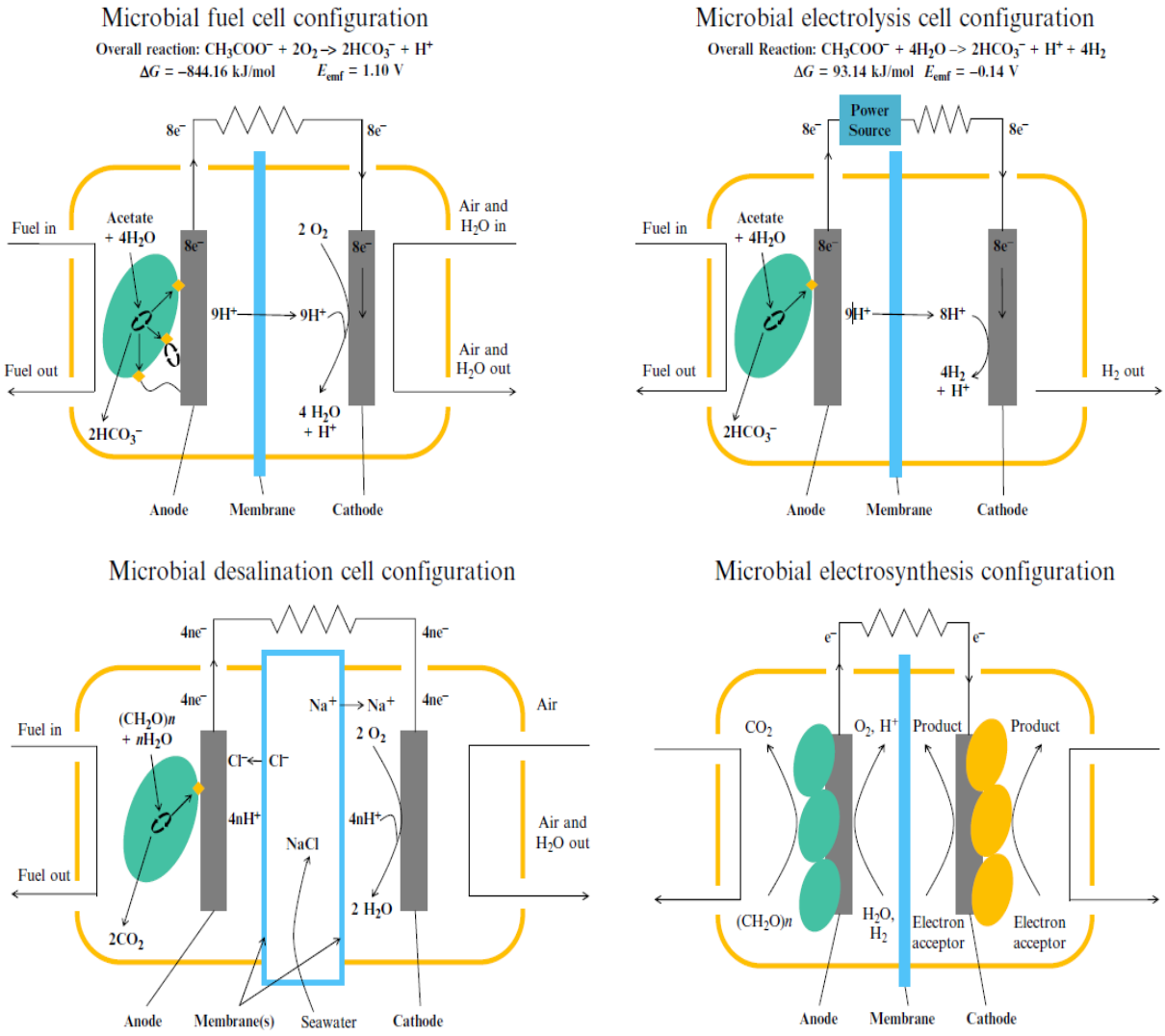


Fig1: Configurations of bioelectrochemical systems: Microbial fuel cell [top left], microbial electrolysis cell [top right], microbial desalination cell [bottom left], and microbial electrosynthesis [bottom right].[26].

Unlike ordinary fuel cell, Microbial fuel cells can operate at natural temperature and pressure. MFCs outperform other wastewater treatment technologies like activated sludge and aerated lagoon [25]. InitIially, the single-step transformation of organic matter as a substrate to electricity ensures improved conversion capacity. Secondly, an MFC could avoid more gas treatment operation due to its CO_2-rich off-gas; finally, single-chamber MFCs do not need any external energy input; further, it can be appropriate for large application in locations with less electrical facilities[27].

Microbial fuel cells perform likewise the hydrogen fuel cells, which have been broadly studied. A part of the energy incorporated in biodegradable substrates may be directly converted into electricity. This transformation became possible via oxidation, at the anode, of the organic components by bacteria as exoelectrogens. These microorganisms, which are present in nature present, remain to the anode in order to form an electroactive biofilm. This biofilm functions as a catalyst for the oxidation reaction but also as the final electron acceptor the anode material. Thus, these electrons circulate in the external circuit defined by a resistor and reach the cathode where the oxygen reduction reaction took place [28].

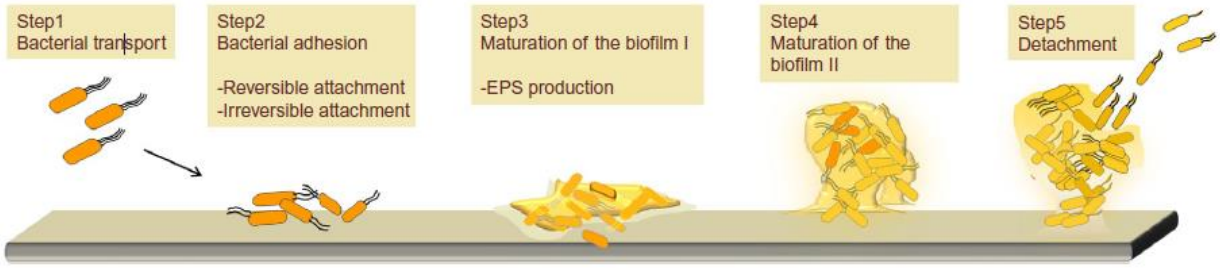


Fig 2: Stages of biofilm development [28].

In a common Microbial Fuel Cell, microbes are used to oxidize the substrate in the anode chamber; afterwards, the electron liberated from the microbes flow to the cathode by the external wire. The anode chamber consists of microorganisms, which are working as a catalyst, and an electrode (anode). It can be alimented with wastewater or growth media called anolyte and redox mediator. During bacterial substrate catabolism, the fundamental protons and electrons extracted combine with oxygen to form H_2O on cathode.

Commonly, electrons migrate to the cathode via a conductive wire connected to an external resistance. The protons which move through the membrane are reduced by the electron acceptors (e.g., O_2 to water) at the cathode [29]. Usual electrode reactions are shown below using acetate as a substrate:

Anode half-cell reaction: $$CH_3OO^- + 2H_2O \xrightarrow{microbes} 2CO_2 + 7H^+ + 8e^- \quad (1)$$

Cathode half-cell reaction: $$2O_2 + 8H^+ + 8e^- \rightarrow 4H_2O \quad (2)$$

$$\Delta G = -847.60\ kJ/mol;\ EMF = 1{:}10\ V \quad (3)$$

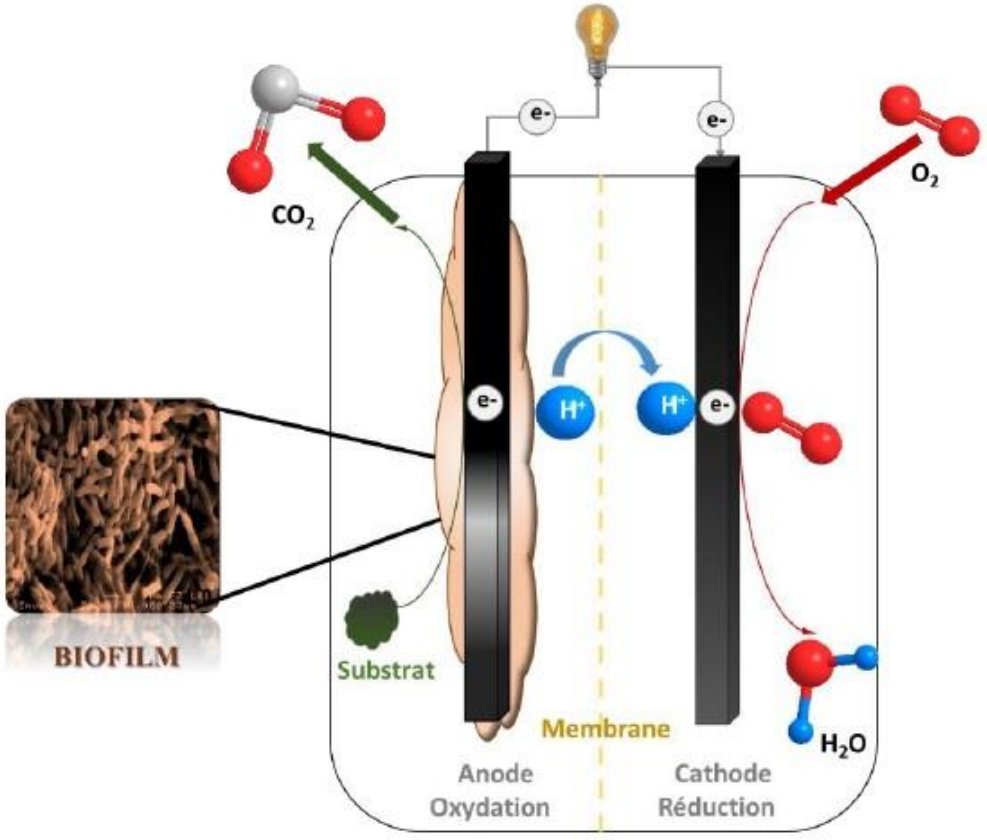


Fig 3: Operating principle of a microbial fuel cell

3.2. Capacity of Wastewater treatment and COD removal

Organic matters are utilized as fuel for electricity generation in MFCs. Although complexorganic substrates improve the growth of various microorganisms, it is much more

difficultto explore the role of each microorganism as the usage efficiency of suchsubstrates differ largely within members of the varied community of microbes. Simple substrates, such as glucose and acetate, which are the very usually used, allow to correlatethe productive output of MFCs; although the hydrolysis products of lignocelluloses fromagriculture waste has also shown to be a satisfying substrate for MFC operation. Furthermore, it is necessary to optimize the concentration of substrate so that it is neither too high to discourage growth nortoo low to restrict microbial growth. When complex wastewater is used assubstrates for microorganisms, organic matters oxidation from wastewater reduces thepollutant load and raise water purity apart from the generation of electricity [30].

Different wastewaters have been utilized as a substrate in the anode chamber of MFCs to confirm the capability of MFCs to degrade complex components from wastewater Generally, complex hydrocarbons are present in wastewater, such as nitrobenzene, phenols, naphthalene, cyanide, ammonia, petroleum hydrocarbons, etc. Furthermore, wastewater contains synthetic organic compounds (pesticide, insecticide, and pharmaceutical drugs), and heavy metals (As^{+}, Cr^{+}, Cu^{+}, etc.). The composition of wastewater indeed varies with the category and source of wastewater. Complex organic matter may be oxidized in MFCs to provide electrons, which could be tapped for electricity generation. In this stage, microorganisms are required and must be exoelectrogenic, and also have the ability to oxidize substrates. So far, MFC application for wastewater treatment still not developed in laboratories or at large scales. Nevertheless, it is obvious that with the growing interest of MFCs around the world, MFCs will be tested for higher-scale goals in the future.

According to FAO, more than 40% of produced wastewater is provided from Agro-Industrial and municipal Wastewater.Recent investigations on electricity generation by MFCs using agro-industries wastewater including effluents of rice mill, palm oil mill, fertilizer producing industries, etc., as substrates is being studied at a laboratory scale [31]. A maximum power density of 1771 mW/m2 was obtained from a cassava mill effluent [32]. A high COD removal efficiency (more than 96.5%) was obtained after the treatment of a palm-oil mill effluent by MFCs [33]. During the treatment of a rice mill effluent, results display that MFCs were better for electricity generation (2.3 W/m3) and a value of COD removal of 96.5% in comparison to proton-exchange membrane MFCs with values of 0.53 W/m3 and 92.6% COD removal [34].

Domestic and municipal wastewater contain complex organic components, they need a powerful technology to eliminate those compounds. In this context, biological degradation is sufficient, and generally an activated sludge procedure is selected. As this technique consumes huge quantities of energy, researchers are now ambitious to develop low-cost MFC technology for domestic and municipal wastewater treatment [35]. A single chamber air cathode MFC attains a maximum power density of 422 mW/m^2 with a 25.8% COD removal ability at the same time of treating domestic wastewater recovered from the Pennsylvania State University Wastewater Treatment Plant (WWTP) [36]. Likewise, the maximum power density performed was 103 mW/m^2 with an 81.4% COD removal efficiency from a municipal wastewater using a single-chambered air cathode MFC [37].

3.3. Separation processes

A common MFC, such as a double chamber MFC, consists of an anodic and cathodic chambers separated by a proton-exchange membrane; whereas, in a single chamber MFC, there is no need for the cathodic chamber since the cathode is exposed directly to air. Separation process plays an important role in MFC. It divides the anodic and cathodic chamber in a double chamber MFC, or the anodic chamber and the cathode in a single chamber MFC. The electrons produced by bacterial metabolic activity are passed through an external circuit while the proton moves via a separator, separating the anode chamber from the one in which the cathode is immersed [38].

Several types of separators have been studied. The types of separator materials may be classified into three categories on the basis of ion exchange membrane, size-selective separators, and salt bridges.

The performances of membranes in MFCS, such as, cation exchange membrane, anion exchange membrane, and Bipolar Membrane (BPM) were compared. The MFC with anion exchange membrane demonstrates improved achievement over others in terms of voltage stability and power generation. The size-selective separators consist of salt bridge, microfiltration membrane (MFM), Ultrafiltration membrane (UFM), porous fabrics, glass fibers, and other coarse pore filter materials, J-Cloth, UCM (ultracentrifugation membranes) etc. In the case of single-chambered MFC, to reduce the resistance of MFC separator cathode assembly (SCA) is used. An SCA consists of a cathode and a membrane

physically or chemically bonded together, also known as membrane cathode assembly (MCA)[39]. Rahimnejad et al. reported application of MCA in a single-chambered air-cathode MFC where membrane is hot pressed directly onto a carbon cloth cathode [40]. The use of separators in MFC may decrease operational costs; furthermore, this type of design has the capacity to simplify reactor configuration by choosing passive airflow.

The need for low-cost membranes inspired investigators to develop clay-based ceramic separators for MFCs. These Proton Exchange Membranes or separators have shown encouraging results regarding power output, coulombic efficiency (CE), and wastewater treatment when compared with Nafion, and a low cost of the fabrication of MFCs [41]. Proton exchange membranes influence an MFC's internal resistance and concentration polarization loss and in turn affect power output of the MFC. Nafion (DuPont, Wilmington, Delaware) is the most used PEM in MFCs since its high selective permeability of protons. Nevertheless, the secondary effects of other cation transports are inevitable during the operations of MFCs, even with Nafion. The PEM surface area has a huge impact on power generation. Internal resistance of the MFC decreases with an increase of the PEM surface area over an approximately large range [42].

The utilization of membranes based on ionic liquids (ILs) has attracted researchers in the MFC field. Their great profit as ecological solvents with a high conductivity and a low amount of vapor pressure make them appropriate for a large range of use such as purification or separation processes, reaction media in biochemistry or chemical catalysis [43-44].

Regarding their use in membranes, they have captured huge importance owing to their characteristics, principally their high ionic conductivity, high stability. Their inclusion as liquid phase in exchange membranes has granted MFCs performance to be increased [39-45].

Methyltrioctylammonium chloride has been selected to develop polymer inclusion membranes that will be utilized as separator in MFCs. A new assembly procedure is developed to obtain a higher compact and effective separator. In a prior study, this ionic liquid has been pointed out to reach modest results of power density when used in polymer exchange membranes as compared with other ionic liquids [45].

3.4. Components and Materials

3.4.1. Anode

The anode material has a preponderant influence on the formation of the electroactive biofilm and the electronic transfer on its surface. By reason of the low electrochemical kinetics and the high price compared to the performances obtained, a lot of researches concern the optimization of this key element of the MFCs. It must have a high electricalconductivity, a low resistance and a large specific surface to allow the adhesion and biofilm formation.

Carbon anodes are extremely used in the field of MFCs (90 to 95% of articles). They are present in various forms: carbon bar, carbon paper, graphite plate, granular-activated carbon, carbon fabric, carbon felt, graphite granule, crosslinked vitreous carbon, carbon brush [46].

Carbon cloth is very flexible and porous than the previously mentioned materials; so, more bacteria can adhere to cloth. Chemical modifications of the anodes can further improve MFC performance [27].

Baudler compared the performance of diverse noble metals (gold, silver, platinum) likewise as different metals (stainless steel, titanium, nickel, cobalt, copper) as anode materials rather than graphite. His tests showed current densities of 1175 $\mu A/cm^2$ and 1119 $\mu A/cm^2$ for gold and silver, respectively, against only 984 $\mu A/cm^2$ with a graphite electrode placed under the same operating conditions, however prices that do not meet the required criteria for a MFC anode[47].

Different materials or deposits have been evaluated as anodes, with the aim of increasing the electron transfer between the microorganisms and the electrode. However, despite the quest to replace platinum as a catalyst in all fuel cells, a team tested this material as an anode catalyst to increase the electron transfer rate between the bacteria and the carbon paper forming the anode.This deposit has significantly increased the current produced.

However, the choice of platinum as catalyst remains questionable as well as the electrochemical reaction generating the bulk of the electrons, these may come partly from the oxidation of water rather than the microbial degradation of the substrate. If the electrons are no longer generated by the microorganisms, it can no longer be in the presence of a microbial fuel cell [48].

Fig 4: Photos of anode materials used in MFC: (A) graphite paper, (B) graphite plate, (C) carbon fabric, (D) carbon mesh, (e) granular graphite, (F) activated carbon granular, (G) carbon felt, (H) crosslinked vitreous carbon, (I) carbon fiber brush

3.4.2. Cathode

The cathode plays an important role in the operation of the MFCs. It must have a high redox potential and an ability to capture ions. Despite the many studies conducted for the development of alternative materials to platinum (as a catalyst), the performance of MFCs is often limited by this electrode. The cathodes are differentiated by the nature of the catalyst, chemical or biological, and by the final acceptor of electrons, oxygen or others [49].

Carbon materials, with or without catalyst, which are most commonly used as cathode are: graphite plates, carbon paper, glassy carbon, carbon felt, graphite granules, carbon cloth and graphite fiber brush [49,50,51]. Air–cathodes are the most ordinarily used configurations in lab-scale MFCs that need catalysts to generate higher power densities.A carbon fabric support appears to be particularly suitable for the case of air cathodes[52].Many studies have been conducted to use air–cathodes without catalysts [120].

Several catalysts, chemical and biological, have been tested. Platinum is often used because it has excellent catalytic capacity, but its cost and availability remain serious drawbacks if wide use was considered. Moreover, the catalytic activity of platinum is easily decreased due to its contamination by the multiple compounds present in the wastewater. Indeed, the cathodic reaction requires the presence of protons, but it turns out that they are not the only cations present in solution (among others K^+, Ca^{2+}, Na^+), these may cause adverse reactions decreasing the potential the cathode and thereby the power of the microbial cell[50,51,53,54]. These factors limit its use and make it unsuitable for its application for the treatment of wastewater. To limit this problem, studies to reduce the amount of platinum deposited on the cathode were conducted, with results that remain quite satisfactory; V.M.Ortiz-Martínez et al.studied the utilizationof mixed manganese oxides with copper and nickel synthesized by co-precipitation in MFC devices fed with industrial wastewater.Among the analyzed materials, the $NiMn_2O_4$ offered interesting results of power performance, achieving 80% (439 mW/m^3) out of the power density obtained with Pt (549 mW/m^3)[55].

3.4.3. Proton exchange membrane

The majority of MFCs is composed of two separate parts. In a two-chamber MFC, the anode and the cathode compartments are separated by a proton exchange membrane. It must be characterized by high ion permeability from the anode to the cathode, and low oxygen diffusion without having a remarkable effect on the internal resistance of the battery; moreover it must ensure the electro-neutrality of the medium by allowing the easy passage of the protons [30].

Overall, Nafion® and Ultrex® are the most used exchange membranes in microbial fuel cells. Recently, ionic liquid based membranes have been tested as proton exchange membrane in MFCs, they have shown a high current density and chemical oxygen demanded removal (i.e. 103.9 mW/m3 and 89.1%, respectively for the ammonium based supported IL membrane), which is similar to conventional PEMs [56].

3.5. Reactor Design

The reactors that constitute the microbial fuel cells have different configurations, both in the form of the reactor itself, the number of chambers it contains (one or two), the use or not of the

proton exchange membrane, cations or other materials with the same function and finally the electrode materials used and the distance separating them. It is possible to combine several batteries to form a stack with some modifications of the systems, this option allows to increase the voltage of the systems and thus the power generated. However, different problems can occur depending on how the batteries are connected.

3.5.1. Double chamber MFC

A common double chamber MFC is composed of an anode chamber and a cathode chamber isolated by a membrane, which permits the proton stream from the anode chamber to the cathode chamber while performing as a physical barrier to the oxygen dispersion or different oxidants from the cathode to the anode chamber. The compartments of the double chamber MFC can be of different shapes and worked in batch or continuous mode. One of the most employed double chamber MFC is H-type MFC. The high internal resistance is a big drawback in this type of reactor which implies a low power generation. This kind of MFC is appropriate for fundamental parameter research such as assessing power generation utilizing new electrode or separator materials, or microbial communitiesinvestigation, etc. [27].

Double chamber MFCs, fabricated in up-stream mode and manufactured as up-stream cylindrical MFC with concentric outside anode chamber and inner U-molded cathode chamber with effluent recirculation, have been reported to be proficient for wastewater treatment as this sort of design can be simply scaled up. The energy prices of pumping fluid around are more noteworthy than their power yields, which limit the use of such apparatus as net power generating devices.

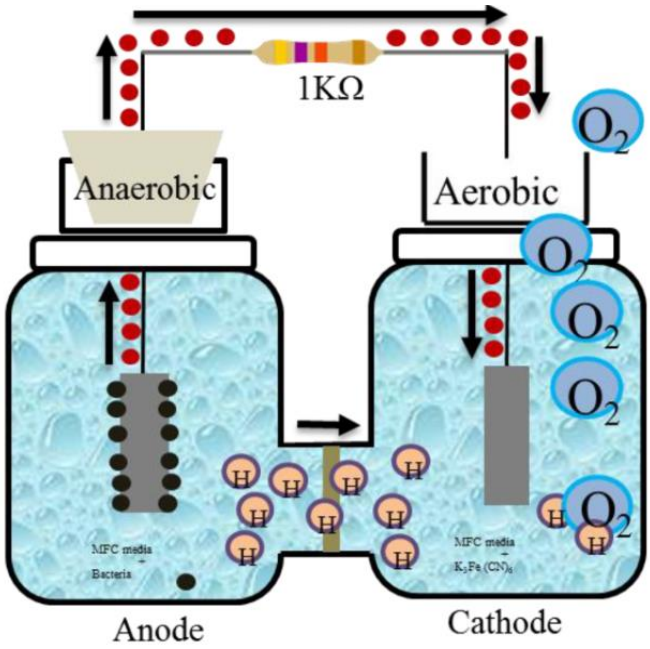


Fig 5: Double chamber MFC

3.5.2. Single-Chamber MFC

Single chamber MFCs can be built by maintaining cathode in direct contact with air. The single chamber air-cathode MFC has various advantages compared to the double chamber MFC: (1) the single chamber MFC don't require cathode chamber; it thus lessens the size and is easy to use, (2) no aerationis required as ambient air can be used (3) reduced internal resistance in single chamber MFC as a consequence higher current amount can be obtained [57]. An up-flow reactor has been experimented as single chamber air-cathode MFC that function in a continuous mode, by a research team in the polytechnic university of Cartagena- Spain [58].

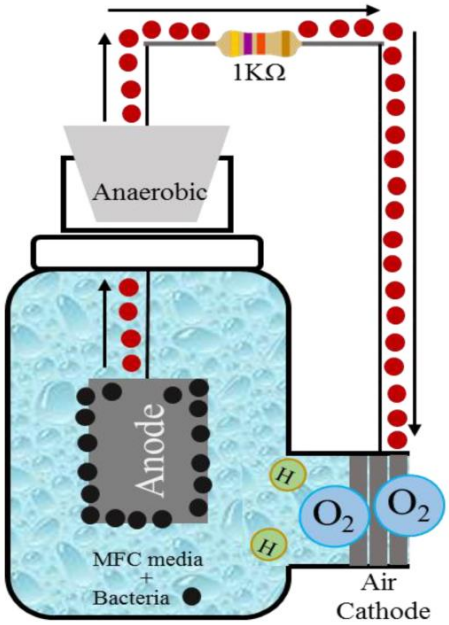


Fig 6: single chamber MFC

3.6. Operational Factors Influencing MFC Performance

3.6.1. Nature of substrate

In MFCs, the substrate is the carbon source and is considered an important factor for electricity production. The substrate can be a pure compound or a more or less complex mixture.

In the case of a pure culture, a pure / simple substrate is chosen, if possible, in order to benefit from an easy degradation by the corresponding bacterium.However, mixtures of organic materials from different sources may be degraded if viable mixed bacterial cultures are used in these media. Acetate is the easiest substrate to degrade by bacteria and is therefore frequently used in MFCs [52, 59, 60], followed by glucose[61], cysteine, and also higher molecular weight compounds such as volatile acids, carbohydrates, proteins, alcohols and cellulose[62, 63].One of the main interests of MFCs lies in the possibility of using waste as a substrate, eliminating it while producing electricity. These sources of waste are very diversified: domesticwastewater, waste from the food industry (beer, corn, rice, starch, yoghurt, chocolate, tincture, meat)[32, 33, 34],water from paper recycling plants[64,65] and leachate from organic and inorganic products[66].Some studies have been carried out with "synthetic" wastewater to overcome the problems of reproducibility of experimental conditions[67].

3.6.2. Temperature effect

MFCs are bioelectrochemical systems that have the great advantage of being effective in a relatively wide temperature range.Temperature changes affect the kinetics (activation energy, mass transfer mechanisms, electrolyte conductivity, ...) and thermodynamics (Gibbs free energy, electrode potential, ...) reactions that have place within MFCs.The type of bacteria that will participate in the electrogenesis will not be the same depending on the temperature of the electrolyte, (psychrotrophic bacteria for temperatures ranging from 25 to 30° C, mesophilic from 30 to 45° C,...). This physical parameter plays a crucial role in the performance of MFCs.

At present, the majority of available PEM for MFCs is Nafion from Dupont however this can't work effectively at temperatures higher than 90° C because of thermal instability.A research group at the Fuel Cell Institute have developed a high temperature composite called Nafion-silicon oxide (SiO_2) -acid (PWA) a composite membranewith low resistance, high proton conductivity, high current density and improvedthermal stability at 90° C than Nafion from Dupont[30]**.**

By increasing temperature in MFC the internal resistance decrease, which can be explained by the fact that ionic conductivityincreases with temperature [68].

3.6.3. pH effect

The pH causes several alterations in concentration of ions. Thus, the performances of MFC dependon it and on proton-motive force, membrane potential and biofilm formation. A pH close to neutral (between 6 and 9) gives the required optimal growth of bacteria.

A major problem with double chambred MFCs is the appearance of a remarkable difference in pH between the two compartments. Gil et al. thus has reported that in a MFC initially supplied with an electrolyte of neutral pH, a significant acidification of the anode compartment, whose pH decreases to a value of 5.4 and conversely an alkalinization of the cathode compartment with pH values equal to 9.5. These results, accompanied by a drop in the electrical performance of the MFCs, seem to result from an insufficient ionic conductivity of the electrolyte and low permeability of the proton exchange membrane, which induces an accumulation of H^+ ions at the anode and disrupts the metabolism of exoelectrogenic bacteria and limits the reduction of

oxygen at the cathode precisely because of a lack of H^+ protons[69]. The use of MFCs with one compartment limits this effect.

pH gradients also appear on the surface of the electrodes and are caused by limitations in ion transport within the MFCs [70]**.** Local acidification of the anode by accumulation of H^+ ions can therefore occur and be harmful to bacteria. This phenomenon can be partially inhibited by adding a phosphate buffer in the electrolyte in very high concentration that will facilitate the transport of protons out of the biofilm.

3.6.4. Conductivity effect

Conductivity is a parameter for measuring ionic mobility in an electrolyte: it is therefore an essential factor in the performance of an electrochemical system. In addition, the resistivity of the membrane is influenced by the conductivity of the solution in which it is immersed: the higher the conductivity of the medium, the lower the membrane resistivity [71]. The field of microbial cells does not currently have a common reference to report on the performance of systems under equivalent conditions.

Many studies have shown a significant increase in power with the increase in electrolyte conductivity. e.g. Cheng et al. reported in their work an increase from 16 W/m^3 to 60 W/m^3 when conductivity increases from 1.7 mS/cm, to 20 mS/cm thanks to the addition of NaCl in the electrolyte [72].

3.6.5. Ionic strength

The effect of ionic strength on microbial fuelcell performance have been investigated by Mohan et al. he has concluded that power density reached its maximum value at an optimum ionic strength [73]. A few bacterial strains have the ability to generate electricity at a high ionic strength.e.g.*Shewanella marisflavi*have been experimented and the results show that it could reduce Fe(III) and produce power at a high ionicstrength of up to 1,488 mM (8% NaCl) using lactate as theelectron donor.Huang et al., found that power production could be improved by an increase in ionic strength due to a decrease in internal resistance [74]. Dlugolecki et al. [75] have demonstrated that when the ionic strength was increased by a factor of 20, the membrane resistance of a proton exchange membrane was reduced by about 93%.As shown in the figure

7,when the anode fluid ionic strength increase, power output increase as well.The total resistance decreased from22.5 Ω at 0.037 Mto 13.0Ωat 0.37 M[76].

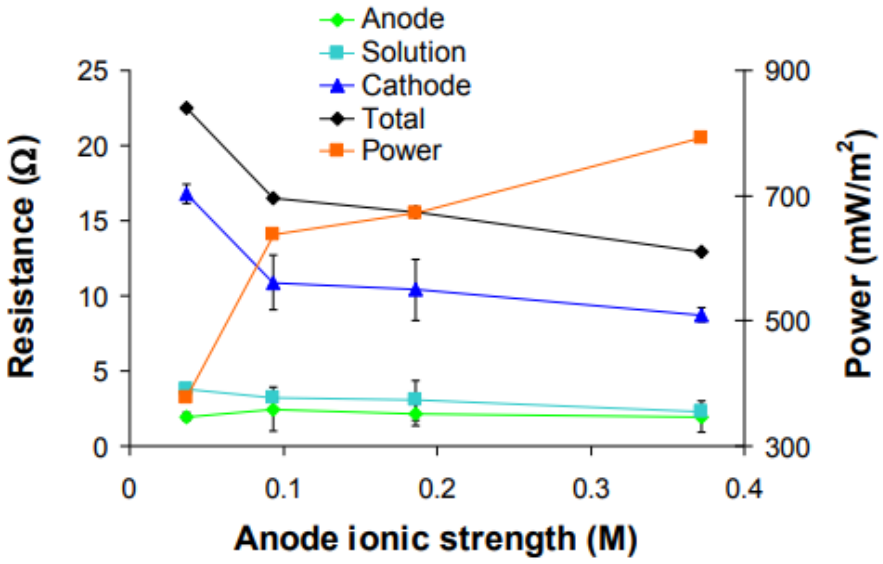


Fig 7: ECM analysis results for EIS data while varying anode fluid strength. [76]

3.6.6. Hydraulic retention time and flow rate

Time is a crucial process leading to the choice of a biofilm of electrochemically active bacterium to maximize MFC performance the anodic biofilm development.[77].Anodic volume is typically considered as a set design part, regulating dilution rate or hydraulic retention time (HRT) is completed through changing the flow rate. However, the recent employed designs of MFCs in several studies can be besides an operating parameter [78, 79].

Since the integration of MFCs in wastewater treatment plants requires a continuous flow supply, some studies have already attempted to correlate the performance obtained with parameters such as the flow rate circulation, Hydraulic Retention Time (HRT) or even shear stress at the anode.

It appears that, even if few studies seem to agree on the precise relationship between the electrical performance of a MFCs and its power supply regime, the use of a continuous flow facilitates the maintenance of stable operating conditions (organic load, pH,...) inside the reactor and reduces the internal resistance of the MFCs by mitigating, among other things, mass transport effects.

Most research also agrees that hydraulic retention time, i.e. the time during which the electrolyte remains inside the cell, is optimal. A short HRT, i.e. a rapid electrolyte change, tends to increase current generation at the expense of a lower COD abatement rate and vice versa [80, 81]**.**

The effect of the organic load concentration of the effluent has also been established since it presents an optimum beyond which a too high concentration leads to a decrease in electrical performance [82].

A long retention time (>10 days) accumulatesmore biomass. For example, for MFCs in fed-batch mode operating for more than ten days, the algal biomass produced is greater than 300 $mg.L^{-1}$. While a system operating continuously[1] and with a retention time of 3 days produces only 128 $mg.L^{-1}$ of biomass[83].

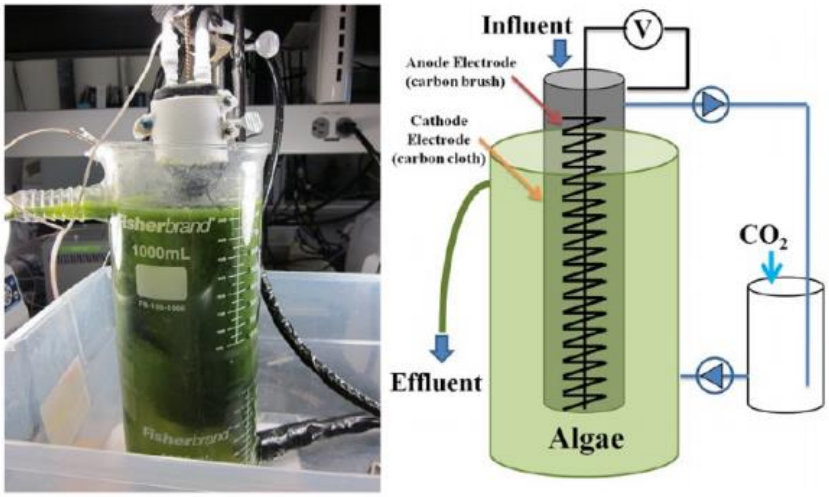


Fig 8: Experimental setup (left) and schematic (right) of the IPB integrated photobioelectrochemical system [83].

[1]An integrated photobioelectrochemical (IPB) system was developed by installing a microbial fuel cell (MFC) inside an algal bioreactor

3.6.7. Ionic liquid selection

Ionic liquids (IL) are generally colorless liquids although those based on $PF6^-$ or $BF4^-$ may have a slight yellow coloration. IL purity is an important parameter for most applications because the presence of impurities can seriously affect its physico-chemical properties [84]. Ionic liquids can be very simply defined as salts melted at low temperature, i.e. salts that are liquid at or near room temperature. It is generally accepted that an ionic liquid has a melting temperature below 100°C. However, many of them are liquid below 25°C. These ionic liquids have unique physico-chemical properties such as very low vapor pressure (and therefore low flammability), very good thermal stability (up to 400°C for in some cases), a liquid state over a wide temperature range, good ionic conductivity (similar to that of organic electrolytes), a very wide electrochemical window, and the ability to dissolve a wide range of chemical compounds ranging from metal cations (transition metals, rare earths, etc.), various organic compounds (volatile organic compounds (VOCs), various synthesizers) to macromolecules (enzymes, cellulose, lignin...)[85].The use of ionic liquids has significantly increased the operational stability of certain enzymes (lipases, catalases, peroxidases, laccases, etc.), in particular those used to catalyse biotransformation reactions on an industrial scale[86].

Ionic liquids have also attracted great interest in biotechnological applications such as biocatalysis, biomass processing, biodegradability and drug delivery [87]. By choosing an appropriate ionic liquid, the oxidized reactive mediator is distributed preferably in the ionic liquid phase, away from the enzyme in the aqueous phase. This minimizes the contact between the enzyme and deactivating species and extends the lifetime of the laccase active. In a recent publication, this strategy was applied to operate a single-compartment microbial fuel cell using a biofilmbacterial at the anode and laccase immobilized in a hydrophobic ionic liquid [OMIM][NTf_2] at the cathode [88]**.**

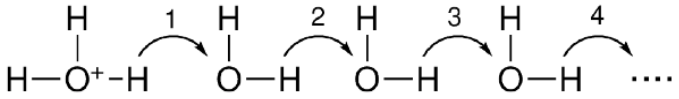
An alternative way to use ionic liquids in this area of MFCs is to use them to form membranes. Recently, new membranes prepared with ionic liquids have been considered as promising competitors to conventional polymers such as Nafion® in MFCs, such as [$P_{I4;I4;I4;1}^+$][TOS^-]-based membrane which outperform other ionic liquids based membrane[39, 45,89].

Nevertheless, the toxicity of ionic liquids on different microorganisms or bacteria has been demonstrated. [90,91],e.g, Wood et al. worked on the toxicity of ionic liquids to E. coli has

shown that ionic liquids have inhibitory effects on the growth of bacteria dependent on the length of the cation's alkyl chain, and, in a lesser extent, on the nature of the anions used.[92].Another study on E. coli and *Staphilococcus aureus* shows that the toxicity of ionic fluid can also increase with loading, as a bicationic system is more toxic[90]**.** However, from the available literature, it can be observed that the publications describe sometimes contradictory observations and results.For example,[BMIM][PF6] at a concentration of 1% is reported to have a toxic effect on the growth of E. coli[93, 94]**.**These apparent contradictions demonstrate the interest of studying the effect of ionic liquids on a bacterial community. Toxicity tests are generally conducted on well-defined strains in solution (planktonic growth mode). However, there is an additional interest in studying the toxic or antimicrobial effect of an ionic liquid on a biofilm.It is the most widespread mode of bacterial growth, where bacterial communities organize and protect themselves through an extracellular matrix.Biofilm provides and maintains a kind of micro-environment favourable to bacterial colonization, which explains the particular resistance of these biofilms to many biocides or antibiotics. In addition, integrating these biofilms into a PCM can allow the toxic effect of ionic liquids on the bacteria constituting the biofilm to be recorded by a simple current measurement.

3.7. Electron Transfer Mechanisms

Protons are transported through the exchange membrane by passive diffusi0n. When themembrane is completely hydrated, protons can be transported viathe Grotthuss mechanism[2]. In this mechanism protons can `hop' from onewater molecule to another, forcing the excess hydrogen atom at the secondwater molecule to `hop' to another molecule, thereby restarting the chain ofevents [95]**.**When a proton binds to a water molecule at one side of themembrane, this will eventually, via several proton `hops', lead to the releaseof a proton on the other side of the membrane. The principle of the Grotthussmechanism is visualized in fig 9.



[2] The Grotthuss mechanism (which is a general name for the proton-hopping mechanism) is the process by which an 'excess' proton or proton defect diffuses through the hydrogen bond network of water molecules or other hydrogen-bonded liquids through the formation of covalent bonds involving neighboring molecules.

Fig 9: The Grotthuss mechanism with the consecutive proton transfer indicated withthe numbered arrows

Microorganisms oxidize different substrates by producing electrons at inside their cells. These electrons are then used in a cascade of reactions via the breathing chain until the last exchange with the final electron acceptor extracellular. It is commonly accepted that this electron acceptor is a molecule soluble as oxygen can be, but also organic acids, ions minerals such as nitrates, sulphates or metal oxides.

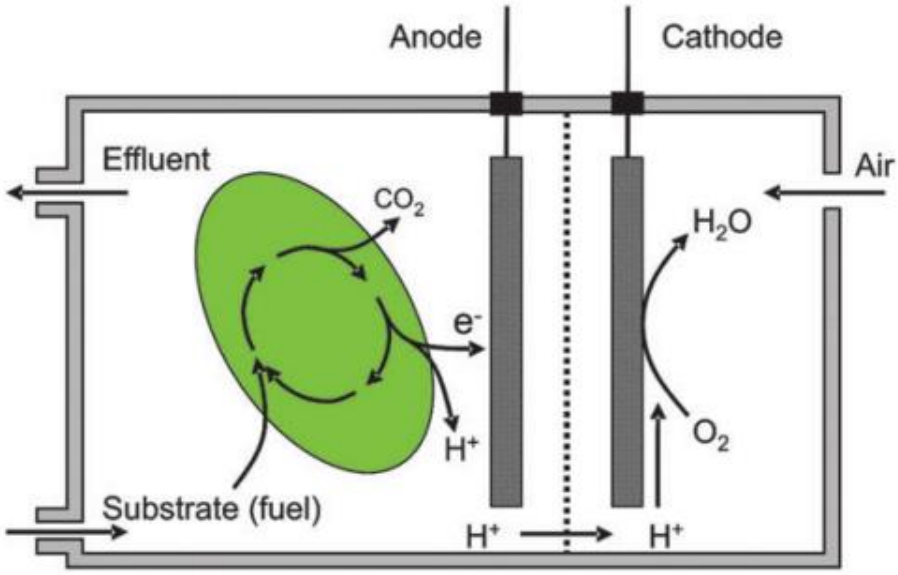


Fig 10: Electron transfer to the anode via oxidation of fermentation products [96].

To avoid the recovery of these metabolites by other processes and in particular aerobic biological processes, reactions are generally carried out in an anaerobic environment. The oxidation reaction on the anode surface is abiotic and therefore requires the presence of an inorganic catalyst such as platinum. The problems of poisoning and deactivation of these catalysts by adsorption of all kinds of compounds considerably reduce the current densities obtained [96].

Significant progress was made when platinum-based electrodes were coated with conductive polymers such as polyaniline. Current densities of about 1.5 mA/cm2 have been obtained. Results have been further improved up to 3 mA/cm2 using electrodes coated with tungsten carbide which is also a less expensive electrocatalyst [97].

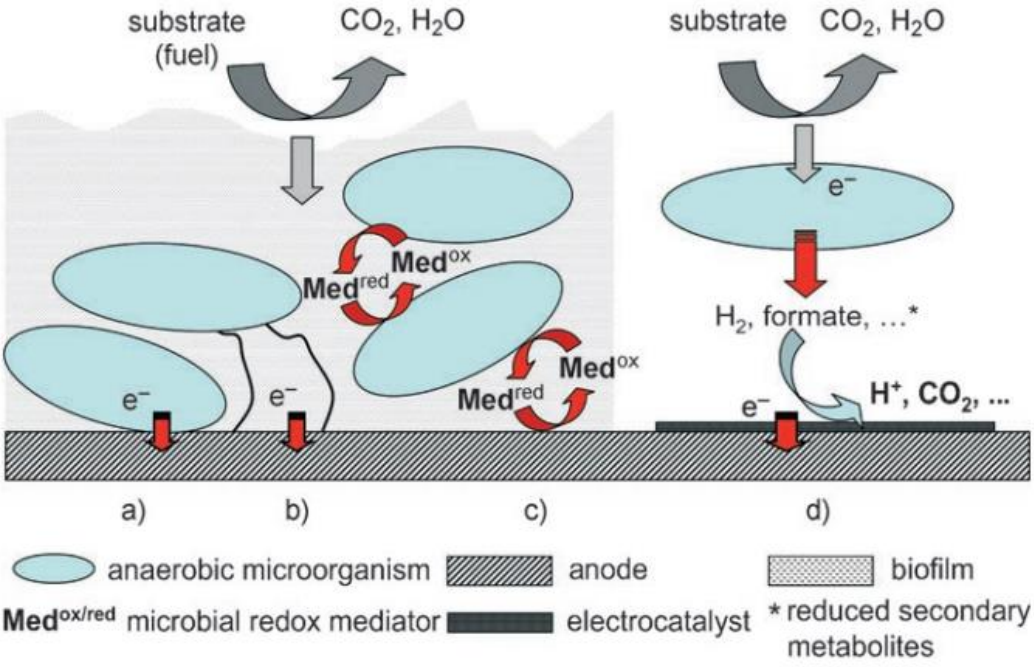


Fig 11: electron-transfer mechanisms in MFCs. Electron transfer via a) cell-membrane-bound cytochromes, b) electrically conductive nanowires, c) microbial redox mediators, and d) oxidation of reduced secondary metabolites. [97].

3.8. Investigation of Ionic Liquid based membrane in ion transport

The proton exchange membrane (PEM) is a key element in the MFC as it carriers proton from the anode to the cathode. Thus, membranes for microbial fuel cells applications needto be extremely proton conductive. Hernández-Fernández et al. illustrated the use of a PEM containing 1-octyl-3-methylimidazolium hexafluorophosphate ([OMIM][PF_6]) and methyl trioctil ammonium chloride ([MTOA][Cl]) as a Proton Exchange Membrane in a microbial fuel cell. The membrane containing 70 wt.% of ammonium-based Ionic Liquidreacheda maximum power up to 450 mW m^{-3} and the percentage of the COD removal was more than 80% [89].

Recently, new membranes prepared with ionic liquids have been considered as promising competitors to conventional polymers such as Nafion® in MFCs. Researchers have shown that the MFC that works with membranes made with [BMIM][NTf2] has a higher energy efficiency than MFC with Nafion®[98].Koók et al. attributed this effectiveness to the involvement of LIs in proton transport betweenanode and cathode compartments of the bioelectrochemical system.

Thus, the amount of ionic liquid immobilized in the membrane is able to influence the power of the MFCs [98].

The inhibition properties of liquids on the formation of biofilm on surfaces or their toxicity can be very advantageously used to solve one of the operational limits of PCMs.Indeed, biofilms can be developed on the ion exchange membranes used in PCMs. This development leads to clogging of the membrane pores, which reduces the transport of ions between the two anodic and cathodic compartments, and consequently increases the internal resistance of the battery. This leads to a decrease in the performance of these MFCs.Further understanding of ion transport selectivity and membrane preparation ways are crucial to enable larger use of ion liquid-based membranes in technical processes for sustainable development.

Baicha et al. 2019 have analyzed nutrients transport through polymer inclusion membranes based ondifferent concentrations of ionic liquid (methyltrioctylammonium chloride) using sodiumhydrogen phosphate (Na_2HPO_4) and Calcium chloride (CaCl2), results shows a high permeation values of Na_2HPO_4 compared to $CaCl_2$ and increase proportionally with the amount ofionic liquid in the membrane[99].

3.9. Practical Applications and Scale-up

The first prototype of a large-scale MFC was developed in Australia (Queensland) in 2005 in a brewery owned by Foster (Figure 12). The prototype consists of 12 modules, each 3 metres high and 1 m3 in volume. The anodes are made of carbon fibre brushes and the cathodes are made of graphite brushes. There is little information on the performance of this biopile. It was reported that the performances were limited by the low conductivity of the brewer's effluent to be treated [36].

Fig 12: Applications for MFCs. (A) Pilot-scale microbial fuel cell constructed by the advanced water management centre at the University of Queensland and Foster's brewery in Yatala, both in Australia

Although the employment of Microbial Fuel Cells for wastewater treatment is in its inception, MFCs as source of energy for environmental sensors are approaching practical use. In distinction to conventional batteries, MFCs powered by organic matters in sediments present advantages as power sources because they could generate energy with no need for recharging. These kinds of MFCs, known as benthic unattended generators (BUGs), are utilized in inaccessible areas essentially in river and ocean sedimentsFig. 13. Technically the operation is very simple; The organisms of Geobacteraceae family can oxidize acetate and other organic matters, and transfer the electrons to a graphite platewhich is used into the anoxic sediment (anode) that is electrically connected to another graphite plate which is overlaid in aerobic water (cathode) where they react with oxygen[101].

A new stacked microbial fuel cell (MFC) with a total volume of 72 Land made of 5 membrane based MFC units reaching a power density of 50.9 $W/m^3$was build up and tested by Wu et al. Nevertheless, this system suffer from electrical current losses in theparallelcircuit[101].

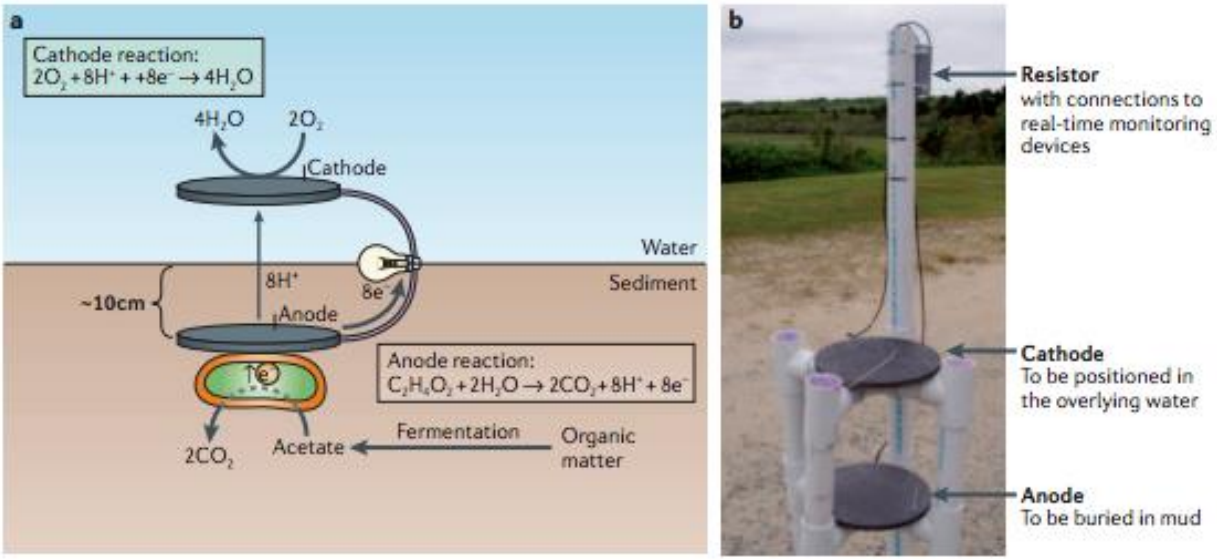


Fig 13: A sediment microbial fuel cell. **a|** A scheme of a sediment microbial fuel cell. **b** |A sediment fuel cell before deployment [64]

Other studies show the possibility of implementation of a large-scale reactors that include up to 1000 L made of 50 modules achieving up to 90% COD removal and up to 60W/m^3 when operated for 1 year on municipal wastewater [103]**.**

3.10. Opportunities and Challenges for MFCs in Wastewater Treatment

One of the very basic and fundamental applications of MFCs is totreat wastewater simultaneously with electricity generation. The task remains difficult as the composition of the wastewater is complex, and the high level of its organic load. The use of MFCs represents an interesting long-term economic alternative to current wastewater treatment processes since they allow remediation while producing electricity. Different kinds of wastewater, including domestic and industrial, are suitable fortreatment by MFC.However, the microbial population present in the wastewater might not always find an ideal substrate to interact with. Another profit that MFCs offer compared to otherprimary treatment processes is the recovery of some valuableproducts such as hydrogen and/or electricity. The related prices with aeration or biomass treatment within the case ofactivated sludge (AS) or trickling filters (TF) are minimal for MFC technology. Economicand system sustainability are necessary issues regarding to operating an MFC-based wastewater treatment system efficiently [65]. As reported by Rabaey, it is possible touse specific microbes as substrates to reach an advantageous result, in the aim

of removing sulfides from wastewater [104]. The COD removal value can exceed 90% in some efficient MFC reactors with columbic efficiency of about 80% [65; 66].

Some studies still hopeful concerning the possibility of anMFC that powers devices or even communities, for example in distant places. Particularly in wastewater treatment, these concepts sound reasonable: it has been presumed that anMFC of 350 m^3 with an output power density of 1 kW m^{-3} would cost practically 2.6 million Euros; the return on investment period for the cost would be around ten years if the reactor was used to convert organic waste to electricity at an effectiveness of 30% in a plant of food processing[105]**.**

In the near future, an MFC will probably be used mainly for wastewater treatment andfurthermore as an energy supplier. Other innovative ideas can also be attainable when the research onMFC is developed, such as valuablechemicals production [106].

4. Microlagae

4.1. What is microalgae?

Microalgae, some of the oldest living organisms, are microscopic photosynthetic organisms that can be found in marine and freshwater environments. They are a diverse group of prokaryotic and eukaryotic photosynthetic microorganisms with a unicellular or simple multicellular structure that allows them to grow rapidly and live in extreme conditions [16]. Due to this simple structure, they harness solar energy quickly and efficiently through photosynthesis. They use sunlight to produce oils or sugars in a more efficient way than crop plants. Moreover, autotrophic production is more efficient than heterotrophic production, and therefore microalgae are commonly used as a substrate for biofuel production. They usually grow in aquatic environments, which provide them with many nutrients in dissolved form, such as CO2 [17].

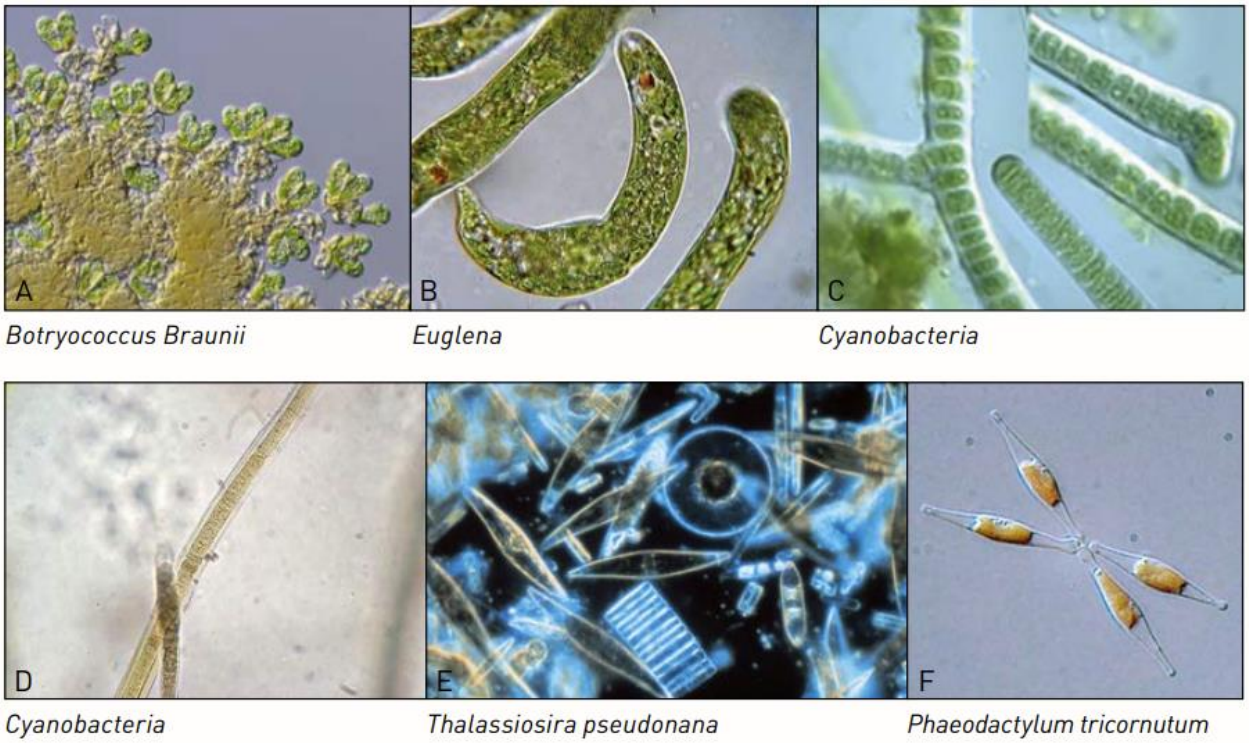


Fig 14: A wide variety of microalgae species present in our planet for 4 billion years., **Source : Wikipédia**, Creative Commons : A) CC-BY-SA-3.0, ja ; B) CC-BY-SA-3.0, Deuterostome ; D) CC-BY-SA-3.0, Kristian Peters ; F) CC-BY-2.5 Image courtesy of Alessandra de Martino and Chris Bowler, Stazione Zoologica

Microalgae can be grouped into different categories based on the pigmentation of their biological structure: i) green algae (Chlorophyta), red algae (Rhodophyta) and diatoms (Bacillariophyta) [19]. They can also be classified into two groups: i) autotrophic, which only requires inorganic compounds to grow, such as CO2, salts (nitrate ion, phosphate) and light. This subcategory can be divided into photoautotrophs, which use solar light as a source of energy and chemoautotrophs, which require an external source of organic compounds as a source of energy. ii) Heterotrophs can also be grouped into photoheterotrophs, which use solar light as source of energy and chemoheterotroph, which oxidizes organic compounds to get energy. However, there are some types ofmicroalgae that can use different sources of energy and carbon, which are called iii) mixotrophic [18,19,22].

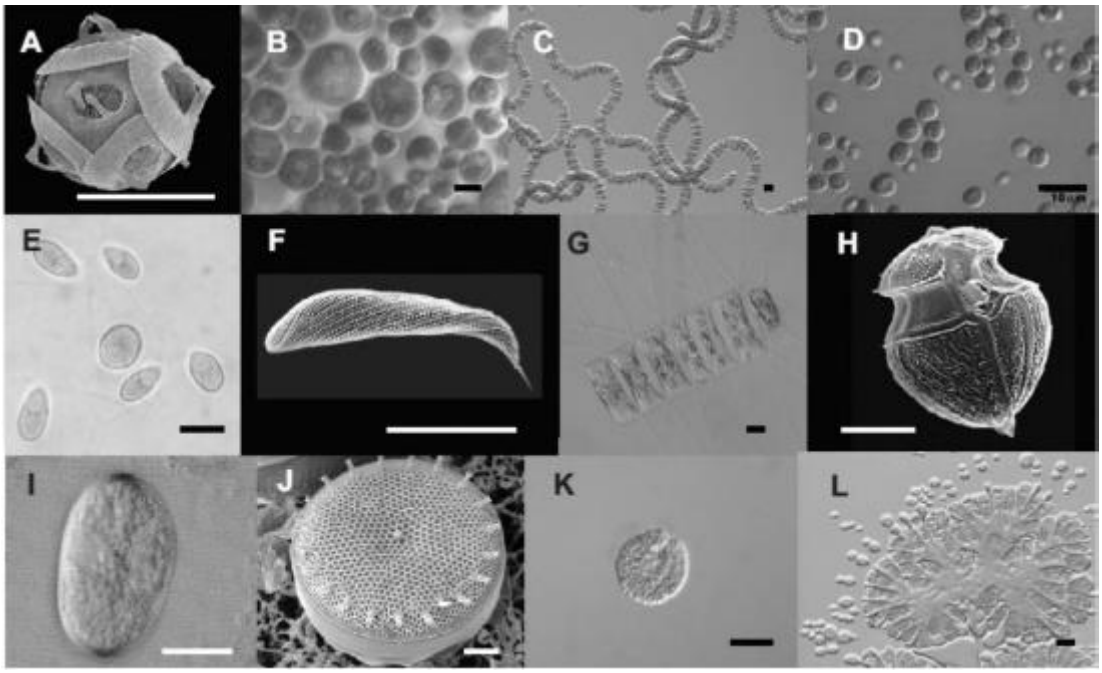


Fig 15: Morphological diversity of microalgae

More than 100,000 different species of microalgae exist around the world, but no more than 30,000 have been investigated and classified, according to their color, size, pigments, cell wall constituencies or metabolism, as suitable for human needs. Microalgae also include the unicellular organisms (phytoplankton) existing in naturalwater, which are an essential source of carbon for aquatic fauna [15,23,24].

4.2. Microalgae for bioenergy

The production of energy from microalgae is the driving force behind the enthusiasm and increasing research activities around the potential offered by these organisms since the beginning of the 21st century.Like the biomass deposits mobilized in bioenergy chains, and because of a metabolic phenotypic identical to higher plants, the work on the energy recovery of microalgae concerns essentially the same chains. Other avenues are also being explored, such as the direct production of hydrogen and the use of these organisms in microbial cells [107,108].

Liquefaction, pyrolysis or hydrogenation of these organisms produce a gaseous biofuel or raw "oil" by thermochemical conversion.Methane and ethanol can be produced by biochemical conversion from, respectively, biomass or fermentation of accumulated sugars in the cell,This conversion process also allows hydrogen to be produced by fermentation of the cell[107].

Finally, intracellular lipids can be extracted by chemical separation to integrate a biodiesel process after transesterification [108]**.** The lipid, fuel ethanol and methanisation sectors are the most interesting and, the most promising avenues for industrial development. Indeed, like other biomass sources, these forms of energy can complement or replace most fossil fuels (diesel, petrol and natural gas) and benefit from the same valorization processes, distribution networks and storage.

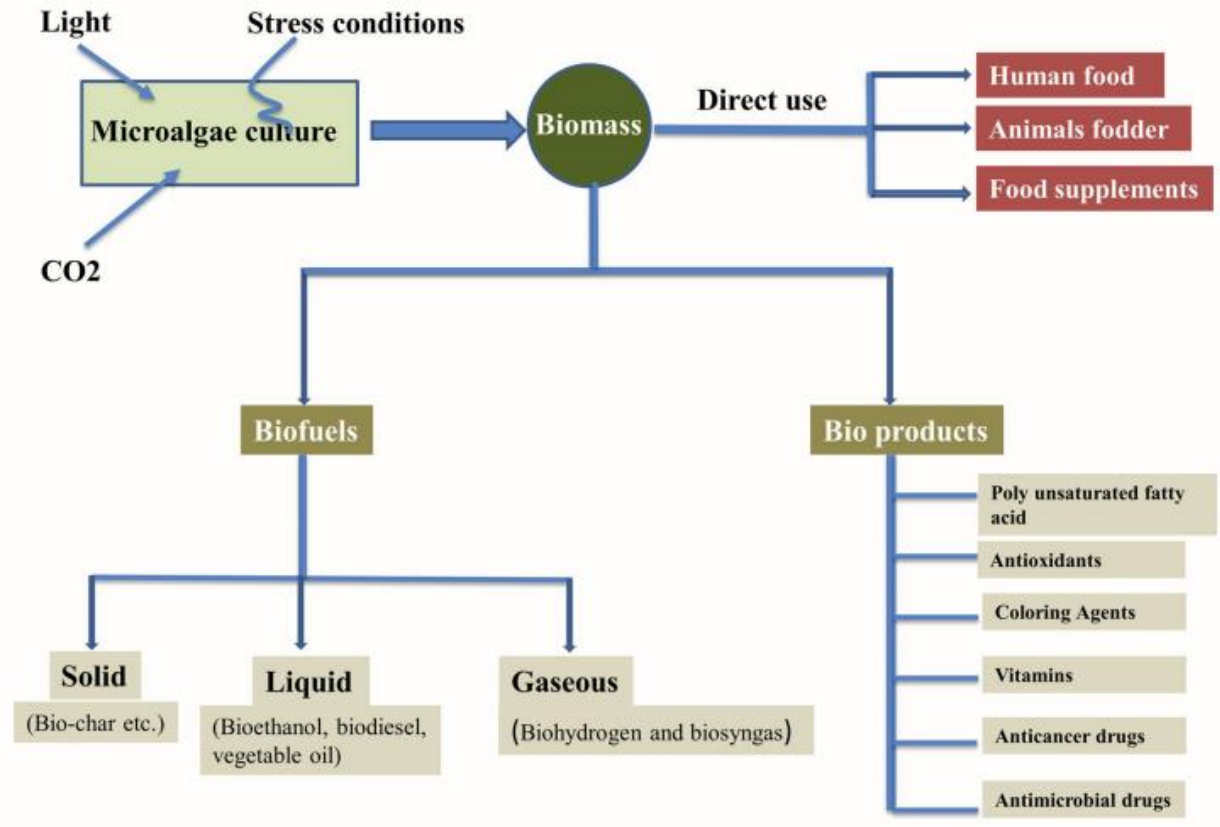


Figure 16: Microalgae Value Chain [109]

The actual focus in research is on microalgae as a feedstock for bioenergy production as a promising raw material to compensate and balance the increasing demands for biofuels, food, feed and valuable chemicals production [109].

4.3. Microlagae for wastewater treatemet

Since the early 1970s, the intention to use algae in the tertiary treatment of municipal water was already present. Treatment process had been considered to reduce the eutrophication potential of these waters [110]. It was found that these algae had an even greater treatment potential than expected and could remove many nutrients from the water, with a higher efficiency than

activated sludge. It therefore seemed economically more interesting to use these microorganisms in secondary rather than tertiary treatment [111]. Aerated ponds have been used for these applications. The unexpected results, in terms of their phytoremediation efficiency and rapid growth rate, have prompted several authors to test the growth of different algal species in specific waste waters, in order to verify their potential for the production of biofuels at more competitive prices.

Several authors report on the efficacy of green microalgae for the treatment of nutrient-laden wastewater. Wang et al [110] have verified in the laboratory the nutrient removal capacity of Chlorella sp. in wastewater municipal. The results obtained have led to the conclusion that these microalgae can easily grow using wastewater as a substrate in a volume of 100 mL. They are are also able to remove significant amounts of metals, phosphorus and nitrates and reduce chemical oxygen demand (COD). The best results of growth and removal were obtained with the medium with the highest nutrient content (N and P mainly) [110]. Other laboratory scale studies have provided similar conclusions with microalgae species and wastewater compositions different [112, 113, 114].

More studies have been carried out on the use of microalgae for phytoremediation of municipal wastewater than for industrial wastewater. The fact that industrial waters contain more compounds that may be toxic to algae than municipal waters may explain the difficulty of applying this treatment. In addition, the composition of industrial wastewater can vary from one production to another, making it difficult to use a standardized process. The few studies carried out on the phytoremediation of waters largely contaminated with dangerous compounds have most often been carried out with bacteria or plants rather than with microalgae. However, the growing interest of microalgae in the 2nd and 3rd generation biofuels sector has contributed to an increase in the number of experiments with these microorganisms.

4.4. Microlagae in MFCs

Research on renewable processes for producing energy has received a lot of attention in previous few years. Standing in such a scenario, the utilization of microalgae to convert greenhouse gases into potential biomass as well as their ability to produce oxygen, assumes strategic importance [115]. Important research is being carried out to use this ability of microalgae and integrate it with microbial fuel cells.

Microalgae can be used as substrate at the anode to removenutrients or capture the CO2 generated in the cathode, among otherpossibilities [106–109].

To capture energy as bioelectricity using photosynthesis in an MFC system, the photosynthetic organisms release electrons and protons and use charge separation, throughout flow chain reactions. During this treatment, synergistic effects between heterotrophic microorganisms and microalgae are happening, in which oxygen is produced by microalgae using solar energy and metabolizing nutrients and bicarbonates produced by heterotrophicmicroorganisms metabolizing organic matter and oxygen. Through this chain, the standard aeration systems might be replaced by the additional environmental and economic sustainable photosynthetic one. Thus, it's assumed that electricity may be produced in MFC by microalgae growing in cathode, where oxygen is produced by the photosynthesis process [117].This assumption has been applied in MFC by using a substrate which is oxidized at the anode and utilizing an micro algal biocathode which acts as a biological electron acceptor and at the same time reducing CO_2 to biomass [116].

Mainly, a mediator is employed for electron shuttling in cathode chamber. The electrons streaming from the anode are transported to the catholyte and reduce the oxidized state mediator, which then penetrates the microalgae cell store. The shuttled electrons are captured by growing microalgae cells, in their metabolic pathways to convert CO_2 into oxygen and biomass (Parlevliet and Moheimani, 2014). The oxidized mediator is released by the cells to the media and therefore the cycle is repeated where the mediator is again reduced by the electrons in the catholyte (Powell et al., 2009). during illumination, the biochemical reactions that take place at the anode and cathode are demonstrated by Eqs (X.1) and (X.2), respectively (Zhou et al., 2012a, b).

$$\boldsymbol{C_6H_{12}O_6 + 6H_2O \xrightarrow{Oxidation} 6CO_2 + 24H^+ + 24e^-}$$

$$\boldsymbol{6CO_2 + 12\,H^+ + 12\,e^- \xrightarrow{Light} -C_6H_{12}O_6 - (biomass) + 3O_2}$$

However, a distinction between photo-microbial fuel cells and microalgae-microbial fuel cells has to be made. Microalgae-MFC canwork in dark and in light conditions, although photo microbial fuelcells can only work with light [107].

4.5. Nutrient and organic matterremoval

Microalgae as an oxygen supplier couldpresent an interesting alternative thatcombines oxygen production in the cathodic chamber and contaminants removal (e.g. nitrate) that are present commonly in domestic and agricultural wastewaters. Domestic wastewater contains a variety of pollutantssuch as nutrients (N and P). It exists various types of technologies of wastewater treatment such as lagooning and activated sludge etc. However, insufficient removals have been reported for these wastewater pollutants because the secondary effluentfrom Wastewater Treatment Plants generally contains high amounts of nitrogen (N) and phosphorus (P), which can lead to nutrientenrichment and eutrophication in the receiving mediums

The microalgae used in the wastewater treatment can efficiently assimilate nitrogen and phosphorus for growth, but also eliminate heavy metals and organic matters (e.g. pharmaceuticals and personal care products (PPCPs)). Zhou et al. studied the capacity of four microalgae: *Chlamydomonasreinhardtii, Scenedesmus obliquus, Chlorella pyrenoidosa* and *Chlorella vulgaris*toremovedifferent pollutants from wastewater due to their high removal effectiveness for contaminants (Nitrogen, phosphorus, organic compounds). Thus, 76.7 to 92.3% of Nitrogen, and67.5 to 82.2% of Phosphoruswere removed. The four microalgae species were also found to be efficient in removing most of the organic matters with>50% removal.

5. Conclusion

The basis of this first chapter is to provide a general background to separation processes, components and Material, reactor Design Investigating the Double chamber MFC and Single-Chamber MFC, a more detailed introduction was given at the beginning of each individual chapter, concerning the purpose of the research that has been conducted there.

The third part was dedicated to the operational Factors Affecting MFC Performance, electron Transfer Mechanisms; investigation of IL based membrane in transport, Practical Applications and Scale-up, and finally resuming theopportunities and Challenges for MFCs in Wastewater Treatment.

The last part entails the study of Microlagae, including its definition, ability to generate bioenergy, and treat wastewater, Microlagae for MFCs, and capacity of organic matter removal was also investigated in detail.

References

[1] M. Kermani et al., "Heterogeneous catalytic ozonation by Nano-MgO is better than sole ozonation for metronidazole degradation, toxicity reduction, and biodegradability improvement," Desalin. Water Treat., vol. 57, no. 35, pp. 16435–16444, 2016.

[2] T. O. Anode, H. Layered, D. Hydroxide, and M. C. Cathode, "Thèse – Tesi di Dottorato – PhD thesis Soliu Oladejo GANIYU Electrochemical Advanced Oxidation Processes for Removal of Organic pollutants from Water : Performance Studies for Sub-stoichiometric Titanium Oxide Anode and Hierarchical Layered Double Hydroxi," 2016.

[3] Unesco and T. United, "Water in a Changing World," World Water, vol. 11, no. 4, p. 349, 2009.

[4] L. Guppy and K. Anderson, "Global Water Crisis: the Facts," 2017.

[5] Bristol Water, "Water Quality Report," 2008.

[6] A. R. Ribeiro, O. C. Nunes, M. F. R. Pereira, and A. M. T. Silva, "An overview on the advanced oxidation processes applied for the treatment of water pollutants de fi ned in the recently launched Directive 2013 / 39 / EU," Environ. Int., vol. 75, pp. 33–51, 2015.

[7] P. Stepnowski and A. Zaleska, "Comparison of different advanced oxidation processes for the degradation of room temperature ionic liquids," vol. 170, pp. 45–50, 2005.

[8] M. T. Bogert, "Chemical Abstracts," Science (80-.)., vol. 25, no. 641, pp. 579–580, 1907.

[9] M. Pirilä, Adsorption and Photocatalysis in Water Treatment. 2015.

[10] A. O. Ibhadon and P. Fitzpatrick, "Heterogeneous Photocatalysis: Recent Advances and Applications," Catalysts, vol. 3, pp. 189–218, 2013.

[11] I. K. Konstantinou and T. a. Albanis, "Photocatalytic transformation of pesticides in aqueous titanium dioxide suspensions using artificial and solar light: Intermediates and degradation pathways," Appl. Catal. B Environ., vol. 42, no. 4, pp. 319–335, 2003.

[12] E. Topkaya, M. Konyar, H. C. Yatmaz, and K. Öztürk, "Pure ZnO and composite ZnO/TiO2catalyst plates: A comparative study for the degradation of azo dye, pesticide and antibiotic in aqueous solutions," J. Colloid Interface Sci., vol. 430, pp. 6–11, 2014.

[13] M. Kralova, P. Dzik, M. Vesely, and J. Cihlar, "Preparation and characterization of doped titanium dioxide printed layers," Catal. Today, vol. 230, pp. 188–196, 2014.

[14] M. Saif and M. S. A. Abdel-Mottaleb, "Titanium dioxide nanomaterial doped with trivalent lanthanide ions of Tb, Eu and Sm: Preparation, characterization and potential applications," Inorganica Chim. Acta, vol. 360, no. 9, pp. 2863–2874, 2007.

[15] M. S. Hamdy et al., "Parameters controlling the photocatalytic performance of ZnO/Hombikat TiO 2 composites," J. Photochem. Photobiol. A Chem., vol. 228, no. 1, pp. 1–7, 2012.

[16] X.-J. Wen, C.-G. Niu, L. Zhang, and G.-M. Zeng, "Novel p–n heterojunction BiOI/CeO 2

photocatalyst for wider spectrum visible-light photocatalytic degradation of refractory pollutants," Dalt. Trans., vol. 46, no. 15, pp. 4982–4993, 2017.

[17] P. Pizarro, C. Guillard, N. Perol, and J. M. Herrmann, "Photocatalytic degradation of imazapyr in water: Comparison of activities of different supported and unsupported TiO2-based catalysts," Catal. Today, vol. 101, no. 3–4 SPEC. ISS., pp. 211–218, 2005.

[18] S. Chiron, A. Fernandez-Alba, A. Rodriguez, and E. Garcia-Calvo, "Pesticide chemical oxidation: State-of-the-art," Water Research, vol. 34, no. 2. pp. 366–377, 2000.

[19] X. Bai, R. Zong, C. Li, D. Liu, Y. Liu, and Y. Zhu, "Enhancement of visible photocatalytic activity via Ag@C3N4core-shell plasmonic composite," Appl. Catal. B Environ., vol. 147, pp. 82–91, 2014.

[20] T. Economist, "WaterStatistics," no. 505.

[21] S. Contreras, E. Pascual, S. Esplugas, J. Gim, and M. Rodrı, "Comparison of different advanced oxidation processes for phenol degradation," Water Res., vol. 36, pp. 1034–1042, 2002.

[22] S. Bougarrani, L. El Azzouzi, S. Akel, L. Latrach, A. Bouziani, and M. El Azzouzi, "Factors influencing Imazapyr herbicide removal from wastewater using photocatalytic ozonation," Acta Chim.Slov., vol. 65, 2018.

[23] K. Guo, D. J. Hassett, T. Gu, "Microbial Fuel Cells: Electricity Generation from Organic Wastes by Microbes," Chapter 9 in Microbial Biotechnology: Energy and Environment edited by R. Arora, ISBN 978-1845939564, pp. 162-189, CAB International, Oxon, United Kingdom. (2012).

[24] Logan, B. E., Hamelers, B., Rozendal, R., Schröder, U., Keller, J., Freguia, S., Rabaey, K. (2006). Microbial fuel cells: Methodology and technology. Environmental Science and Technology, 40(17), 5181-5192. https://doi.org/10.1021/es0605016

[25] B. E. Logan, D. Call, S. Cheng,H. V. M. Hamelers, T. H. J. A. Sleutels, A. W. Jeremiasse, R. A. Rozendal. Microbial Electrolysis Cells for High Yield Hydrogen Gas Production from Organic Matter.Environ. Sci. Technol. 2008, 42, 23, 8630-8640

[26] J. R. Beegle, A. P. Borole, Exoelectrogens for Microbial Fuel Cells, Progress and Recent Trends in Microbial Fuel Cells. 2018. 193- 230.

[27] S. Pandit, K. Chandrasekhar, R. Kakarla, A. Kadier, V. Jeevitha. Basic Principles of Microbial Fuel Cell: Technical Challenges and Economic Feasibility. Microbial Applications Vol.1. 2017. 165- 188.

[28] R. Veerubhotla, J. L. Varanasi, D. Das. Biofilm Formation Within Microbial Fuel Cells. Progress and Recent Trends in Microbial Fuel Cells.2018, 231-242

[29] S. VenkataMohan, G. Velvizhi, J. Annie Modestra, S. Srikanth. Microbial fuel cell: Critical factors regulating bio-catalyzed electrochemical process and recent advancements. Renewable and Sustainable Energy Reviews 40 (2014) 779–797

[30] S. Sevda, X. Dominguez-Benetton, K. Vanbroekhoven, H. De Wever, T.R. Sreekrishnan, D. Pant. High strength wastewater treatment accompanied by power generation using air cathode microbial fuel cell. Applied Energy 105 (2013) 194–206

[31] H. Lin, X. Wu, B. Hu, J. Zhu, Microbial electrochemical systems for agro-industrial wastewater remediation andrenewable products generation: a review, Arch. Microbiol. Biotechnol.1 (1) (2014). 20.

[32] P. Kaewkannetra, W. Chiwes, T.Y. Chiu, Treatment of cassava mill wastewater and production of electricitythrough microbial fuel cell technology, Fuel 90 (8) (2011) 2746–2750.

[33] J. Cheng, X. Zhu, J. Ni, A. Borthwick, Palm oil mill effluent treatment using a two-stage microbial fuel cellssystem integrated with immobilized biological aerated filters, Bioresour. Technol. 101 (2010) 2729–2734.

[34] M. Behera, P.S. Jana, T.T. More, M.M. Ghangrekar, Rice mill wastewater treatment in microbial fuel cellsfabricated using proton exchange membrane and earthen pot at different pH, Bioelectrochemistry 79 (2)(2010) 228–233.

[35] S. Puig, M. Serra, M. Coma, M.D. Balaguer, J. Colprim, Simultaneous domestic wastewater treatment andrenewable energy production using microbial fuel cells (MFCs), Water Sci. Technol. 64 (4) (2011) 904–909.

[36] Y. Ahn, B.E. Logan, Effectiveness of domestic wastewater treatment using microbial fuel cells at ambient andmesophilic temperatures, Bioresour.Technol. 101 (2) (2010) 469– 475.

[37] N. Mehrdadi, N.G. Bidhendi, H.R. Tashauoei, B. Vakili, M. Asadi-Ghalhari, Producing renewable energy frommunicipal wastewater treatment using a bio-electrochemical system, J. Saf. Environ. Health Res. 1 (1) (2016)23–26.

[38] Leong JX, Daud WRW, Ghasemi M, Liew KB, Ismail M (2013) Ion exchange membranes asseparators in microbial fuel cells for bioenergy conversion: a comprehensive review. RenewSustain Energy Rev 28:575–587. doi:10.1016/j.rser.2013.08.052

[39] V.M. Ortiz-Martínez, M.J. Salar-García, F.J. Hernandez-Fernandez, A.P. de los Ríos. Development and characterization of a new embedded ionic liquid based membrane-cathode assembly for its application in single chamber microbial fuel cells. Energy 93 (2015) 1748e1757

[40] Rahimnejad M, Bakeri G, Najafpour G, Ghasemi M, Oh S-E (2014) A review on the effect ofproton exchange membranes in microbial fuel cells. Biofuel Res J 1:7–15. doi:10.18331/BRJ2015.1.1.4

[41] A.N. Ghadge, M.M. Ghangrekar, Development of low cost ceramic separator using mineral cation exchanger toenhance performance of microbial fuel cells, Electrochim. Acta 166 (2015) 320–328.

[42] Z. Du, H. Li, T. Gu, A state of the art review on microbial fuel cells: a promising technology for wastewatertreatment and bioenergy, Biotechnol. Adv. 25 (2007) 464–482.

[43] Hernandez-Fernandez FJ, Perez de los Ríos A, Salar-García MJ, Ortiz-Martínez VM, Lozano-Blanco LJ, Godínez C, et al. Recent progress and perspectivesin microbial fuel cells for bioenergy generation and wastewatertreatment. Fuel Process Technol 2015;138:284e97.

[44] Martak J, Schlosser S, Vlckova S. Pertraction of lactic acid through supported liquid membranes containing phosphonium ionic liquid. J Membr Sci2008;318:298e310.

[45] M.J. Salar-García, V.M. Ortiz-Martínez, A.P. de los Ríos, F.J. Hernandez-Fernandez. A method based on impedance spectroscopy for predicting the behavior of novel ionic liquid-polymer inclusion membranes in microbial fuel cells. Energy 89 (2015) 648-654

[46] Y. Y. Yu, D. Zhai, R. Si, J. Sun, X. Liu, Y. C. Yong. Three-Dimensional Electrodes for High-Performance Bioelectrochemical Systems. Int. J. Mol. Sci. 2017, 18, 90; doi:10.3390/ijms18010090

[47] A. Baudler, I. Schmidt, M. Langner, A. Greiner and U. Schröder, Energy Environ. Sci., 2015, DOI: 10.1039/C5EE00866B.

[48] M. T. Noori, P. Chatterjee, M. M. Ghangrekar, C. K. Mukherjee. Low-Cost Solutions for Fabricationof Microbial Fuel Cells: Ceramic Separator and Electrode Modifications. 2018

[49] Li, Y., A. Lu, H. Ding, S. Jin, Y. Yan, C. Wang, C. Zen, and X. Wang, Cr(VI) reduction at rutile-catalyzed cathode in microbial fuel cells. Electrochemistry Communications, 2009.11(7): p. 1496-1499.

[50] Ter Heijne, A., D.P.B.T.B. Strik, H.V.M. Hamelers, and C.J.N. Buisman, Cathode Potential and Mass Transfer Determine Performance of Oxygen Reducing Biocathodes in Microbial Fuel Cells. Environmental Science & Technology, 2010. 44(18): p. 7151-7156.

[51] Zhang, L., C. Liu, L. Zhuang, W. Li, S. Zhou, and J. Zhang, Manganese dioxide as an alternative cathodic catalyst to platinum in microbial fuel cells. Biosensors and Bioelectronics, 2009.24(9): p. 2825-2829.

[52] Logan, B., S. Cheng, V. Watson, and G. Estadt, Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells. Environmental Science and Technology, 2007. 41(9): p. 3341-3346.

[53] Roche, I., K. Katuri, and K. Scott, A microbial fuel cell using manganese oxide oxygen reduction catalysts. Journal of Applied Electrochemistry, 2010. 40(1): p. 13-21.

[54] Cheng, S., H. Liu, and B.E. Logan, Power densities using different cathode catalysts (Pt and CoTMPP) and polymer binders (Nafion and PTFE) in single chamber microbial fuel cells. Environmental Science and Technology, 2006. 40(1): p. 364-369.

[55] Ortiz-Mart´ınez VM, Touati K, Salar-Garc´ıa MJ, Hernandez-Fernandez FJ, de los R´ıos AP, Mixed transition metal-manganese oxides as catalysts in MFCs for bioenergy generation from industrial wastewater, Biochemical Engineering Journal (2019), https://doi.org/10.1016/j.bej.2019.107310

[56] F.J. Hernández-Fernández A. Pérez de los Ríos, F. Mateo-Ramírez, C. Godínez, L.J. Lozano-Blanco, J.I. Moreno, F. Tomás-Alonso. New application of supported ionic liquids membranes as proton exchange membranes in microbial fuel cell for waste water treatment. Chemical Engineering Journal 279 (2015) 115–119

[57] L. Zhuang, Y. Zheng, S. Zhou, Y. Yuan, H. Yuan, Y. Chen. Scalable microbial fuel cell (MFC) stack for continuous real wastewater treatment. Bioresource Technology 106 (2012) 82–88

[58] M.J. Salar-García, V.M. Ortiz-Martínez, Z. Baicha, A.P. de los Ríos,F.J. Hernandez-Fernandez. Scaled-up continuous up-flow microbial fuel cell based on novel embedded ionic liquid-type membrane-cathode assembly. Energy 101 (2016) 113e120

[59] Zhang, E., W. Xu, G. Diao, and C. Shuang, Electricity generation from acetate and glucose by sedimentary bacterium attached to electrode in microbial-anode fuel cells. Journal of Power Sources, 2006. 161(2): p. 820-825.

[60] Chae, K.J., M.J. Choi, J.W. Lee, K.Y. Kim, and I.S. Kim, Effect of different substrates on the performance, bacterial diversity, and bacterial viability in microbial fuel cells. Bioresource Technology, 2009. 100(14): p. 3518-3525.

[61] Rabaey, K., G. Lissens, S.D. Siciliano, and W. Verstraete, A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. Biotechnol Lett, 2003. 25(18): p. 1531-5.

[62] Bennetto, H.P., Delaney, G. M., Mason, J. R. , Roller, S. D. , Stirling, J.L. & Thurston, C. F, The sucrose fuel cell: Efficient biomass conversion using a microbial catalyst. Biotechnol.Letts, 1985. 7: p. 699-705.

[63] Rezaei, F., D. Xing, R. Wagner, J.M. Regan, T.L. Richard, and B.E. Logan, Simultaneous cellulose degradation and electricity production by Enterobacter cloacae in a microbial fuel cell. Applied and Environmental Microbiology, 2009. 75(11): p. 3673-3678.

[64] D. R. Lovley. Bug juice: harvesting electricity with microorganisms. Nature Reviews-Microbiology.2006. (4) 497-508

[65] Y. P. Wang, X.W. Liu, W.W. Li, F. Li, Y.K. Wang, G.P. Sheng, R. J. Zeng, H.Q. Yu.A microbial fuel cell–membrane bioreactor integrated system for cost-effective wastewater treatment. Applied Energy 98 (2012) 230–235

[66] S. Puiga, M. Serraa, M. Comaa, M. Cabréa, M. D. Balaguera, J. Colprima. Microbial fuel cell application in landfill leachate treatment. Journal of Hazardous Materials 185 (2011) 763–767

[67] Venkata Mohan, S., G. Mohanakrishna, B.P. Reddy, R. Saravanan, and P.N. Sarma, Bioelectricity generation from chemical wastewater treatment in mediatorless (anode) microbial fuel cell (MFC) using selectively enriched hydrogen producing mixed culture under acidophilic microenvironment. Biochemical Engineering Journal, 2008. 39(1): p. 121-130.

[68] Gonzalez del Campo, a., Lobato, J., Cañizares, P., Rodrigo, M., andFernandez Morales, F. (2013). Short-term effects of temperature and COD in a microbial fuel cell.Applied Energy, 101:213–217.

[69] Gil G. C., Chang I. S., Kim B. H., Kim M., Jang J. K., Park H. S., KimH. J.Operational parameters affecting the performannce of a mediator-less microbial fuelcell.Biosensors and Bioelectronics, vol. 18, 2003, p. 327–334.

[70] Oliot M., Etcheverry L., Bergel A.Removable air-cathode to overcome cathode biofouling in microbial fuel cells. BioresourceTechnology, vol. 221, 2016, p. 691–696.

[71] Mauritz, K. a, and Moore, R.B. (2004). State of understanding of nafion. Chemical Reviews 104, 4535–4585.

[72] Cheng, S., Liu, H., and Logan, B. E. (2006). Increased power generation in a continuous flow mfc with advective flow through the porous anode and reduced electrode spacing. Environmental Science & Technology, 40(7):2426–2432. PMID: 16646485.

[73] M. Hosseinpour, M. Vossoughi, I. Alemzadeh. An efficient approach to cathode operational parameters optimization for microbial fuel cell using response surface methodology

[74] J. Huang, B. Sun, X. Zhang. Electricity generation at high ionic strength in microbial fuel

cell by a newly isolated Shewanella marisflavi EP1. Appl Microbiol Biotechnol (2010) 85:1141–1149

[75] Dlugolecki, P.; Anet, B.; Metz, S.J.; Nijmeijer, K.; Wessling, M. Transport limitations in ion exchange membranes at low salt concentrations. J. Membr. Sci. 2010, 346, 163–171.

[76] D. Aaron, C. Tsouris, C. Y. Hamilton, A. P. Borole. Assessment of the Effects of Flow Rate and Ionic Strength on the Performance of an Air-Cathode Microbial Fuel Cell Using Electrochemical Impedance Spectroscopy.

[77] G. Buitrón, I. López-Prieto, I. T. Zúñiga, A.Vargas. Reduction of start-up time in a microbial fuel cell through the variation of external resistance. Energy Procedia 142 (2017) 694–699

[78] J. You, J. Greenman, I. Ieropoulos. Novel Analytical Microbial Fuel Cell Design for

Rapid in Situ Optimisation of Dilution Rate and Substrate Supply Rate, by Flow, Volume Control and Anode Placement. Energies 2018, 11, 2377; doi:10.3390/en11092377

[79] W. L. Cheng, C. Erbay, R. Sadr, A. Han. Dynamic Flow Characteristics and Design Principles of Laminar Flow Microbial Fuel Cells. Micromachines 2018, 9, 479; doi:10.3390/mi9100479

[80] K.Y. Kim, W. Yang, B. E. Logan. Impact of Electrode Configurations on Retention Time and Domestic Wastewater Treatment Efficiency Using Microbial Fuel Cells WaterResearch (2015), doi: 10.1016/j.watres.2015.05.021.

[81] K. Y. Kim, W. Yang, P. J. Evans, B. E. Logan. Continuous treatment of high strength wastewaters using air-cathode microbial fuel cells. Bioresource Technology 221 (2016) 96–101

[82] H. Moon, I. S. Chang, Jae Kyung Jang, Byung Hong Kim. Residence time distribution in microbial fuel cell and its influence on COD removal with electricity generation. Biochemical Engineering Journal 27 (2005) 59–65

[83] Li Xiao, Erica B. Young, John A. Berges, Zhen He. Integrated Photo-Bioelectrochemical System for Contaminants Removal and Bioenergy Production. Environ. Sci. Technol. 2012, 46, 11459−11466

[84] Akihiro Noda, Kikuko Hayamizu, Masayoshi Watanabe. Pulsed-Gradient Spin-Echo 1H and 19F NMR Ionic Diffusion Coefficient, Viscosity, and Ionic Conductivity of Non-Chloroaluminate Room-Temperature Ionic Liquids. J. Phys. Chem. B 2001, 105, 4603-4610

[85] Tom Welton. Ionic liquids: a brief history. Biophysical Reviews (2018) 10:691–706

[86] Kyoko Fujita, Kenichi Murata, Miyuki Masuda, Nobuhumi Nakamura and Hiroyuki Ohno. Ionic liquids designed for advanced applications in bioelectrochemistry. RSC Advances, 2012, 2, 4018–4030

[87] Yuanyuan Zhang, Xiang Chen, Jingbo Lan, Jingsong You, and Lijuan Chen. Synthesis and Biological Applications of Imidazolium-Based Polymerized Ionic Liquid as a Gene Delivery Vector. Chem Biol Drug Des 2009; 74: 282–288

[88] Sihem Haj Kacem, Said Galaia, Antonia Pérez de los Ríos, Francisco José Hernández Fernández, IssamSmaali. New efficient laccase immobilization strategy using ionic liquids for biocatalysis and microbial fuel cells applications. 2018 (93) 174-183

[89] F.J. Hernández-Fernández, A.P. de los Ríos, F. Mateo-Ramirez, M.D. Juarez, L.J. Lozano-Blanco, C. Godinez, New application of polymer inclusion membrane based on ionic liquids as proton exchange membrane in microbial fuel cell, Separation and Purification Technology (2015), doi: http://dx.doi.org/10.1016/ j.seppur.2015.12.047

[90] Z.Zheng, Q. Xu, J. Guo, J. Qin, H. Mao, B.Wang, F.Yan." Structure−Antibacterial Activity Relationships of Imidazolium-Type Ionic Liquid Monomers, Poly(ionic liquids) and Poly(ionic liquid) Membranes: Effect of Alkyl Chain Length and Cations". ACS Appl. Mater. Interfaces 2016, 8, 12684−12692.

[91] E.Diaz, V. Monsalvo, J. Palomar, Angel F. Mohedano. "Ionic Liquids: Bacterial

Degradation in Wastewater Treatment Plants".Encyclopedia of Inorganic and Bioinorganic Chemistry, Online © 2011–2016 John Wiley & Sons, Ltd.

[92] N.Wood, J.Screening, "Ionic liquids for use in biotransformations with whole microbial Cells". Green Chem., 2011, 13, 1843.

[93] S. Mok Lee, W-J. Chang, A.R. Choi, Y.M. Koo, "Influence of Ionic Liquids on the Growth of Escherichia coli" Korean J. Chem. Eng., 22(5), 687-690 (2005)

[94] F.Ganske, U.T. Bornscheuer, "Growth of Escherichia coli, Pichia pastoris and Bacillus cereus in the presence of the ionic liquids [BMIM][BF4] and [BMIM][PF6] and organic solvents" Biotechnology Letters (2006) 28: 465_469

[95] C.J.T. de Grotthuss, "Memoir on the decomposition of water and of the bodies that it holds in solution by means of galvanic electricity". Biochimica et Biophysica Acta 1757 (2006) 871-875

[96] Schröder U, Anodic electron transfer mechanisms in microbial fuel cells and their energy efficiency Phys. Chem. Chem. Phys., 2007, 9, 2619–2629 | 2619

[97] M.Rosenbaum, F.Zhao, U.Scheder, F.Scholz. Interfacing Electrocatalysis and Biocatalysis with Tungsten Carbide: A High-Performance, Noble-Metal-Free Microbial Fuel Cell. Chem. Int. Ed. 2006, 45, 6658 –6661

[98] L.Koók, N.Nemestóthy, P.Bakonyi, G.Zhen,G. Kumar, X.Lu, L.Su, G.D.Saratale, S-H.Kim, L.Gubicza, "Performance evaluation of microbial electrochemical systems operated with Nafion and supported ionic liquid membranes", Chemosphere (2017).

[99] Z. Baicha, M.J. Salar-García, V.M. Ortiz-, F.J. Hernández-Fernández, A.P. de los Ríos, D.P. Maqueda Marín, J.A. Collado, F. Tomás-Alonso, M. El Mahi; "On the Selective Transport of Nutrients through olymer Inclusion Membranes Based on Ionic Liquids"Processes 2019, 7, 544

[100] Y. Ahn, B.E. Logan, Effectiveness of domestic wastewater treatment using microbial fuel cells at ambient andmesophilic temperatures, Bioresour.Technol. 101 (2) (2010) 469– 475.

[101] Kelly C. Wrighton and John D. Coates. Microbial Fuel Cells: Plug-in and Power-on Microbiology. Microbe. 2009 (4), Number 6, 281-287

[102] Shijia Wu, Hui Li, Xuechen Zhou, Peng Liang, Xiaoyuan Zhang, Yong Jiang, Xia Huang. A novel pilot-scale stacked microbial fuel cell for efficient electricity generation and wastewater treatment. Water Research 98 (2016) 396-403

[103] Liang, P., Duan, R., Jiang, Y., Zhang, X., Qiu, Y., Huang, X., One-year operation of 1000-L modularized microbial fuel cell for municipal wastewater treatment, Water Research (2018), doi: 10.1016/j.watres.2018.04.066.

[104] Rabaey K, Van de Sompel K, Maignien L, Boon N, Aelterman P, Clauwaert P, De Schamphelaire L, Pham HT, Vermeulen J, Verhaege M, Lens P, Verstraete W. Microbial fuel cells for sulfide removal. Environ Sci Technol. 2006 Sep 1;40(17):5218-24.

[105] Yonggang Yang, Guoping Sun, Meiying Xu. Microbial fuel cells come of age. J Chem Technol Biotechnol 2011; 86: 625–632

[106] Derek R. Lovley. Powering microbes with electricity: direct electron transfer from electrodes to microbes.Environmental Microbiology Reports (2011) 3(1), 27–35

[107] Z. Baicha, M.J. Salar-García, V.M. Ortiz-Martínez, F.J. Hernández-Fernández, A.P. de los Ríos, N. Labjar, E. Lotfi, M. Elmahi. A critical review on microalgae as an alternative source for bioenergyproduction: A promising low cost substrate for microbial fuel cells. Fuel Processing Technology 154 (2016) 104–116

[108] Qiang Hu, Milton Sommerfeld, Eric Jarvis, Maria Ghirardi, Matthew Posewitz, Michael Seibert, Al Darzins. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. The Plant Journal (2008) 54, 621–639

[109] Muhammad Imran Khan, Jin Hyuk Shin, Jong Deog Kim. The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. Khan et al. Microb Cell Fact (2018) 17:36

[110] Liang Wang, Min Min, Yecong Li, Paul Chen,Yifeng Chen, Yuhuan Liu, Yingkuan Wang, Roger Ruan. Cultivation of Green Algae Chlorella sp. in Different Wastewaters from Municipal Wastewater Treatment Plant. Appl Biochem Biotechnol (2010) 162:1174–1186

[111] N. E Y. Tam & Y. S. Wong. Wastewater Nutrient Removal by Chlorella pyrenoidosa

and Scenedesmus sp. Environmental Pollution 58 (1989) 19-34.

[112] Giulia Samorı`, Chiara Samorı`, Franca Guerrini, Rossella Pistocchi. Growth and nitrogen removal capacity of Desmodesmuscommunis and of a natural microalgae consortium in a batc culture system in view of urban wastewater treatment: Part I. water research 47 (2013) 791-801

[113] Iago Teles Dominguez Cabanelas, Jésus Ruiz, Zouhayr Arbib, Fábio Alexandre Chinalia, Carmen Garrido-Pérez, Frank Rogalla, Iracema Andrade Nascimento, José A. Perales. Comparing the use of different domestic wastewaters for couplingmicroalgal production and nutrient removal. Bioresource Technology 131 (2013) 429–436

[114] Sunja Cho, Nakyeong Lee, Seonghwan Park, Jaecheul Yu, Thanh Thao Luong, You-Kwan Oh, Taeho Lee. Microalgae cultivation for bioenergy production using wastewaters from a municipal WWTP as nutritional sources. Bioresource Technology 131 (2013) 515–520

[115] J. Popp, Z.Lakner, M.Harangi-Rákos, M.Fári. The effect of bioenergy expansion: Food, energy, and environment. RenewableandSustainableEnergyReviews32(2014)559–578

[116] S.P. Singh, P. Singh, Effect of CO_2 concentration on algal growth: a review, Renew. Sust.Energ. Rev. 38 (2014) 172–179.

[117] S. Pandit D. Das, Role of Microalgae in Microbial Fuel Cell. Ch. 17. Algal Biorefinery: An Integrated Approach, DOI 10.1007/978-3-319- 22813-6_17

[118] Wei Y, Van Houten R. T., R.Borger A, Eikelboom D. H, YaoboFan. Minimization of excess sludge production for biological wastewater treatment. Water Research 37, 18, (2003) 4453-4467.

[119] Canfield R.E.,(1963). J. Biol. Chem. 238, 2698-2707

[120] Firas Khaled. Contribution to electrical valorization of microbial fuel cells. Micro and nanotechnologies/ Microelectronics. Université de Lyon, 2016. English. NNT : 2016LYSEI003. tel-01494668

Chapter II:
Factors influencing wastewater treatment in microbial fuel cell: Practical case

1. Introduction

Waste treatment and electricity generation are both key issues for sustainable modern societies. Microbial fuel cells (MFCs) are attracting increased attention, driven by the demands for clean and renewable energy resources, and especially for their potential to directly recover electricity from various wastewaters [1]. All types of wastewater containing organic matter can be treated by this process, including domestic wastewater, brewery effluent, and much else. Several plants are in operation and have shown good results. Use of MFCs for wastewater requires a design which allows the wastewater to flow through the cell over the anode surface. Various configurations have been adopted for this purpose, including the tubular MFC where the cathode is placed on the outside of the tube and the anode occupies the full internal space. Wastewater flows through the anode from one end to the other [2-3].

The electricity generation efficiency and wastewater treatment are affected by MFC architecture, electrode design, and feeding flow.

Many types of architectures, such as micro-size MFC [6], vertical MFC using up-flow feeding, horizontal MFC using up-flow feeding [7-9] or down-flow feeding [10], baffled stacking MFC [11], and tubular MFC [12], have been used to enhance the efficiency of The electricity generation efficiency and wastewater treatment.

In this study, the potential of microbial fuel cell in treating an industrial oil wastewater collected from a primary treatment stage of the ACEITES ESPECIALES DEL MEDITERRANEO S.A (AEMEDSA) factory in Cartagena City, Spain.

In order to evaluate the efficiency of wastewater treatment through the MFC system, the effluent from the reactor was examined with regard to, COD (Chemical Oxygen Demand), total P (Phosphorus), TSS (Total Suspended Solids), SO_4^{2-} (sulfate), Cl^- (Chloride), NO_3^- (Nitrate), HPO_4^{2-} (Hydrogen phosphate), Fe^{2+} (Iron) and pH according to the standard methods in the textbook of standard methods for water and wastewater examination [6]. Total suspended solids, hardness, alkalinity and ion analysis were performed using AFNOR standard methods.

Flow rate was also investigated during this study in order to evaluate the efficiency of wastewater treatment through the MFC systemsince biofilm requires sufficient contact time with organic substrates in order to both absorb and degrade the substrates.

2. Material and methods

2.1. Wastewater characteristics

Effluents from the food and agricultural industries are complex fuels, as already explained in the first chapter; due to their organic matter loadings. They contain a wide variety of organic components, making them suitable for applications as source in microbial fuel cells.

Industrial oil wastewater was collected fromthe output of a primary treatment stage of the ACEITES ESPECIALES DEL MEDITERRANEO S.A (**AEMEDSA**) factory in Cartagena City, Spain and was kept at 4 °C in a fridge before their use as substrate of organic matter and microorganisms in the continuous flowexperiments, being the initial COD (chemical oxygen demand) 1280 mg L^{-1}.Some characteristics of this wastewaterare shown inTable 1.

The Industrial oil wastewater was utilized as fuel for MFC tests without additions of whatever other nutrient or trace metals. The injected wastewater before inoculation to the anode MFC chamber were deoxygenated with gas of N_2 during all the period of the experiments to remain in anaerobic condition. The tank with wastewater was placed on a mixing device with 50 rpm in view to prevent decantation.

The sampling operation was done each 24h by a plastic syringe at the outlet of the reactor by taking a 10 mL aliquot. The sample was centrifuged at 5000 rpm within 5 min and then filtrated (standard membrane filter of 0.45 microns).

Table II-1: Characteristic of AEMEDSA factory wastewater in Cartagena

Parameters	Value
pH	8,44
Electrical Conductivity (mS/cm)	14,58
Redox Potential (mV)	112
COD (mg/l)	1276
TTS (mg/l)	1534
SO_4^{2-} (mg/l)	7397
Fe (II) (ppm)	1285
Cl^- (mg/l)	726
NO_3^- (mg/l)	9.17
Total Phosphorus (mg/l)	15,96

2.2. Continuous flow Rectors configuration

Two single-chamber air-cathode MFCs reactor wereconstructed: a) a vertical one on the basis of a cylindrical polyvinylchloride (PVC)-methacrylate plastic tube, with a total volume of 1.7 L. Fig1 shows the main components of the vertical reactor. b) a horizontal one on the basisof polyvinylchloride (PVC) plastic tube with a total volume of 0.88 L. Fig 3 shows the composition of the horizontal reactor.

2.2.1 Vertical reactor:

A PVC-filter incorporating carbon granules is stored inside the methacrylate housing cylinder measuring 25 x 5 Cm, with a plastic cap on the top of the reactor containing three orifices. The first orifice is used to supply the MFC with influent by a flexible tube that reaches the bottom of the housing fixed to its wall. The second orifice is utilized to pump out the treated wastewater (effluent) from the top of the reactor. In this manner, wastewater is supplied at the bottom of

the reactor and then streams to the top whereas being treated. Finally, the third orifice located in the middle of the cap houses a carbon bar of 23 Cm insert across the body of the reactor, being in contact with graphite granules (anode).

The membrane-cathode assembly covers a window on the methacrylate housing of 18 x 20 cm, which is the total area of the membrane exposed to air and to the water that flows through the reactor simultaneously. Finally, a copper wire is wrapped around the reactor (in contact with the membrane-cathode assembly) and connects the cathode (carbon cloth) to the anode (carbon bar/graphite granules) through an external resistance of 1000 Ω.

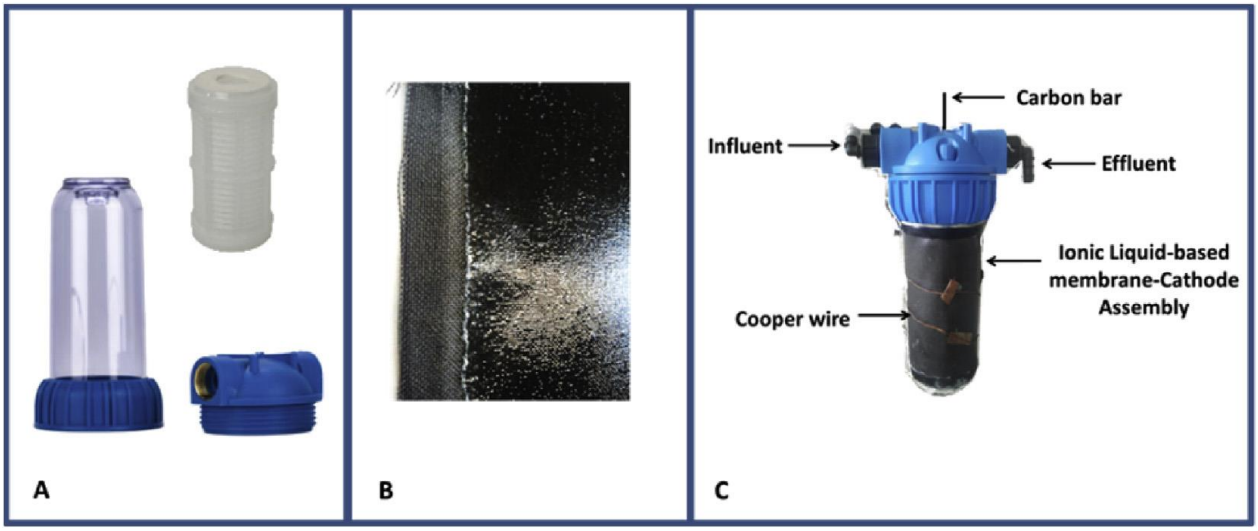


Fig. 1A) Components of the continuous MFC; B) Embedded IL-based membrane-cathode assembly; C) Final design of the single-chamber air-cathode microbial fuel cell.

.

.

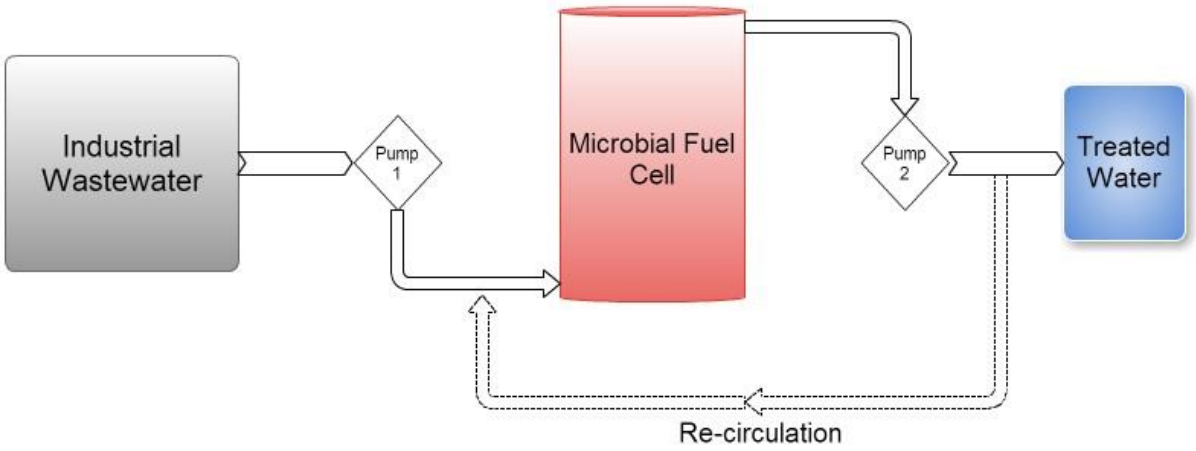


Fig 2: Scheme of the MFC vertical reactor operated in a continuous flow

2.2.2 Horizontal reactor:

Carbon granules are incorporated in a PVCperforated tubemeasuring 22 x 3 Cm, which is included in a cylinder tube containing 12 small windows with two plastic capsat two endsof the reactor containing one hole, The first hole which is situated in the low level of the reactor is used to feed the MFC with wastewater. The second hole which is in the highest point of the reactor is used to collect the treated wastewater. Thus, wastewater is fed from the bottom and flows to the top of so wastewater will be treated.

Four carbon bars of 8 Cm insert across four orifices in the body of the reactor, being in contact with graphite granules (anode).

The membrane-cathode assembly covers all the reactor (expect the caps) on the methacrylate housing of 10 x 18 cm, which is the total area of the membrane exposed to air and to the water that flows through the reactor simultaneously. Finally, a copper wire is wrapped around the reactor (in contact with the membrane-cathode assembly) and connects the cathode (carbon cloth) to the anode (the four carbon bars/graphite granules) through an external resistance of 1000 Ω.

Fig3: Components of the horizontal reactor, embedded membrane-cathode assembly and the final design of the single-chamber air-cathode microbial fuel cell

2.3. Procedure, inoculation, and operation

Two single-chamber air-cathode MFCs operating in continuous mode were set up by using an embedded membrane-cathode assembly based on Triisobutyl(methyl) phosphonium tosylate, $[P^{+}_{I4,I4,I4,1}][TOS^{-}]$. The systems were matured during 15 days until the development of biofilm took place around the graphite granules.

Therefore, the reactorswere nourished at a constant feed flow rate of 0.25 mL min^{-1} until becoming stable (=168 h). At that time, the two systems were emptied, the carbon granules replaced by new ones and filled with fresh wastewater. After the reactors matured, the feed flow rate was changed and fixed at 0.35 mL min^{-1} and the same process was carried out. The evolution of chemical oxygen demand and voltage response (Metrohm Autolab PGSTAT302N, The Netherlands) were monitored for process control.

2.4. Membrane cathode preparation and assembly

The method to prepare polymer inclusion membranes embedded into carbon cloth cathodes dwells of pouring a homogenous solution of ionic liquid (70%), polyvinyl chloride (PVC, 30%) and an appropriate amount of solvent directly into a borosilicate rectangle (18 x 20 cm) placed on a rectangular piece of carbon cloth (19 x 21 mm)with waterproofing treatment of 5 % and a thickness of 0.5 mm (Fuel CellEarth, USA) for the vertical reactor. Regarding the horizontal reactor, the same mixture of ionic liquid, PVC and solvent was used into borosilicate rectangle (10 x 18 cm)placed on a rectangular piece of carbon cloth (11 x 19 cm) with waterproofing treatment of 5 % and a thickness of 0.5 mm (Fuel CellEarth, USA).

The cathode with the membrane mixture is left to dry overnight to allow the complete evaporation of the solvent. The platinum was sprayed 0.5 mg Pt/cm^2)blended with distilled water, isopropyl alcohol and a small amount of polytetrafluoroethylene, which acts as a binder (60 wt% dispersion in water, Sigma AldricheFlucka), Once dry, the homogeneous polymer inclusion membrane solution is poured over the same face of the cathode. When the membrane solution is poured over the carbon cloth, the solution streams by gravity through the cloth fibers to the other side of carbon cloth, which becomes covered in a bigger amount of membrane (plastic appearance) [13].

A HITACHI S-3500N scanning electron microscope (SEM) and an energy dispersive X-ray BRUKER AXS (EDX) analyzer with high vacuum and variable pressure mode are used to study the morphological appearance, overall chemical composition and distribution of the chemical elements present in the two membranes assemblies. This characterization by SEM-EDX is performed for polymer inclusion membranes based on ionic liquids in their fresh state and directly after use.

2.5. Analytical methods

2.5.1. Electrochemical characterization of Polymer inclusion membranes

The studied PIMwas characterized in a two MFC configuration. Internal resistancevalues were calculated from Nyquist plots, which in turnswere obtained with the AC ImpedanceSpectroscopy method, FRA(frequency response analysis) (Autolab PGSTAT302N, MetrohmAutolab B.V., Utrech, The Netherlands) Electrical impedance measurements ranged from 1 MHZ to 0.1 Hz with a perturbation voltage amplitude of 0.05 V [8].

2.5.2. Polarization and power curves calculation

Polymer inclusion membranes were studied in MFCs using wastewater as substrate, andthe results in terms of maximum power density were linked to theionic conductivity appearing from electrochemical impedancespectroscopy technique (*EIS*). Polarization curves present how MFCs maintains voltage well as a function of the current (*V vs. I*) as long as power curves describe the power densityin functionof the current (*P vs. I*). Both were obtained by measuring thevoltage of the system in a range of external load resistances (R_{ext}), from 1100 kΩ to 0.9 Ω, starting at OCV (OpenCircuit Voltage) conditions. For each load, the currentdensity was first calculated by the formula$I=E/R_{ext}$ and then standardized by theanode volume. The power density can be first calculated as $P=E_2/R_{ext}$, or as $P = I_2/R$, and then standardized by the anodevolume.

From the power curve, the internal resistance of a given MFC can becalculated. As reported by Logan [7], external and internal resistance areequal whenpower density is maximum ($R_{ext} = R_{int}$). The internalresistance of the system is known if the $R_{ext}$is calculated from thepower curve as $R_{ext} = E/I$, where *E* and *I* are voltage and current at maximum power density.

2.5.3. Chemical oxygen demand effect and measurements

Concentration of the wastewater chemical oxygen demand (COD) necessarily influences the wastewater treatment and power generation in MFC operated in continuous mode. Current output and effectiveness of treatment are directly proportional to the concentration of substrate which can be measured in terms of COD in an MFC. Higher COD yields maycauses substrate inhibition [14].

COD (Chemical oxygen demand) values was measured withSpectroquant COD Cell Tests, Photometric method (Merck, Germany) and COD Spectroquant Nova 30 spectrophotometer (Merck, Germany), using the method described in APHA **[5,6]**. It was measured at the beginning and after 24h during 5 days for each flow.

For the measurement of COD, the photometric method was used with the MERCK COD TUBES IN TESTS (*Fig 4*) for a range of measurements from 25 to 1500 mg/l. The digestion of 3 ml of sample filtered with MILLEX 0.45 filter μm (MILLIPORE) is carried out during two hours at 150ºC in a Velp®Scientifica ECO 16 model digestion thermoreactor with capacity for 14 digestion tubes of 16 mm diameter and 10 ml capacity. A measurement was then made on a MERCK spectrophotometer model NOVA 30 (*Fig 5*). This spectrophotometer has a large

number of programs, but when the tube is inserted, it automatically recognizes the appropriate program for the introduced cell.

Soluble CODremoval $(COD)_R$ is defined as the ratio between the total COD loadconsumed in the process and the initial COD load, being the CODconsumed (COD_R) the difference between the initial chemical oxygendemand $[COD]_0$ and the chemical oxygen demand at a giventime [COD]:

$$COD_R\ (\%) = ([COD]_0 - [COD]).100/[COD]_0 \qquad (1)$$

Fig 4: MERCK cuvette test for COD

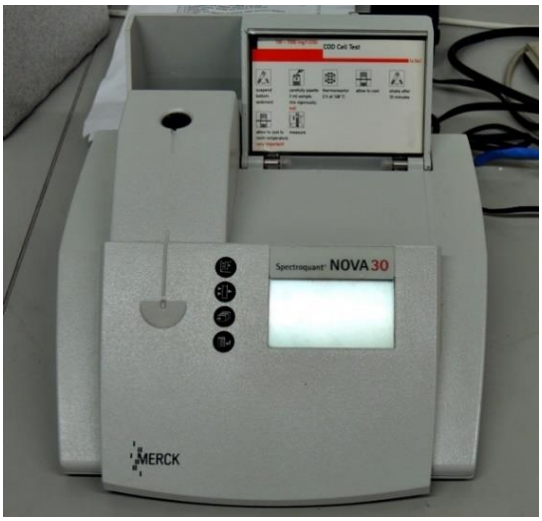


Fig 5: MERCK spectrophotometer model NOVA 30

2.5.4. Wastewater treatment parameters

In order to evaluate the efficiency of wastewater treatment through the MFC system, besides COD (Chemical Oxygen Demand) the effluent from the reactor was examined with regard to, total nitrogen, total P (Phosphorus), TSS (Total Suspended Solids), SO_4^{2-} (sulfate), Cl^- (Chloride), NO_3^- (Nitrate), HPO_4^{2-} (Hydrogen phosphate), Fe^{2+} (Iron) and pH according to the standard methods in the textbook of standard methods for water and wastewater examination **[6].** Total suspended solids, hardness, alkalinity and ion analysis were performed using AFNOR standard methods.

2.5.5. Hydraulic Retention Time (HRT)

In terms of HRT, biofilm requires sufficient contact time with organic substrates in order to both absorb and degrade the substrates. The MFC is performing in a continuous flow mode by feeding

wastewater into the bottom of the vertical reactor using a peristaltic pump (Ismatec™ MS-4/12 Reglo Digital Pump Flow Rate: 0.001 to 24mL). The flow rates were adjusted to obtain HRTs of 360 mL/day or 504 mL/day, and these were slow enough to warm the wastewater to ambient temperature in the constant temperature room (30 °C).

3. Results and discussion

3.1 Horizontal reactor:

The ionic-liquid based PIMs in this study were tested asseparator in a new horizontal configuration of MFCs fed with wastewater and their polarization and power curve was obtained. Fig 6. This curve describe power density and voltage, respectively, power and current densities are depended to the anode volume. The internal resistance of the MFCcan be calculated by power curve, as explained above. The power density at 0.11 V was 42,78 mW m^{-3}.

After 48 hours of the experiment, the voltage achieved the lowest value, and the reactor stop working because of membrane degradation. Thus, water start leaking through the membrane and the power shut down. (Table 2-3)

Table II-2: Voltage results of the horizontal reactor after 2 days of experiment

Day	***Hour***	***Voltage (mV)***
First day	***09:00***	***152***
	13:00	***126***
	17:00	***114***
	21:00	***80***
Second day	***09:00***	***37***
	13:00	***39***
	17:00	***41***
	21:00	***47***

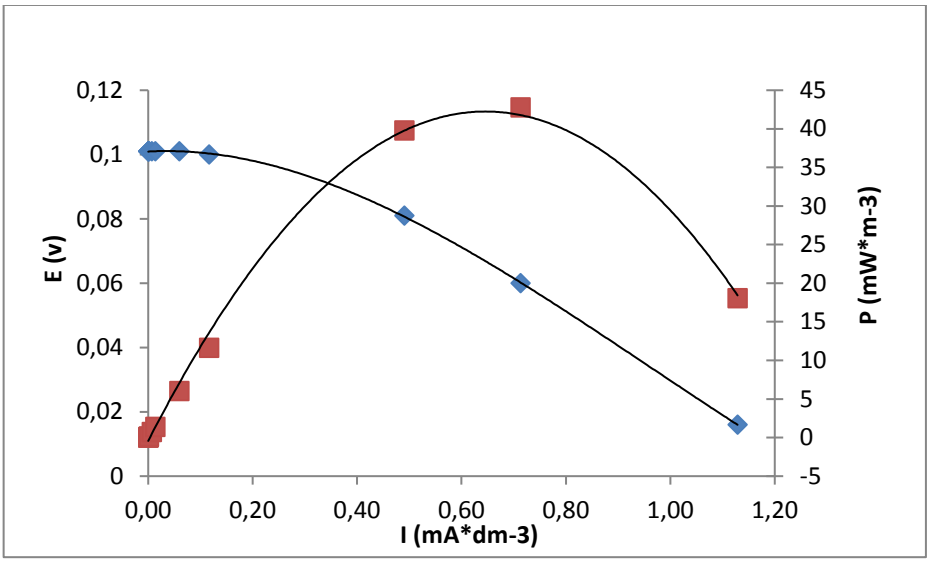


Fig 6: Polarization curve of the horizontal reactor MFC using an embedded cathode membrane based on IL $[P_{I4;I4;I4;1}^{+}][TOS^{-}]$ for the first day

Table II-3: COD Value of the horizontal reactor MFC

	COD Value (mg/L)
Initial value	***1280***
After one day of treatment	***1832***

As shown in table 3, COD value after one day of treatment is higher than the initial value, which prove that an important part of the membrane was dissolved in the wastewater. The reasons of membrane degradation are discussed in the next section.

3.1.1 Membrane degradation and effect of $Fe^{2+}$and Carbon

Membrane degradation can be caused by various phenomena: mechanical, thermal stresses or chemical degradation. Mechanical degradations are pointed out as the major cause of failure during the first hours of operation. These degradations can occur upstream, during the manufacture of the membrane or during assembly (positioning, tightening...). The areas under the channels, which are particularly sensitive, are subject to inhomogeneous mechanical stresses, contributing to the weakening of the membrane [12].

IL based membrane was exposed to the wastewater containing Fe^{2+}in a concentration of 250 ppm at 27 °C for 48 h. It has been proved that the exposure of a membrane to different concentrations of Fe^{2+} is a key factor in membrane degradation. At low concentrations of Fe^{2+},

the principal degradation product is fluoride ion and a small number of polymers. As the Fe^{2+} concentration increases, the radicals may attack fewer active bonds such as C–O in the polymer. Despite, the X-ray fluorescence spectra discussed below show a large loss of fluorine from the membrane with the highest Fe^{2+} concentration, the low F^{-} emission rate cannot account for this large loss [15].

Based on the experimental results, the degradation of the membrane can occur due to the attack of defects in the main chain such as "residual" C–H bonds that exist in the wastewater, in carbon-cloth and in the graphite granules. In fact, C–F bonds are responsible for the chemical stability membranes and are thereby improbable to be attacked extensively by free radicals. However, defects such as C–H or C=C bonds, which may develop in the polymer during the experiment, are enough to launch radical reactions and ultimately degrade the membrane [9].

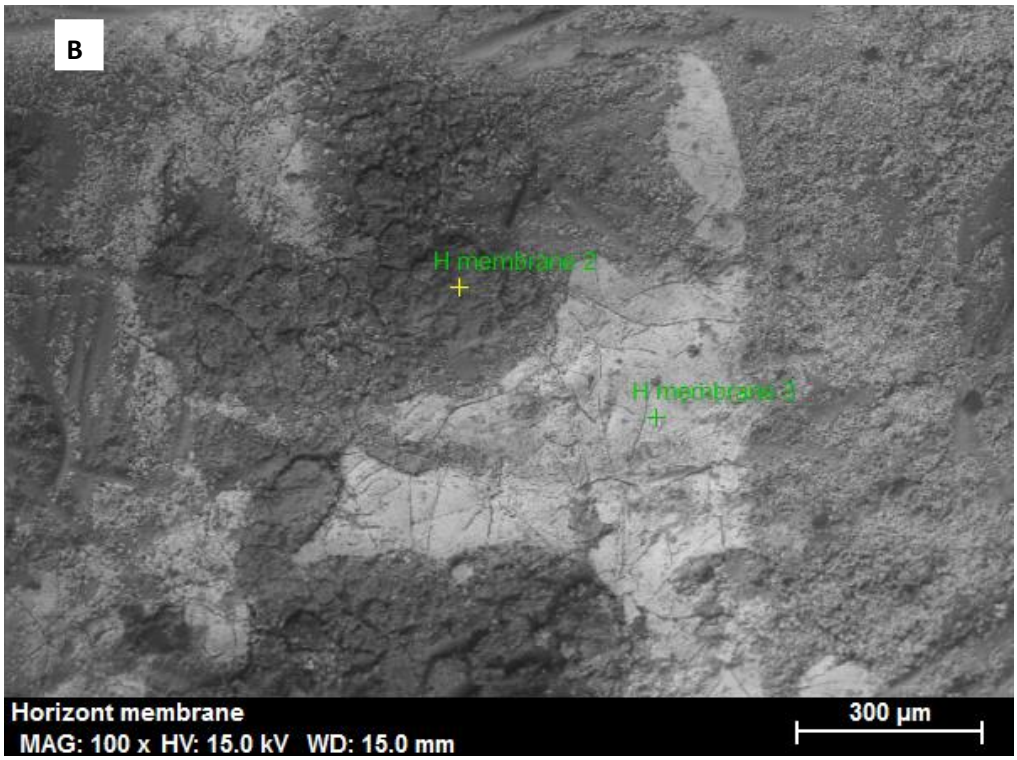


Fig 7: A: PIM before experimentation in a MFC; B: PIM after experimentation in a MFC

The carbon-centered radical is not stable; therefore, it can be subjected to further radical reaction with hydroxyl radicals or even Oxygen and water to generate more stable peroxyl radicals or ester structures and thereby produce fluoride ions and polymer fragments [15].

Regarding our experiment, the horizontal reactor had a small distance between the tube containing carbon granules and carbon graphite bar compared to the vertical one.
In horizental case, carbon is precipitad in the bottom of reactor result of gravity in direct contact with the menmbrane. Wastewater is then charged by carbon particulesdue to its contactwith carbon granules and PIM.

Unlike the vertical reactor, which the PIM is in contact only with effluent in the half of the reactor and where the precipated carbon is not in direct ontact with membrane.

SEM images were taken before and after the experiment as shown in Fig 7. In these images the membrane cathode assembly before use is shown left (A), and the right image shows the membrane cathode assembly after the experiment (B).

3.1.2 Pollutants adsorption by membrane

Even if the membrane was degraded partially, it has been tested by X-ray fluorescence and it shows another aspect of this membrane. The percentage of many pollutants have decrease after only 48 h of treatment. Thus, the membrane has adsorbed some pollutants such as: Na^+, Mg^+, Ca^+, Al^-, Cl^-, and also some heavy metals e.g. Cu, Cd, Cr.

Sulek et al. have reported that aluminum and chromium contaminated membrane are able to produce a great loss in performance due to their high valence. Thus, this hypothesis held true only for aluminum [11].

Chloride adsorption have been resulted in a loss in electrochemical surface area. The presence of Cl^- in the fuel stream leads to an initial sudden decline of the cell performancemeasured by cell voltage at a constant current density [10].

Table II-4: Results of X-Ray Fluorescence test in the membrane of the horizontal reactor in the end of treatment process by MFC.

Elements	Blank	H Vacuum	V Vacuum	H Helium	V Helium
C	74,6800	58,4000	69,3300	59,1900	69,7400
Na	0,0091	2,6150	0,5630	2,3000	0,6100
Mg	0,0055	0,2080	0,0495	0,1570	0,0515
Al	n.d.	0,1390	0,1060	0,1950	0,1220
Si	0,0140	0,0461	0,0191	0,0430	0,0277
P	5,1570	1,0380	1,4570	1,3390	1,3580
S	5,0510	0,9818	n.d.	0,5356	n.d.
Cl	14,6400	35,2700	26,3000	34,4500	25,7400
K	n.d.	0,0401	0,0110	0,0341	0,0130
Ca	0,0062	0,1970	0,2040	0,2200	0,1620
Mn	n.d.	n.d.	n.d.	n.d.	0,0021
Fe	0,0056	0,0030	0,2505	0,0285	0,3068
Cu	0,0122	0,0437	0,0175	0,0170	0,0507
Zn	0,0167	0,0027	n.d.	0,0023	0,0019
Br	0,0034	0,0021	0,0057	0,0040	0,0092
Sr	n.d.	0,0047	0,0043	0,0027	n.d.
Pt	0,3950	1,0230	1,6750	1,4700	1,7960
Total (%)	99,9957	100,0062	99,9926	99,9882	99,9909
Mass (g)	0,57	0,65	0,40	0,28	0,21

Note: the membrane adsorbs Cl^- and Na^+, Fig 8 shows the formation of salt during and after treatment in the membrane assembly.

Fig 8: Salt formation on the membrane assembly during and after treatment

3.2 Vertical reactor:

Polarization and power curves for the studied continuous vertical MFCs were measured when the voltage response of the systems werestable. In contrary to the horizontal reactor, the vertical one has performed in good conditions in both flows (0.25 ml.min^{-1}and 0.35 ml.min^{-1}) till the end of experiment with a small amount of leaking drops through the membrane during the second and the third day.

The first flow: 0.25 ml.min^{-1}

Table II-5: Effluent concentration and COD removal by the vertical reactor using the first flow (*0.25 ml.min^{-1})*

Time (h)	Effluent concentration (mg/l)	Removal (%)
0	***1276,33***	***0,00***
24	***1158,33***	***9,25***
48	***983,33***	***22,96***
72	***711,33***	***44,27***
96	***579,00***	***54,64***
120	***522,00***	***59,10***
144	***512,00***	***59,89***
168	***502,33***	***60,64***

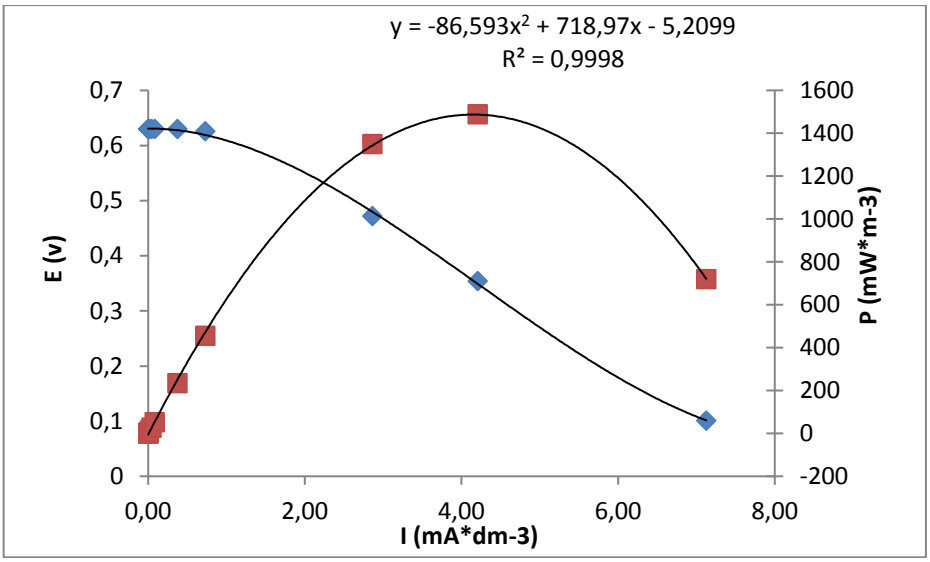


Fig 9: Polarization curve of the vertical reactor MFC working in the first flow for the last day of treatment

Power curves and polarization for the continuous MFCs studied were measured when the voltage response of the systems werestable. As shown in Fig 9, power density at 0.35 V was 1,49 Wm^{-3}.

The second flow: 0.35 ml.min^{-1}

Table II-6: Effluent concentration and COD removal by the vertical reactor using the second flow (*0.35 ml.min^{-1}*)

Time (h)	Effluent concentration (mg/l)	Removal (%)
0	***1276,33***	***0,00***
24	***1188,33***	***6,89***
48	***1023,33***	***19,82***
72	***818,67***	***35,86***
96	***722,67***	***43,38***
120	***626,67***	***50,90***
144	***618,67***	***51,53***
168	***607,67***	***52,39***

3.2.1 IL-based membrane stability:

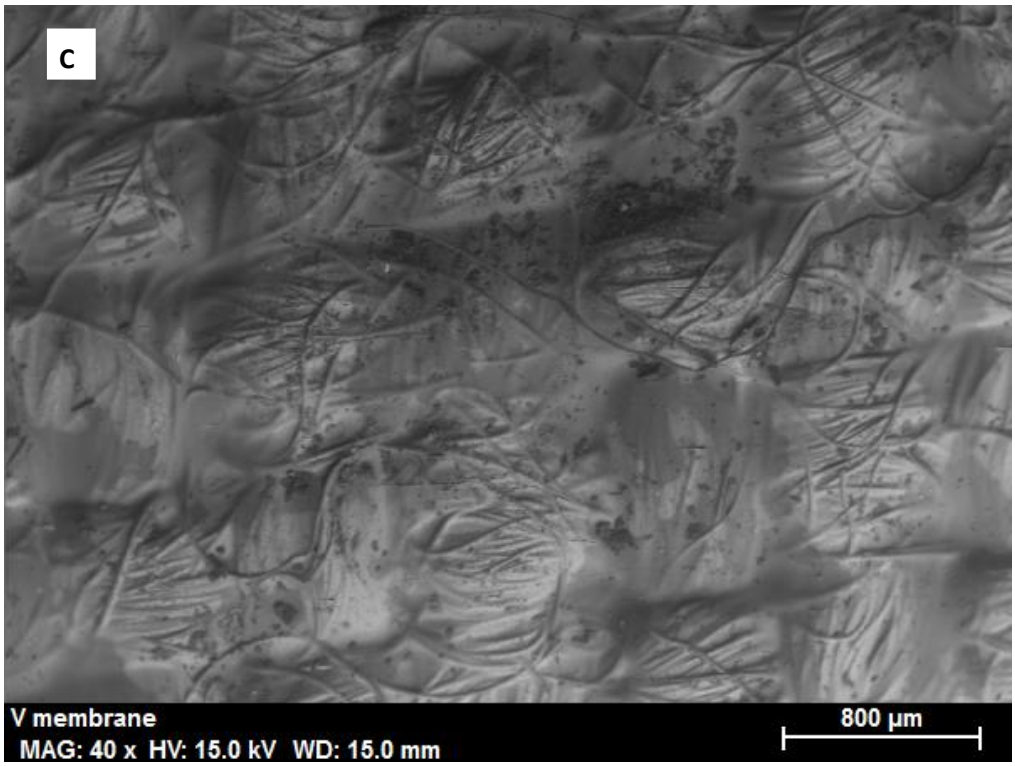
The membrane stability was studied before using it in the MFC. After the membrane-cathode assemblies was prepared the weights of the $[P^{+}_{I4,I4,I4,1}][TOS^{-}]$ assemblies was 21.3 g. After 20 days of application in distilled water the membrane-cathode was dried and weighed giving a weight of 16.6 g, which is almost 22% of the totalweight. It must be observed that this weight reduction is due to the quantity of ionic liquid that goes from the membrane to the water. The selected ionic liquids are not toxic and contain carbon chains and ammonium atoms which can be utilized by microorganisms as a power source in case of being discharged to the medium.

3.2.2 Wastewater treatment by a continuous flow Microbial Fuel Cells (MFC):

Physical analysis of industrial wastewater is carried out analyzing many parameters pH, EC, Eh, COD, TSS, SO_4^{2-}, Cl^{-}, NO^{3-}, TP, HPO_4^{2-}, Fe^{2+} (Table 1). Results obtained after 168 h of treatment is show, below:

Table II-7: Comparison of physicochemical parameters after treatment in microbial fuel cells (MFCs) using two different flows.

Parameters	Initiale Value	Reactor- First flow (0.25 $ml.min^{-1}$)		Reactor- Second flow (0.35 $ml.min^{-1}$)	
		Effluent concentration (HLR-25)	Removal (%)	Effluent concentration (HLR-35)	Removal (%)
pH	8,44	7,03	N/A	7,51	N/A
Electrical Conductivity (mS/cm)	14,58	11,42	N/A	13,31	N/A
Redox Potential (mV)	112	52,00	N/A	38,00	N/A
COD (mg/l)	1276	502,33	60,64	607,67	52,39
TTS (mg/l)	1534	515,76	66.38	636,70	58,49
SO_4^{2-} (mg/l)	7397	3176,00	57,06	4072,00	44,95
Cl^- (mg/l)	726	408,00	43,80	528,00	27,27
NO_3^- (mg/l)	9.17	6,64	27,59	8,02	12,54
Total Phosphorus (mg/l)	15,96	12,63	20,86	14,64	8,27
HPO_4^{2-} (mg/l)	11.93	9,52	20,20	10,75	9,89
Fe (II) (ppm)	1285	318,00	75,25	629,00	51,05



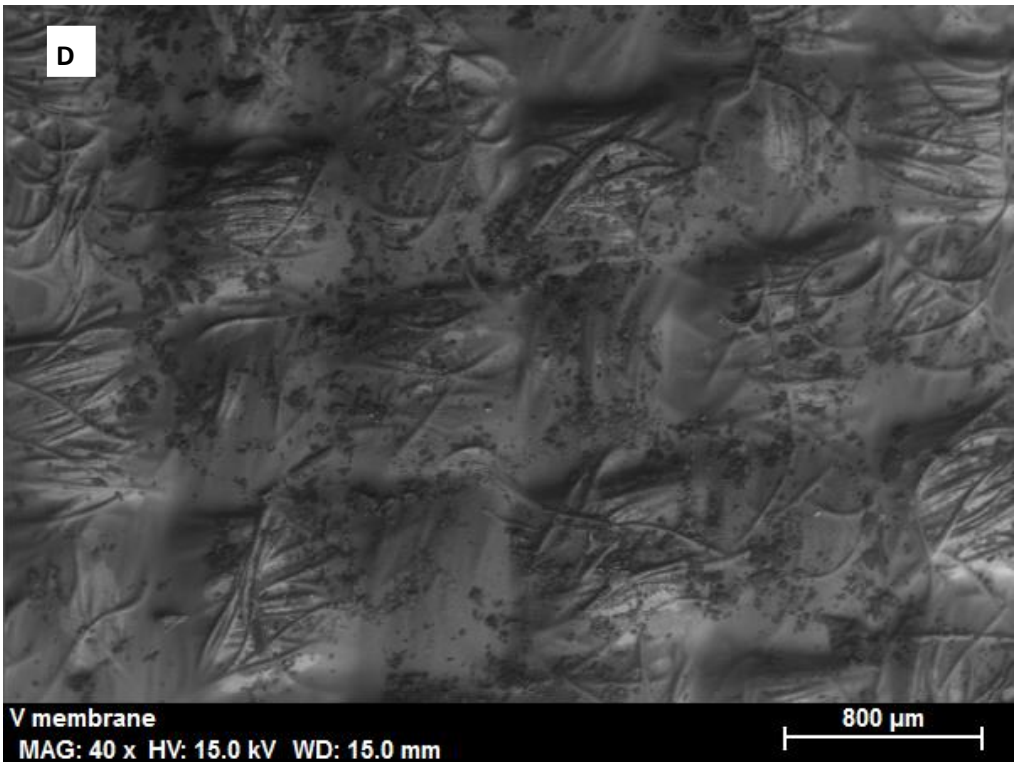


Fig 10: Scanning electron microscopy on both sides of each membrane-cathode assembly designed.

C: PIM before experimentation in the vertical MFC; ***D***: PIM after experimentation in thevertical MFC

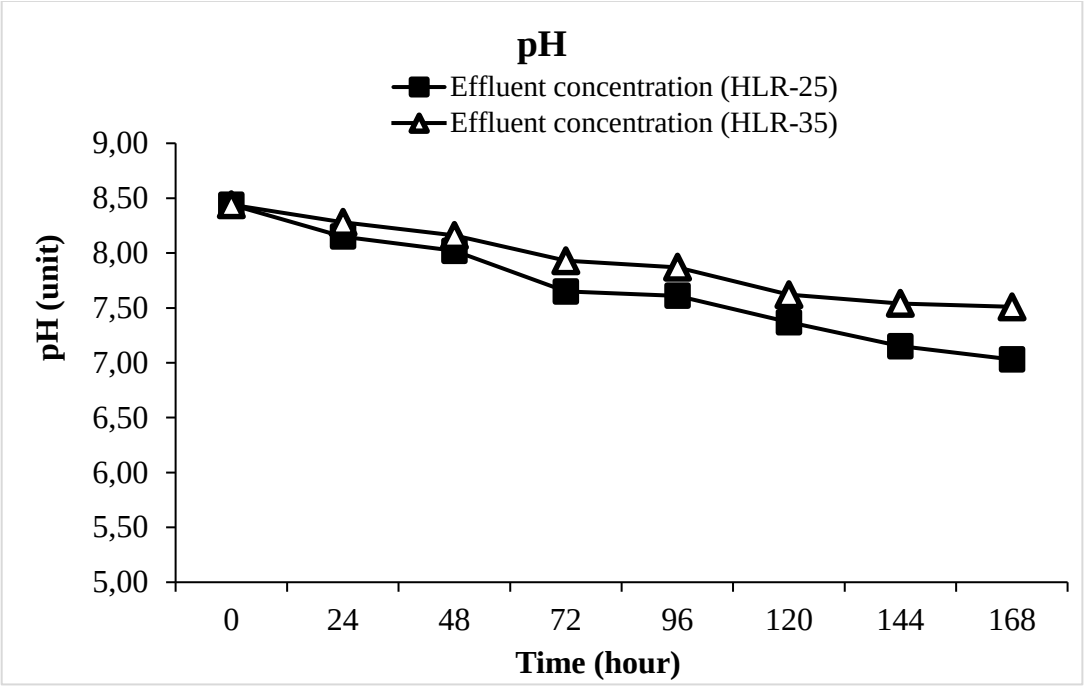


Fig 11: pH variation of the effluent concentration during the treatment by two flows

There is slight reduction in pH, the pH of the solution for the first flow (0.25 $ml.min^{-1}$) varied between 8.44 and 7.03, while the pH variation for the second flow is varied between 8.44 and 7.51. Biological processes such as nitrification, in which ammonia present in the wastewater is oxidized biologically to nitrite then transformed by oxidation as well to nitrate, provide protons and thus lower the pH.

Fig 11 shows the variation of pH in two different flows, this variation could be due to the fact that the non electrogenic microorganisms present in the wastewater consumed the substrate under anaerobic conditions producing acids as mentioned by Ren et al. (2018) [16]. Despite this variation, the pH was between 7 and 8.4, which is the range suitable for electrogenic microorganism's development.

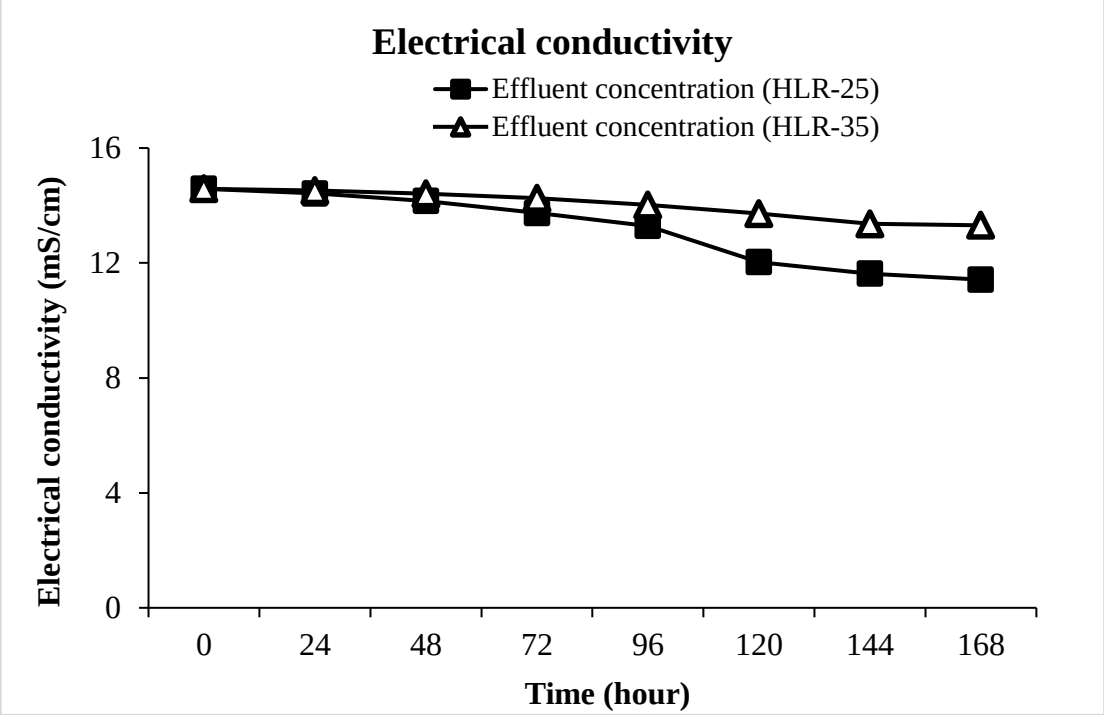


Fig 12: Electrical conductivity variation of the effluent concentration during the treatment by two different flows

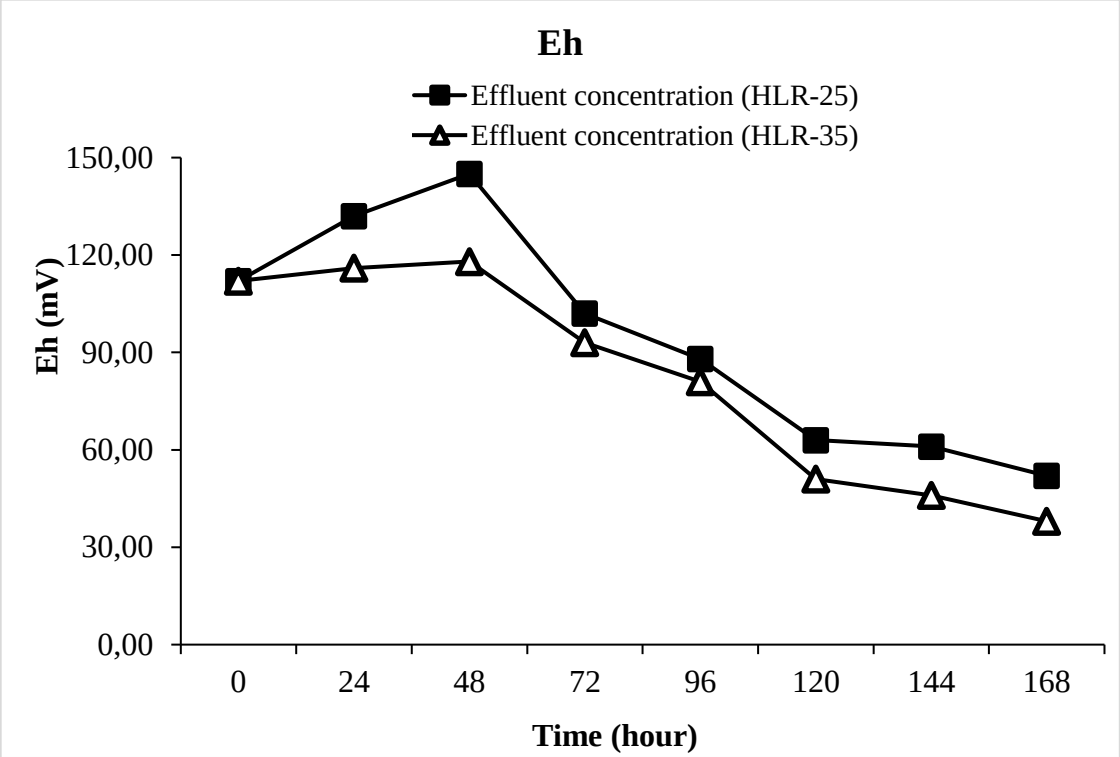


Fig 13: Redox potential variation of the effluent concentration during the treatment by two different flows

Electric conductivity of domestic and industrial wastewater is a major challenge that still face large scale application of the microbial fuel cells technology [17]. Biofilms in microbial fuel cells (MFCs) have been identified to express electrical conductivity further strengthen the hypothesis that interactions through electrical currents are a crucial component of these environment. Electrical conductivity decreased slightly in the reactor performing by the HLR 0.25ml.min^{-1}, while it is almost stable in the other reactor. This decrease in the conductivity would raise the internal resistance of these systems.

Considering the oxidation states between oxidized and reduced electro active sections mostly differ by one electron, the presence of compensating small, mobile counter ions is necessary in supporting electro neutrality of the redox polymer meanwhile super exchange [18]. The redox potential reached a maximum of 145 mV by the HLR of 0.25 ml.min^{-1} after 48 h of treatment. The HLR of 0.35 ml.min $^{-1}$ reached also the highest value after 48 of operation by 118 mV.

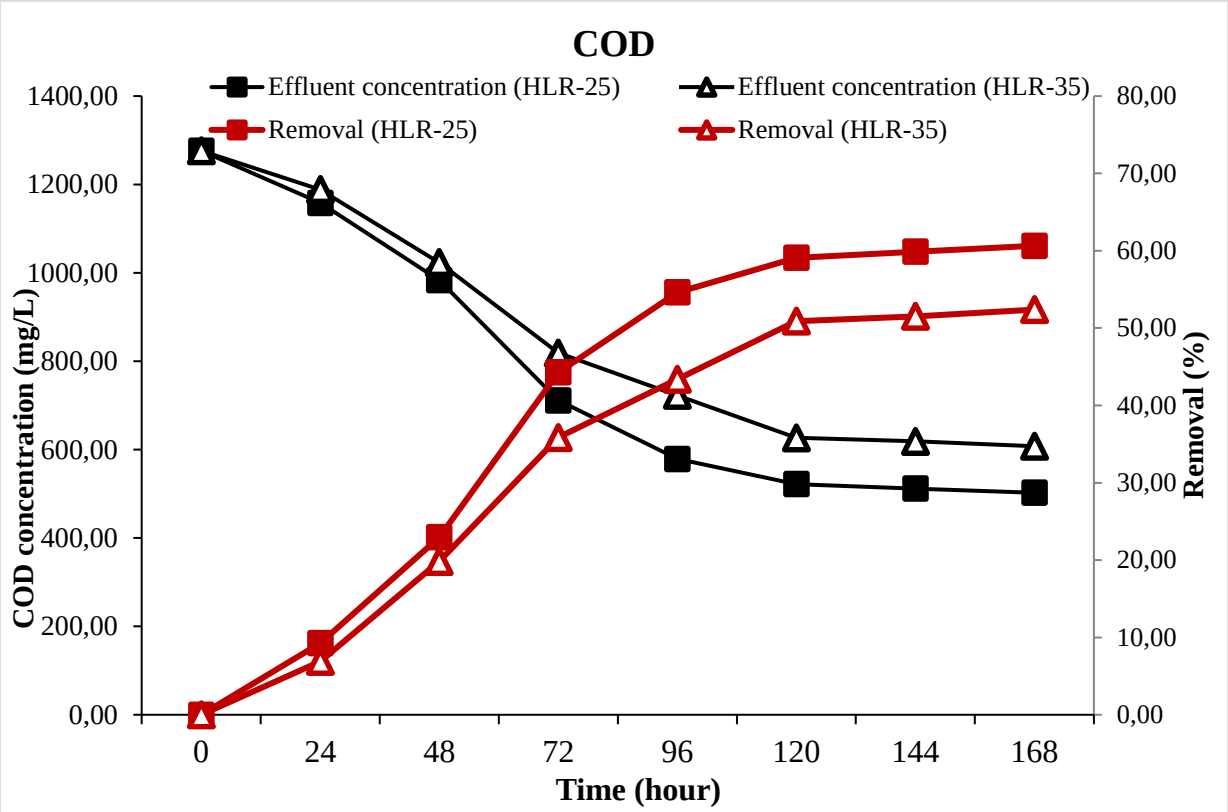


Fig 14: COD variation of the effluent concentration, and COD removal during the treatment by two different flows

Figure 14 shows that the flow has an influence on the removal of organic matter expressed in COD. Where the second flow (0.35 $ml.min^{-1}$) has a low removal compared to the first flow (0.25 $ml.min^{-1}$). According to Feng et al. (2009), if the temperature is reduced slightly, this would affect the removal of organic matter and therefore the production of electric current. This responds to the behavior of the two MFCs performing in the two different flows. Then it is concluded that during the entire electricity generation process the temperature with which one initially works must be kept constant, since if it is varied it could negatively influence the efficiency of the MFC [19].

Slow biodegradation rates demonstrate less COD is consumed in a given period of time, in this manner the exchange of electrons to the anode is slower, which normally results in lower anode potentials and low amount of produced energy.

The final value of COD is 502,33 mg/L at a flow rate of 0.25 $ml.min^{-1}$, representing a wastewater conversion of 60,64 % giving the best rate compared with the flow rate of 0.35 $ml.min^{-1}$ which had a value of 52,39 %.

Regarding the levels of TSS, it's shown in the Fig 15 that there is big reduction in TSS after treatment the appearance and color also changed during treatment. In other studies, is was been observed an increase in the TSS and a low removal rate in this parameter is linked to the presence of graphite granules in the reactor, which would raise the presence of graphite dust in the solution [28]. TSS removal in this experiment was 66.38% and 58,49 % for the first and second flow respectively. The second result was similar to the results from Tian et al. (2017) who reached a TSS removal of 57% associated to substrate biodegradation in MFCs, indicating that this value is low an improvement of the hydrolysis of substrate was necessary to obtain a higher removal[20]. Various studies shows MFCs are able of removing TSS. Wang et al. (2015) reported that a MFC treating wastewater achieved 50% removal of TSS, without undergoing any pretreatment [21].

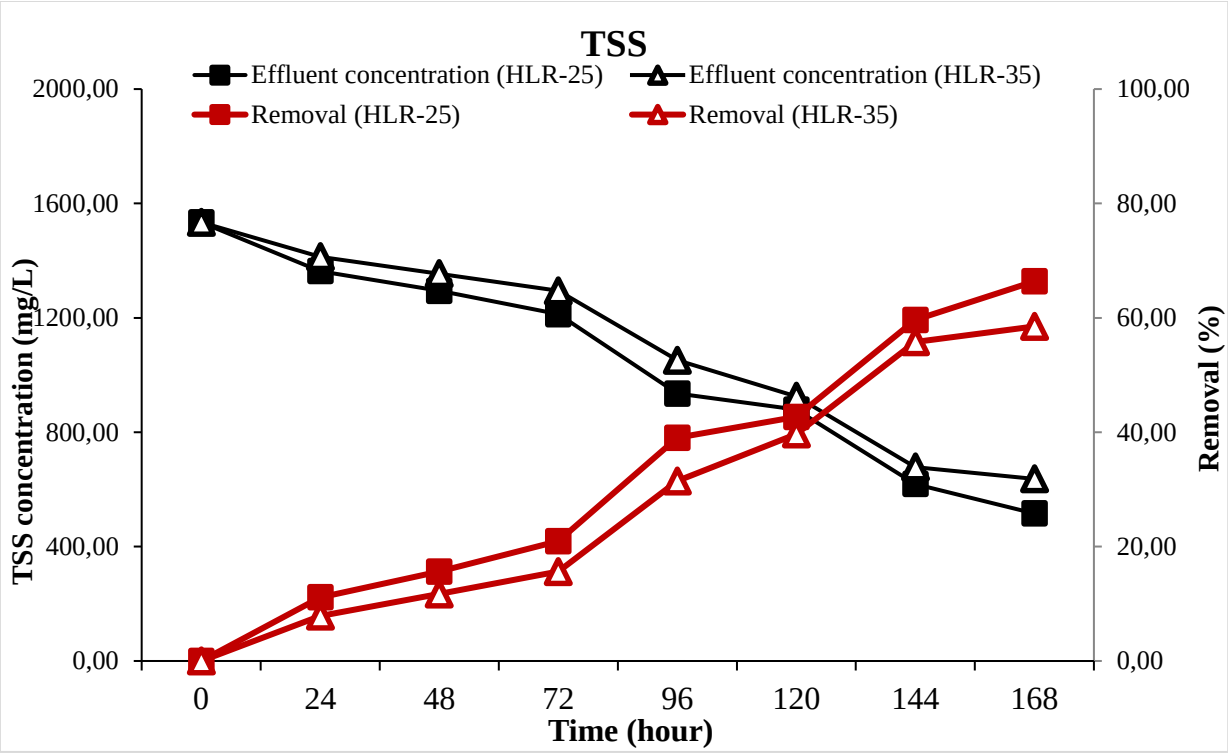


Fig 15: TSS variation of the effluent concentration and percentage removal during the treatment by two different flows

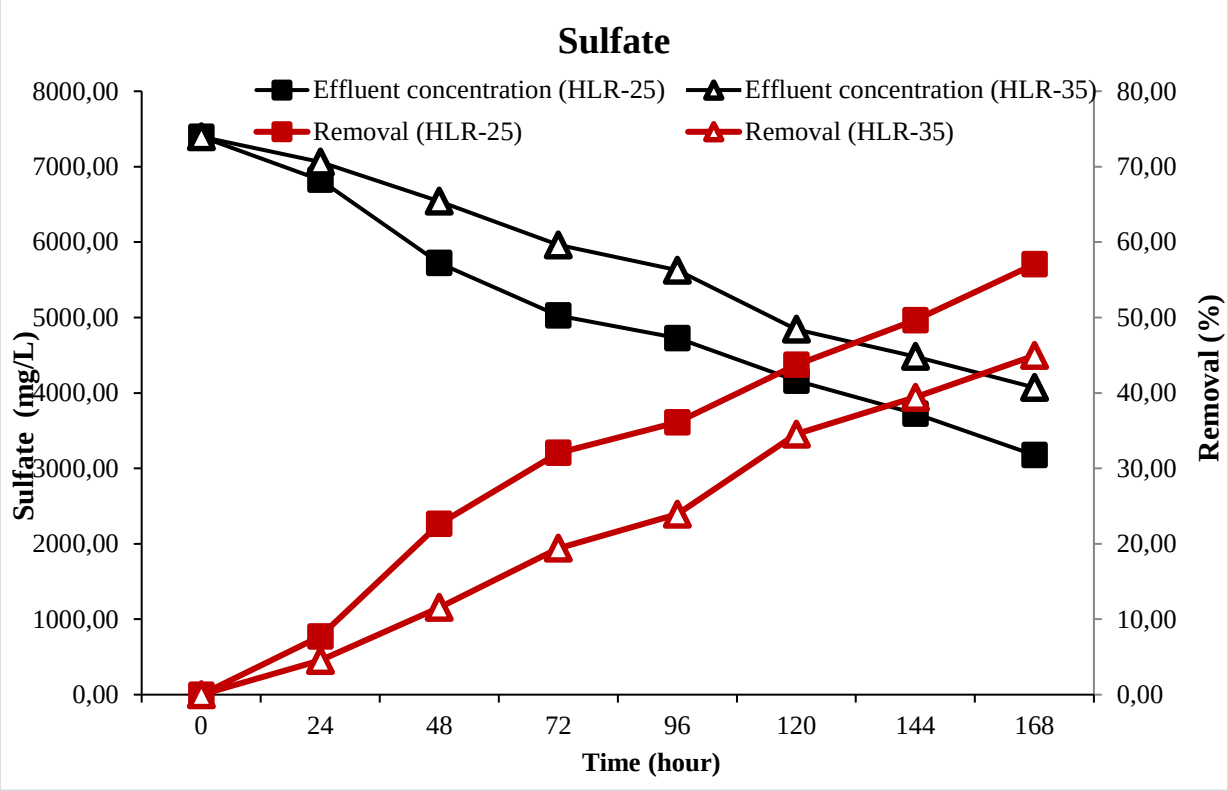


Fig 16: SO_4^{2-} variation of the effluent concentration and percentage removal during the treatment by two different flows

Wastewater coming from oil industry contain a high amount of hydrocarbons. Microbial degradation of hydrocarbons depends largely on the presence of terminal electron acceptors (TEAs) e.g. NO_3^-, SO_4^{2-}, O_2. Sulfate reducing bacteria can reduce sulfur compounds as sulfate to sulfide by sulfate reducing bacteria and then be eliminated by MFCs.

The concentration of sulfate in the effluent from the two reactors was evaluated and the results are shown in Fig. 16. After 7 days operation, the highest removal rate of sulfate was 57,06 % was achieved by the flow of 0.25 $ml.min^{-1}$. The other flow achieved 44,95 % .Some microorganisms present in wastewater have the capacity to utilize sulfate in wastewater as the possible electrons receiver and then sulfate is reduced to sulfide that is active electrochemically in the anode and it's oxidized on the electrode surface causing loses of its electros electrons, then it's reduced to sulfate which induces a low sulfate removal rate [22]. This class of MFCs has a high current density compared to other classes and doesn't require other heterogeneous mediators, because sulfate or sulfides proceed as a mediator.

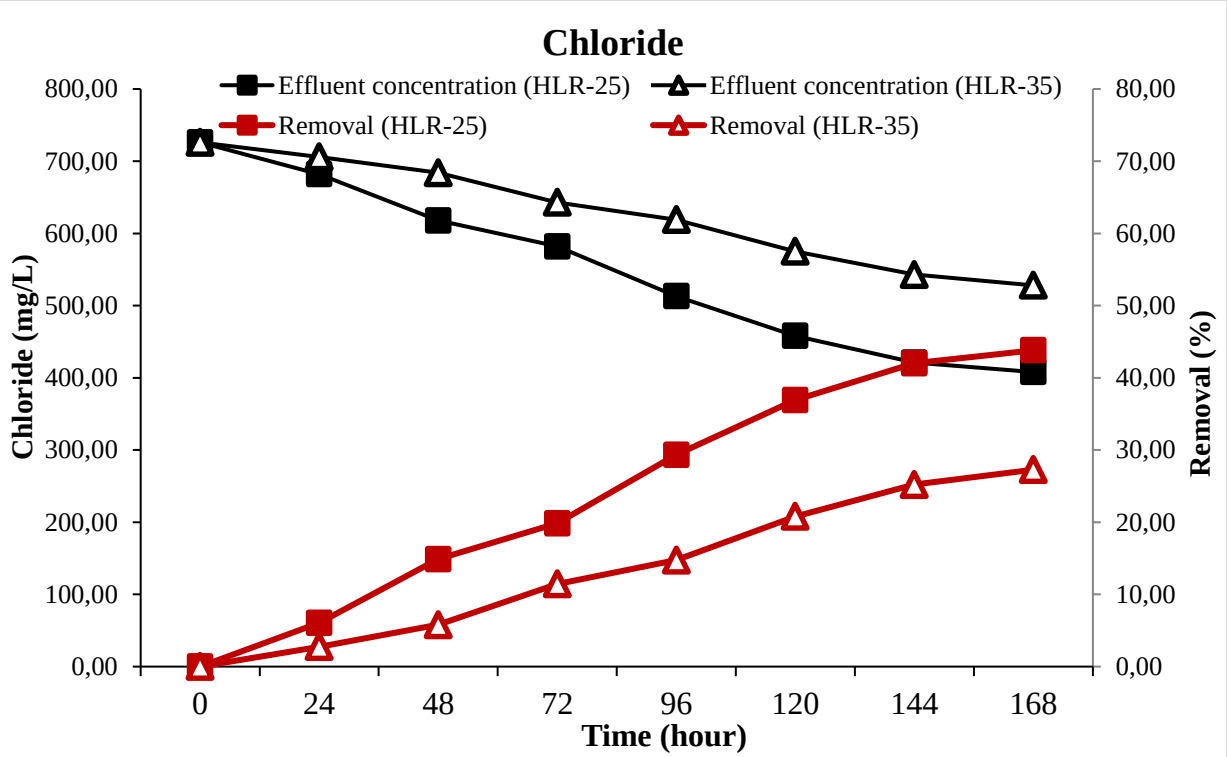


Fig 17: Cl^- variation of the effluent concentration and percentage removal during the treatment by two different flows

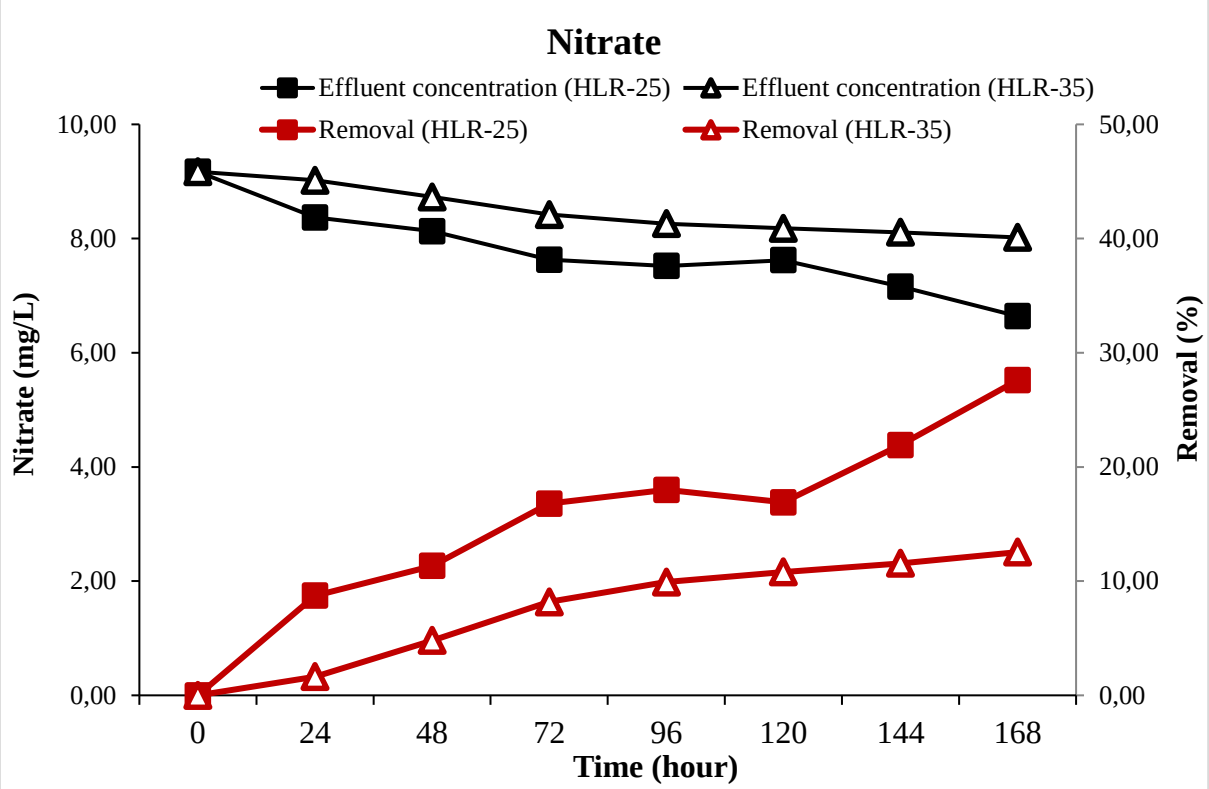


Fig 18: NO_3^- concentration and Removal by two different flows

Nitrogen exist in domestic and industrial wastewater principally in the form of ammonium (NH_4^+) and/or nitrate (NO_3^-). Organic nitrogen removal is mainly preferred over physicochemical methods such as adsorption, ion exchange, and chemical treatment owing to its lower value and the requirements of chemical/energy [23].

During the treatment process, NO_3^- was reduced electrochemically using the provided electrons by wastewater, which explain the decrease in concentration of NO_3^-. A significant removal of NO_3^- by the first flow (27,59 %) compared with 12,54 % given by the second flow (Fig. 18). The highest nitrogen removal effectiveness was only 27,59 % due to the higher NO3 amount in the substrate (9,17 mg NO_3^-. L^{-1}). Insignificant levels of NO_2^- were measured under all the applied conditions (<0.3 mg NO_2^- . L^{-1}).

Chlorides are generally present in industrial wastewater, that can be associated to dissolution of salts deposits of effluent from chemical industries, etc. The experimental data demonstrates that chlorides content has decreased from 726mg/l to 408mg/l. The chlorides removal may be associated to the availability of biodegradable substrate in wastewater leading to competitive inhibition in microorganisms.

The HLR 0.25 ml.min^{-1}gave the best removal rate by 43,8 % against 27,27 % by the HLR of 0.35 ml.min^{-1} as shown in the Fig 17. Only few studies have focused on chloride ions in microbial fuel cells. Sophia et al. (2016) identified the removal of sodium ions (Na^+) as 83.3% and chloride ions (Cl^-) as 57.8% using the shells of coconut as a carbon source to power production improvement [24].

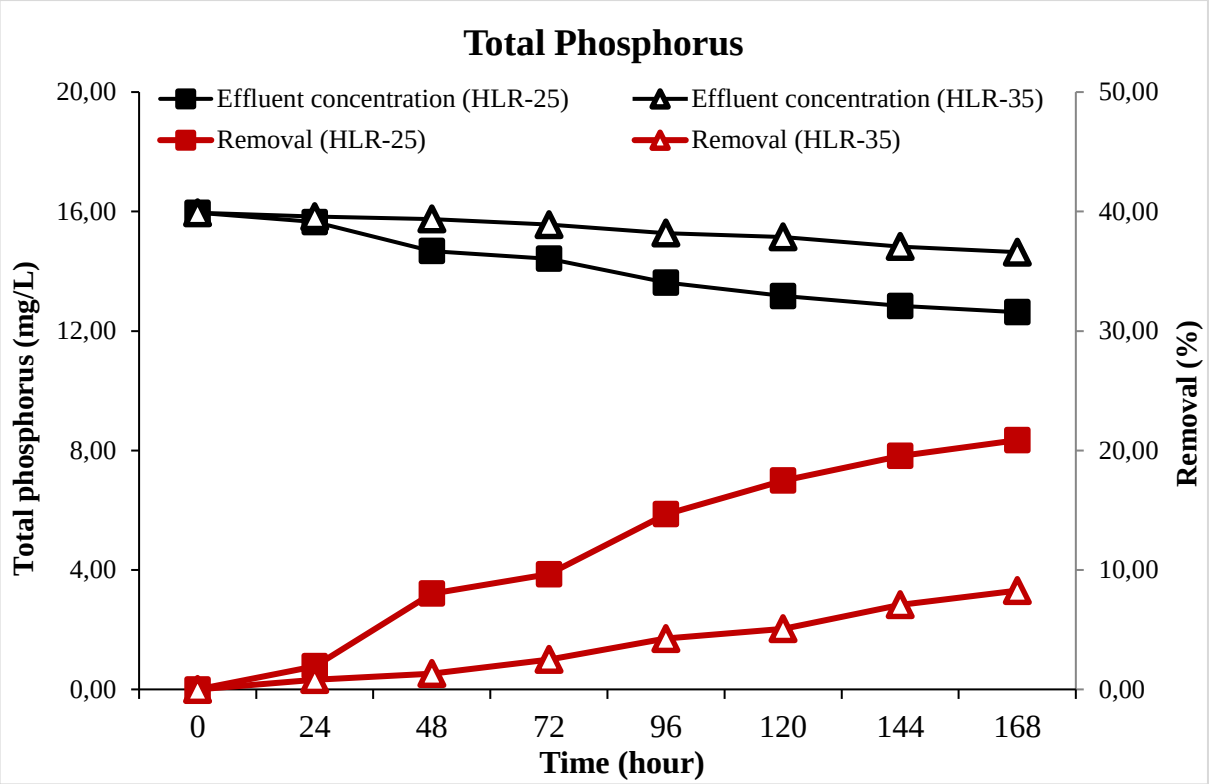


Fig 19: Total phosphorus concentration and Removal by two different flows

The phosphorus is present in the membrane based on ionic liquid "triisobutyl methyl phosphonium tosylate". Membrane degradation is already discussed in section 3.2. The membrane degradation will affect the removal of total phosphorus by providing phosphorus components to the medium. Some researches present a maximum removal efficiency of total phosphorus of 31.18%, and after 30 days of treatment the removal percentage is almost 0% [25].

In our experiment, the average concentration of phosphorus in wastewater was 16 mg/l and after 7 days of treatment its removal rate was 20.86% by the reactor operating in 0.25 ml.min^{-1} flow, while the removal by the second flow was only 8,27 % (Fig. 19). Likewise, the other

explanation of this aspect is possibly owing to the low Redox potential that drives to stimulation of microorganisms for releasing phosphorus or transforming the organic phosphorus in the wastewater to orthophosphate.

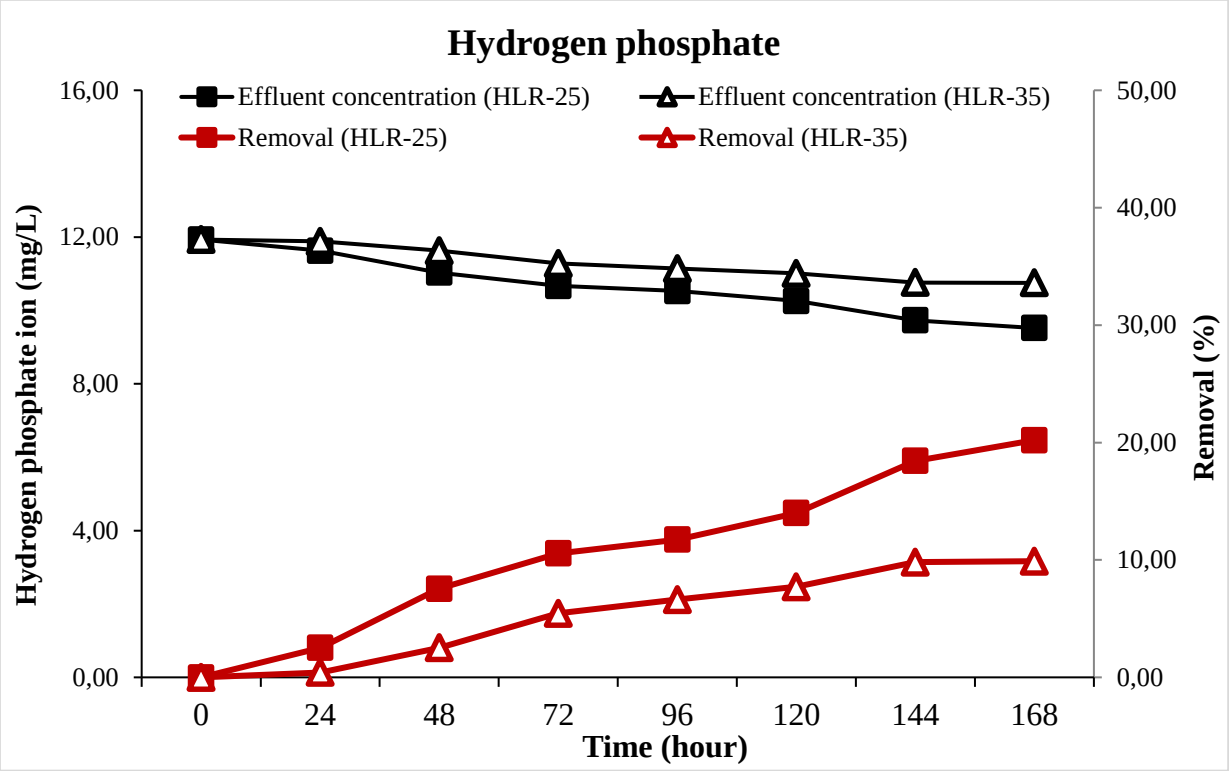


Fig 20: Hydrogen phosphate ion concentration and Removal by two different flows

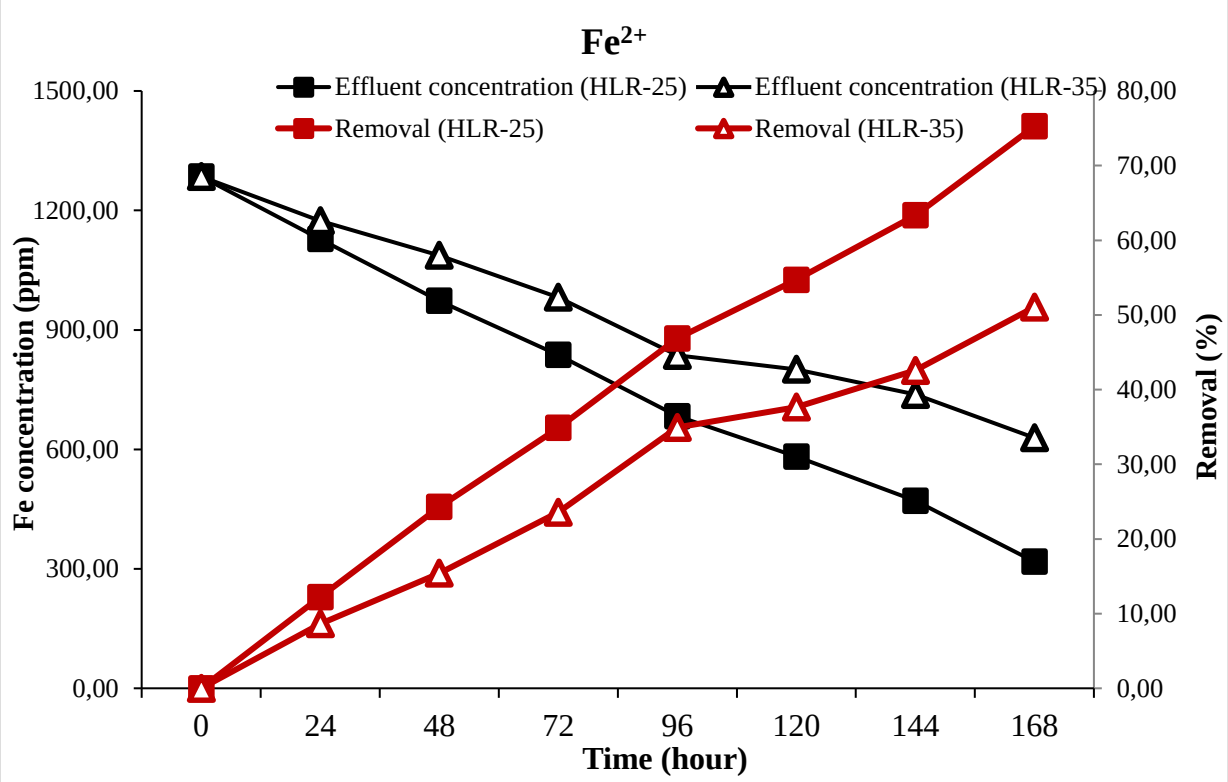


Fig 21: Iron (II) ion concentration and Removal by two different flows

Notable evolution in phosphate removal was noted in the end of experiment (20.20%) for the HLR of 0.25 ml.min-1, while the HLR of 0.35 ml.min registered only 9.89 % (Fig. 20). The removal of phosphate was probably inhibited by localization of the pH evolution and complexes amounts of carbonate and magnesium [26].

The iron phosphate present in the wastewater was reduced and then orthophosphate liberated:

$$3e^- + 3H^+ + Fe^{(III)}PO_4 \leftrightarrow H_3PO_4, H_2PO_4^-, HPO_4^{2-}, PO_4^{3-}$$

The major limitation for productive orthophosphate amounts is the restricted accessibility of the reduction in the phosphate containing particles, Meanwhile, it presents proved satisfactory adsorption by the membrane in presence of F^{2+} as discussed in the section 3.2. Fischer et al. proved the possibility of phosphate recycling and recovery from digested sewage sludge, it offers a supplementary alternative to the common sewage disposal and reuse practices.

The MFC technology excellently fits the larger conceptual need to treat phosphate present in wastewater in a decentralized way [27].

4. Conclusion

Ionic-liquid based PIMs were tested as separator in a new horizontal and vertical configurations of MFCs fed with wastewater.

It has been concluded that the effectiveness of treatment is directly dependent to the HRT, pH, T° operated in continuous mode, and the highest performance of wastewater treatment was reached for the flow of 0.25 $ml.min^{-1}$.

For horizontal configuration of MFCs, COD value was found to be higher than the initial value after one day of treatment, which proves that an important part of the membrane was dissolved in the wastewater. This membrane degradationis mainly attributed to the small distance between the tube containing carbon granules and carbon graphite bar, in that case, carbon is precipitated in the bottom of reactor result of gravity in direct contact with the membrane.Wastewater is then charged by carbon particles due to its contact with carbon granules and PIM.Althoughthe membrane was degraded partially, the percentage of many pollutants and heavy metals have decrease considerably after only 48 h of treatment.

In contrary to the horizontal reactor, the vertical one has performed in good conditions in different conditions.

the efficiency of wastewater treatment through the MFC system, the effluent from the reactor was examined with regard to, COD (Chemical Oxygen Demand), total P (Phosphorus), TSS (Total Suspended Solids), SO_4^{2-} (sulfate), Cl^- (Chloride), NO_3^- (Nitrate), HPO_4^{2-} (Hydrogen phosphate), Fe^{2+} (Iron) and pH, Total suspended solids, hardness, alkalinity and ion analysis.The following parameters were examined for both flows (0.25 $ml.min^{-1}$and 0.35 $ml.min^{-1}$).

It has been concluded that wastewater treatment reached the highest performance for the flow of 0.25 $ml.min^{-1}$

References

[1] J.-C. Boeglin, Traitements biologiques des eaux résiduaires. Ed. Techniques Ingénieur,1998.

[2] J.-C. Bertrand, P. Bonin, P. Caumette, J.-P. Gattuso, G. Grégori, R. Guyoneaud, X. Le Roux, R. Matheron, and F. Poly, "Les cycles biogéochimiques," in Ecologie Microbienne, Microbiologie des milieux naturels et anthropisés, puppa ed., 2011.

[3] A. Mulder, A. A. Graaf, L. A. Robertson, and J. G. Kuenen, "Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor," FEMS Microbiology Ecology, vol. 16, no. 3, pp. 177–184, 1995.

[4] R. J. Seviour, T. Mino, and M. Onuki, "The microbiology of biological phosphorus removal in activated sludge systems," FEMS Microbiology Reviews, vol. 27, pp. 99–127, Apr. 2003.

[5] A. Larrosa, L.J. Lozano, K.P. Katuri, I. Head, K. Scott, C. Godinez. On the repeatability and reproducibility of experimental two-chambered microbial fuel cells. Fuel 2009;88:1852-7.

[6] APHA, AWWA, and WPCF, Standard Methods for the Examination of Water and Wastewater. 20th ed. 1998: American Public Health Association. Washington, DC.

[7] B.E. Logan, B. Hamelers, R. Rozendal, U. Schroder, J. Keller, S. Freguia et al. Microbial fuel cells: methodology and technology. Environ Sci Technol 2006;40: 5181e92.

[8] M.J. Salar-García, V.M. Ortiz-Martínez, A.P. de los Ríos, F.J. Hernandez-Fernandez. A method based on impedance spectroscopy for predicting the behavior of novel ionic liquid-polymer inclusion membranes in microbial fuel cells. Energy 89 (2015) 648-654

[9] A. Alentiev, J. Kostina, G. Bondarenko, Desalination 200 (2006) 32.

[10] H. Li, H. Wanga, W. Qian, S. Zhang, S. Wessel, T. Tommy, H. Cheng, J. Shen, S. Wu; Chloride contamination effects on proton exchange membrane fuel cell performance and durability. Journal of Power Sources 196 (2011) 6249– 6255

[11] M. Sulek, J. Adams, S. Kaberline, M. Ricketts, J. R. Waldecker. In situ metal ion contamination and the effects on proton exchange membrane fuel cell performance. Journal of Power Sources 196 (2011) 8967– 8972

[12] Wu, Jinfeng, et al. "A review of PEM fuel cell durability: degradation mechanisms and mitigation strategies." *Journal of Power Sources* 184.1 (2008): 104-119.

[13] V.M. Ortiz-Martínez, M.J. Salar-García, A.P. de los Ríos, F.J. Hernandez-Fernandez, J.A. Egea, L.J. Lozano. Developments in microbial fuel cell modeling. Chem Eng J 2015; 271:50-60

[14] S. Pandit, K. Chandrasekhar, R. Kakarla, A. Kadier, V. Jeevitha. Basic Principles of Microbial Fuel Cell: Technical Challenges and Economic Feasibility. Microbial Applications Vol.1. 2017. 165- 188

[15] C. Chen G. Levitin, D. W. Hess, T. F. Fuller. XPS investigation of Nafion® membrane degradation. Journal of Power Sources 169 (2007) 288–295

[16] Y. Ren, J. Chen, X. Li, N. Yang & X. Wang. (2018). Enhanced bioelectricity generation of air-cathode buffer-free microbial fuel cells through short-term anolyte pH adjustment. *Bioelectrochemistry*, 120: 145-149.

[17] R.A. Rozendal, H.V.M. Hamelers, K. Rabaey, J. Keller, C.J.N. Buisman. Towards practical implementation of bioelectrochemical wastewater treatment. Trends in Biotechnology 2008. 26, 450–459.

[18] C. Li, K.L. Lesnik, Y. Fan, H. Liu. Redox Conductivity of Current-Producing Mixed Species Biofilms. PLoS ONE 2016. 11(5): e0155247. doi:10.1371/ journal. pone.0155247

[19] Y. Feng, H. Lee, X. Wang & Y. Liu. Electricity generation in microbial fuel cells at different temperature and isolation of electrogenic bacteria. *In Power and Energy Engineering Conference-APPEEC 2009. Asia-Pacific*, 1-5.

[20] Y. Tian, X. Mei, Q. Liang, D. Wu, N. Ren, D. Xing. Biological degradation of potato pulp waste and microbial community structure in microbial fuel cells. RSC Adv. **2017**, 7, 8376–8380.

[21] H. Wang, J.D. Park, Z.J. Ren. Practical energy harvesting for microbial fuel cells: A review. Environ. Sci.Technol. **2015**, 49, 3267–3277.

[22] L. Wei, H. Han, J. Shen, Effects of temperature and ferrous sulfate concentrations on the performance of microbial fuel cell, Int. J. Hydrogen Energy (2013).

[23] J. Park, Y. Yoo. Biological nitrate removal in industrial wastewater treatment: which electron donor we can choose. Applied Microbiology and Biotechnology 2009. 82, 415–429.

[24] A.C. Sophia, V.M. Bhalambaal. Microbial water desalination and bio-electricity generation – role of biomass carbon. Curr Sci. 2016; 111:6.

[25] H. J. Mansoorian, A. H. Mahvi, A. J. Jafari & N. Khanjani, (2016). Evaluation of dairy industry wastewater treatment and simultaneous bioelectricity generation in a catalyst-less and mediator-less membrane microbial fuel cell. *Journal of Saudi Chemical Society*, *20*(1), 88–100. https://doi.org/10.1016/j.jscs.2014.08.002

[26] R.D. Cusick, B.E. Logan. Phosphate recovery as struvite within a single chamber microbial electrolysis cell. Bioresour. Technol. 2012, 107, 110–115.

[27] F. Fischer et al. Microbial fuel cell enables phosphate recovery from digested sewage sludge as struvite. Bioresource Technology 102 (2011) 5824–5830

[28] H. Addi, F. Mateo-Ramírez, V. M. Ortiz-Martínez, M. J. Salar-García, F. J. Hernández-Fernández, A. P. de los Ríos, C. Godínez, E. M. Lotfi, M. El Mahi, L. J. Lozano Blanco. Treatment of Mineral Oil Refinery Wastewater in Microbial Fuel Cells Using Ionic Liquid Based Separators. Appl. Sci. 2018, 8, 438; doi:10.3390/app8030438

Chapter III: Nutrients transports through polymerinclusion membranes based on ionic liquids

1. Introduction

Over the years, membrane-based processes have attracted much interest because they can be performed under moderate conditions and their energy needs are significantly low [1]. Reverse osmosis, ultrafiltration, microfiltration, pervaporation or gas permeation are a few examples of the most common membrane-based processes. In all these cases, the performance of the separation process might be improved by tailoring the membrane properties for the selective separation of a specific compound. One of the current trends for improving the membranes properties involves the use of ionic liquids (ILs) [1].

Ionic liquids are molten salts, which remain liquid at temperatures below 100 °C. They usually consist of an organic cation (imidazolium, pyrrolidinium, pyridinium, etc) and an inorganic (hexafluorophosphate, tetrafluoroborate, chloride, etc) or organic anion (dicyanamide, bistrifluoromethylsulfonylimde, tosylate, etc.). Their unique properties make them very attractive for a wide variety of chemical processes such as separation or catalysis. They exhibit good chemical and thermal stability along with near-zero vapor pressure. They are considered as environmentally friendly solvents whose properties can be tailored by varying the anion and the cation of their structure in order to be adapted to a specific process [2][3].

ILs have successfully employed as extraction agents for metal ions, organic compounds or macromolecules and in liquid/liquid systems [4][5][6][7]. However, their immobilization in different kinds of matrixes provides a dual benefit: i) to obtain stable materials with a minimal content of IL and ii) the opportunity to reuse the IL at the end of the process. Other benefits of these types of membranes are their simple synthesis process and their low energy requirements. Regarding the membrane properties, the use of ionic liquid in its structure allows us to tailor them in order to be adapted to a specific and selective separation process [8][9]. IL-based membranes can be mainly grouped into: i) supported ionic liquids membranes (SILMs), ii) polymer ionic liquid inclusion membranes (PILIMs), iii) polymerized ionic liquid membranes (PyILMs) and iv) other ionic liquid/polymer composite materials [10]. SILMs consist of immobilizing the IL into a porous material by using capillary forces. The main drawback of these membranes is the lack of long-term stability caused by the leakage of the IL from the porous. To overcome this limitation, the IL can be immobilized into a polymer matrix where it is completely retained, giving rise to PILIMs. Both the long-term stability as well as the selectivity of this type of membranes has promoted their application in a broad domain. For instance, IL-based membranes have shown promising results in gases separation and metal

extraction [11] [12]. Another recent use of PILIMs is as separators in microbial fuel cells (MFCs) [13]. Microbial fuel cells use bacteria to convert the chemical energy stored in a specific substrate into electrical energy. So far, different kinds of synthetic substrates have been explored, however complex feedstock such as wastewater is most interesting due to its need for treatment and availability. The main advantage of MFCs over other technologies is that they are able to produce electricity and treat wastewater simultaneously [14]. However, in order to facilitate the commercialization of this technology is necessary to reduce the overall cost of the devices as well as improving the energy harvesting. The use of IL-based membranes addresses both challenges by replacing the expensive and sometimes low-efficient commercial membranes [15]. In recent years, MFCs technology was combined with algae as the oxygen supplier. In this case, bacteria degrade the organic matter in the anodic chamber while algae grow in the cathodic compartment, providing the oxygen necessary for completing the redox reaction on the cathode. Furthermore, algae are able to capture carbon dioxide and the algal biomass produced could be used for the production of added-value compounds [16]. This novel approach could improve the power performance and the wastewater treatment efficiency of MFCs. Moreover, the transport of specific compounds from the anode to the cathode might also help to the algae growth, reducing the nutrients added to the cathode chamber. The selective transport of nutrients through the membrane will allow us to design a more efficient system for both bioenergy productionand wastewater treatment.

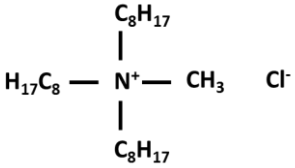
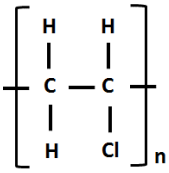
In this work, the transport of two nutrients, specifically calcium chloride ($CaCl_2$) and sodium hydrogen phosphate (Na_2HPO_4), through polymer inclusion membranes based on ammonium-type ionic liquid was evaluated. The effect of the ionic liquid membrane composition and the nature of the nutrient on the permeability of the membrane was analyzed in-depth. Although the main objective of this work is the application of the results to the design of a double-chamber MFC, the conclusions of the present work might be applied to any field in which the recovery or selective separation of the studied nutrients are of interest.

2. Materials and Methods

2.1. Preparation of polymer inclusion membranes based on ionic liquids

Casting method was used in order to prepare polymer inclusion membranes based on methyltrioctylammonium chloride, [MTOA$^+$][Cl$^-$]. This ionic liquid was purchased from Sigma-Aldrich-Fluka (purity >97%). This technique allows us to immobilize the ionic liquid in a polymeric matrix, in this case, polyvinyl chloride (PVC) (Sigma-Aldrich-Fluka). The method consists of preparing a mixture based on the selected ionic liquid, the polymer and an adequate amount of tetrahydrofuran (THF). The solution is stirred until the polymer is totally dissolved. Then, it is poured on a glass ring overnight in order to allow the solvent to evaporate. The final weight of the membranes was fixed at 0.3 g and two different amounts of ionic liquids were used (70 % w/w and 30 % w/w) [17].Table 1 depicts the chemical structure of the ammonium-based ionic as well as the polymer used to synthesize the membranes.

Table III-1. Structure of both the ionic liquid and the polymer used to prepare the membranes.

Compound	Structure
Methyltrioctylammonium Chloride	
Polyvinyl Chloride	

2.2. SEM-EDX and elemental mapping characterization

The morphological appearance and the chemical composition of the membranes were analyzed, as well as the distribution of the elements of interest on their surface by using a scanning electron microscope (SEM) Hitachi S-3500N and a Bruker AXS for energy-dispersive X-Ray (EDX).

The ionic liquid-based membranes were characterized by SEM-EDX and elemental mapping before and after being used for the selective transport of the nutrients.

2.3. Ions transport studies

The transport of CaCl2 and Na2HPO4through the PILIMs was evaluated. The transport studies were performed at 25°C by using a glass diffusion cell with two independent compartments of 250 mL eachand separated by the PILIMs(see Fig. 1). In each experiment, pure $CaCl_2$ and Na_2HPO_4 were used in order to prepare two different feeding solutions with a final concentration of 1 $g.L^{-1}$. Pure water was used as the receiving solution in both cases. Substrates, solvents and other chemicals were purchased with the highest purity available from Sigma-Aldrich-Fluka. Both compartments were mechanically stirred to avoid concentration polarization conditions at the membrane interface. The transport of the anions and cations contained in the feeding phase across the IL-based membranes was analyzed during 168 h. Samples were taken at different time intervals during the operating time andtheir composition was analyzed by ionic chromatography (850 Professional IC, Metrohm).

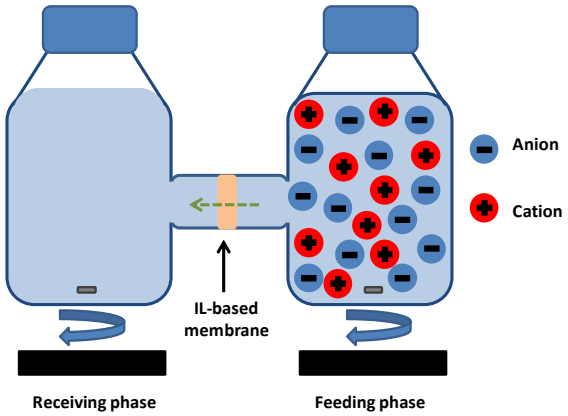


Figure 1. Scheme of the glass diffusion cell set-up used for the nutrient analysis through IL-based membranes.

2.4. Ionic chromatography

Ion detection was performed by using an 861 Advanced Compact IC module. Cations Na^{+} and Ca^{+2} were determined by a Metrosep C4-250 column whereas Cl^{-} and HPO_4^{-2} anions were detected by a Metrosep A Supp 5-250 column.

2.5. Calculation of permeability

The transport of the nutrients through the IL-based membranes was analyzed in terms of permeability. The average permeability ($\bar{P}$) was calculated from the slope of equation 1, which was derived from the application of the solution-diffusion model (See Fig. 2) to the transport of nutrients through PILIMs[18].

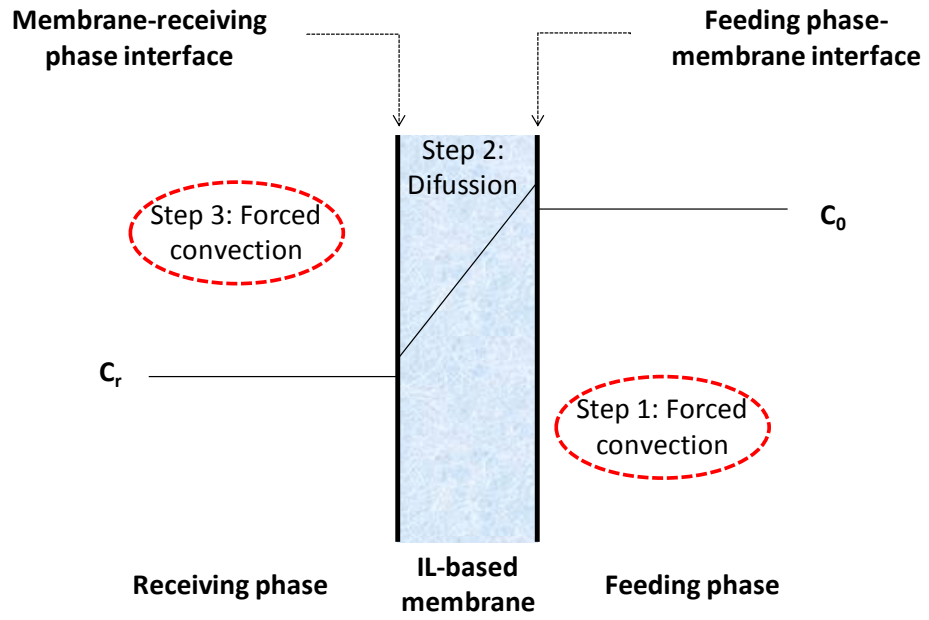


Figure 2. Transport of nutrients through PILIMs membranes based on solution-diffusion model.

$$ln\left(\frac{C_0-2C_r}{C_0}\right)=\frac{-2\bar{P}A}{V}t \qquad \textit{Equation 1}$$

where C0 is the initial solute concentration in the feeding phase (mol.L^{-1}), Cr the solute concentration in the receiving phase (mol.L^{-1}), A the membrane area (cm^2), V is the volume of the compartments (mL) and t the run time (h). All experiments were carried out in duplicate and the mean values are reported.

In order to evaluate the ability of the different membranes to separate the target compounds, another parameter named average permselectivity of the membrane is defined as follows:

$$r\bar{P}=\frac{\sum_i^n rP_i}{n} \text{with} \quad rP_{i>1} \qquad \textit{Equation 2}$$

Where rPi is the membrane permselectivity between two compounds and n is the number of the possible combinations of compounds. rPi can be rewritten as follows:

$$rP_i=\frac{P_A}{P_B} \qquad \textit{Equation 3}$$

The average permselectivity ($r\bar{P}$) of the membranes indicates its efficiency for the separation of a specific compound [18].

3. Results and Discussion

3.1. Polymer ionic liquid inclusion membrane characterization before and after being used as separators

Before the preparation of the PILIMs (30 and 70 % w/w), their respective morphology was studied by SEM. SEM micrographs (see Fig. 3A and 3B) showed a smooth surface. Figures 3A' and 3B' show the characterization of the membranes by elemental mapping, demonstrating the homogeneous distribution of the N (in green) and Cl (in red) along the membrane surface. Figures 3A and 3B also show some stains on the external surface of the membranes. These stains might be an excess of IL since they are highlighted in green color in the mapping images. This color represents the nitrogen and it is only present in the IL structure and not in the PVC (see Fig. 3A' and 3B').The EDX spectra of the membranes (see Fig. 4A and 4B) presented the characteristic peaks assigned to chlorine (Cl) and nitrogen (N) [19]. The presence of these chemical elements corresponds to the chemical formulation of PVC (Cl) and $[MTOA^+][Cl^-]$ (N and Cl), respectively.

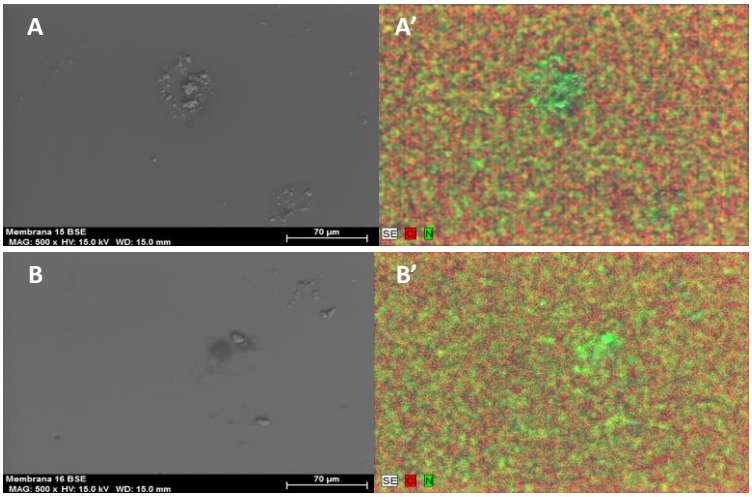


Figure 3. SEM and elemental mapping of membranes containing 70 $\%_{w/w}$ of IL (A and A', respectively) and membranes containing 30 $\%_{w/w}$ of IL (B and B', respectively) before being used.

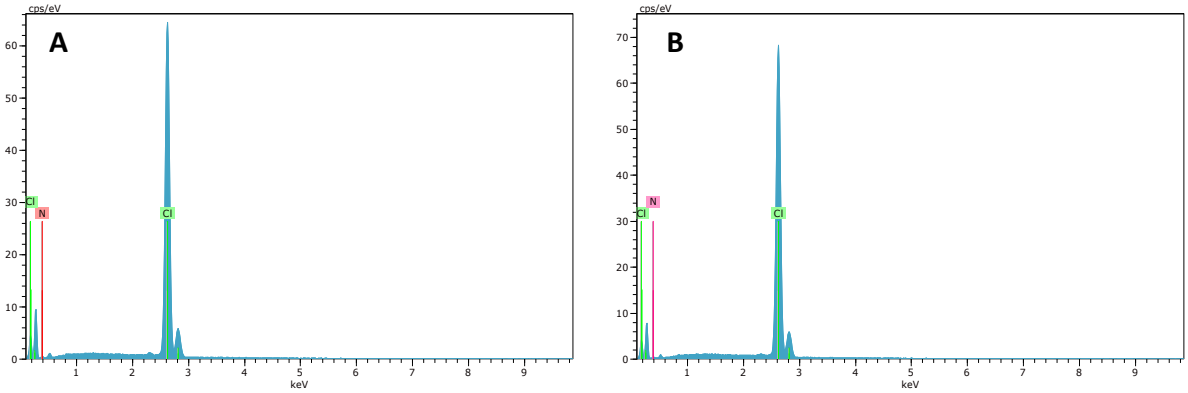


Figure 4.EDX analysis of membranes containing 70 $\%_{w/w}$ (A) and 30 $\%_{w/w}$ of IL (B) before being used.

Figures 5, 6, 7 and 8 show the SEM-EDX and elemental mapping of the membranes containing 30 and 70 %w/w of ionic liquid after being used as separators for each feeding solution ($CaCl_2$ and Na_2HPO_4, respectively).

As can be seen in Figures 6 and 8, the characteristic peaks of the membrane (N for $[MTOA^+][Cl^-]$ and Cl for PVC) appeared after using it as separator which indicates the adequate stability of the membrane. In addition to the characteristic peaks of the membranes, other signals belonging to the nutrients studied are also observed. Those are calcium (Ca) and chlorine (Cl) when the nutrient studied is $CaCl_2$ (see Fig. 5A', 6A', 7A' and 8A'), and sodium (Na), phosphorus (P) and oxygen (O) in the case of the nutrient Na2HPO4 (see Fig. 5B', 6B', 7B' and 8B'). These results might be due to the transported compounds are absorbed in the membrane structure. The SEM images of the PILIMs after being used in the diffusion cell are shown in Figures 5 and 7 (30 % w/w and 70 % w/w of ionic liquid, respectively). In general, it is observed that the smooth surface of the membranes is kept after being used in the diffusion cell. However, as previously commented, the mapping analysis shows some salt deposits such as $CaCl_2$ when the transport of this nutrient was studied through the membrane with 30 % w/w and 70 % of ionic liquid (see Fig. 5A' and 7A', respectively).

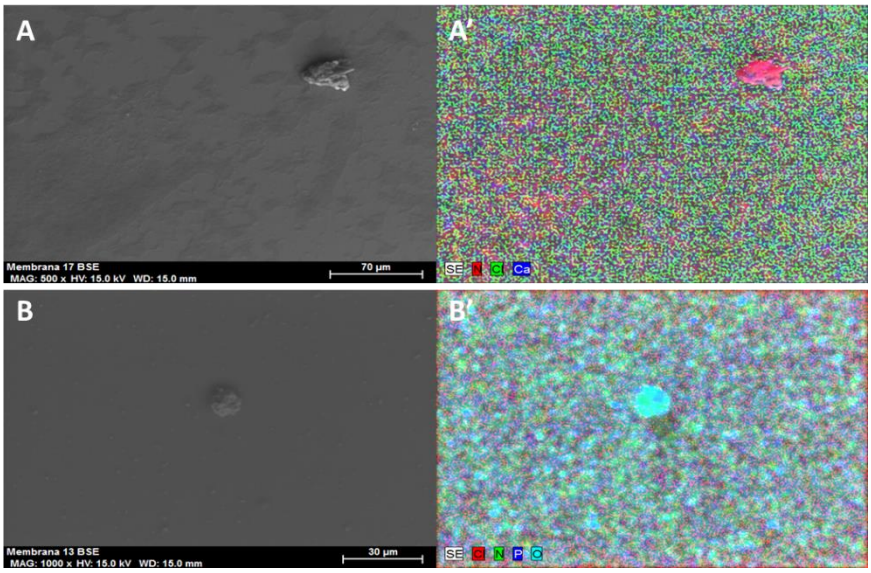


Figure 5.SEM of membranes containing 30 % $_{w/w}$ of IL after being used as separators for each feeding solution: $CaCl_2$ (A) and Na_2HPO_4 (B), and elemental mapping: $CaCl_2$ (A') and Na_2HPO_4 (B').

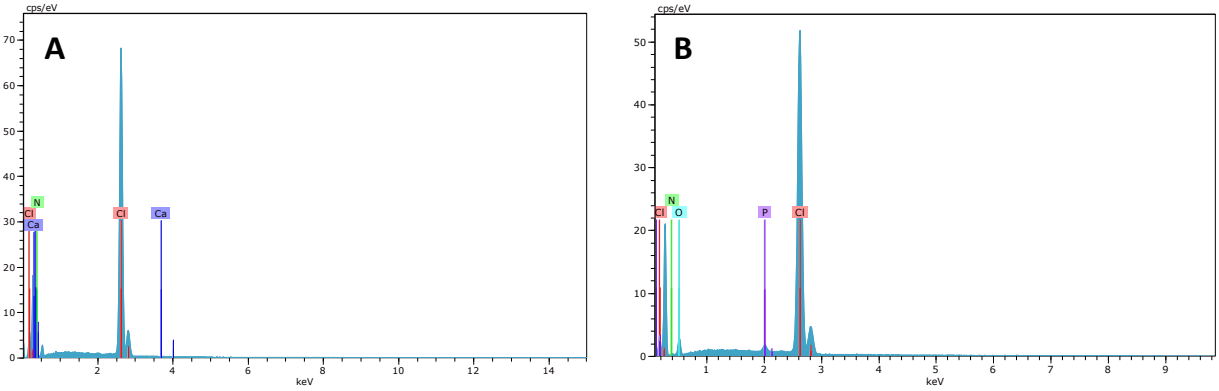


Figure 6.EDX analysis of membranes containing 30 % $_{w/w}$ of IL after being used as separators for each feeding solution: $CaCl_2$ (A) and Na_2HPO_4 (B).

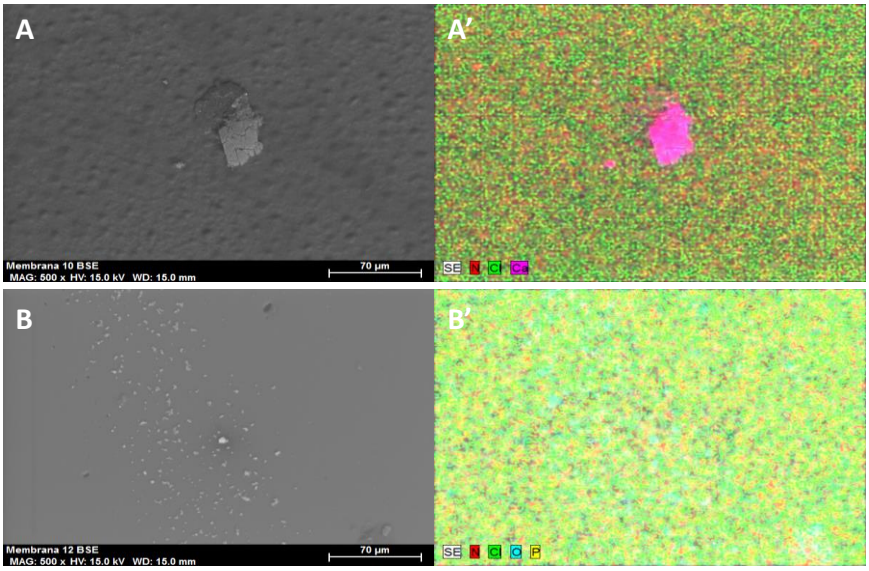


Figure 7.SEM of membranes containing 70 % $_{w/w}$ of IL after being used as separators for each feeding solution: $CaCl_2$ (A) and Na_2HPO_4 (B), and elemental mapping: $CaCl_2$ (A') and Na_2HPO_4 (B').

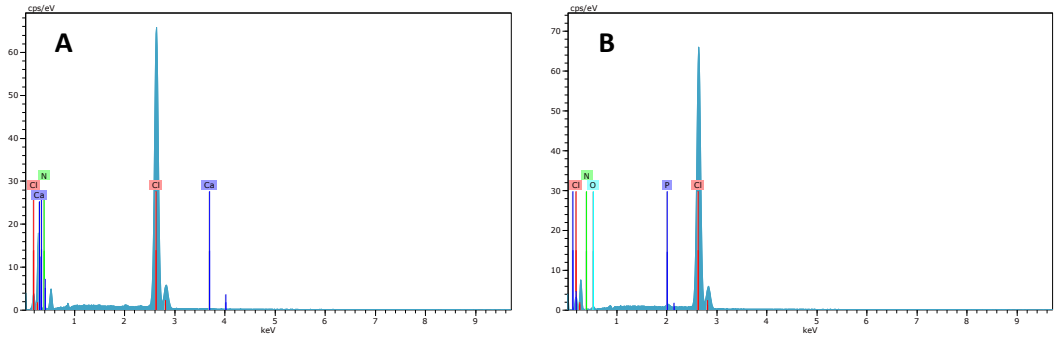


Figure 8.EDX analysis of membranes containing 70 % $_{w/w}$ of IL after being used as separators for each feeding solution: $CaCl_2$ (A) and Na_2HPO_4 (B).

3.2. Transport studies of $CaCl_2$ and Na_2HPO_4 through polymer inclusion ionic liquid membranes.

The permeability of $CaCl_2$ and Na_2HPO_4 through polymer inclusion membranes based on $[MTOA^+][Cl^-]$ was determined. The experimental concentration of the anions and cations in the feeding and receiving phases as a function of the time was monitored and the permeability of the species was calculated by using the concentrations in the receiving phase (see Fig. 9). In this way, the permeability values were calculated from the slopes of the plot of ln[(C0−2Cr)/C0] for each compound versus time using Equation (1). As an example, Figure 10 shows the plots used to calculate the permeability of each compound through the membrane based on 70 % w/wof $[MTOA^+][Cl^-]$. The permeability values for each compound through the different PILIMs are shown in Table 2.

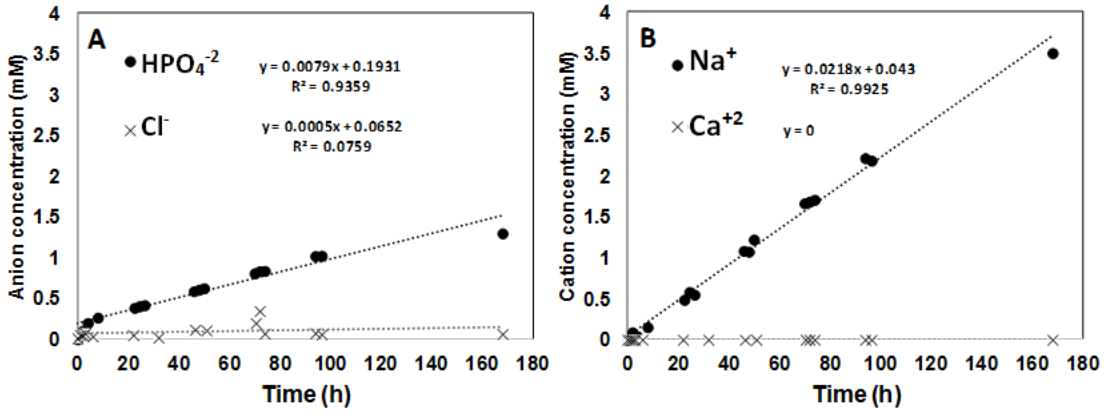


Figure 9. Time courses for $CaCl_2$ and Na_2HPO_4 in the receiving phase using a PILIM based on 70 % $_{w/w}$of $[MTOA^+][Cl^-]$.

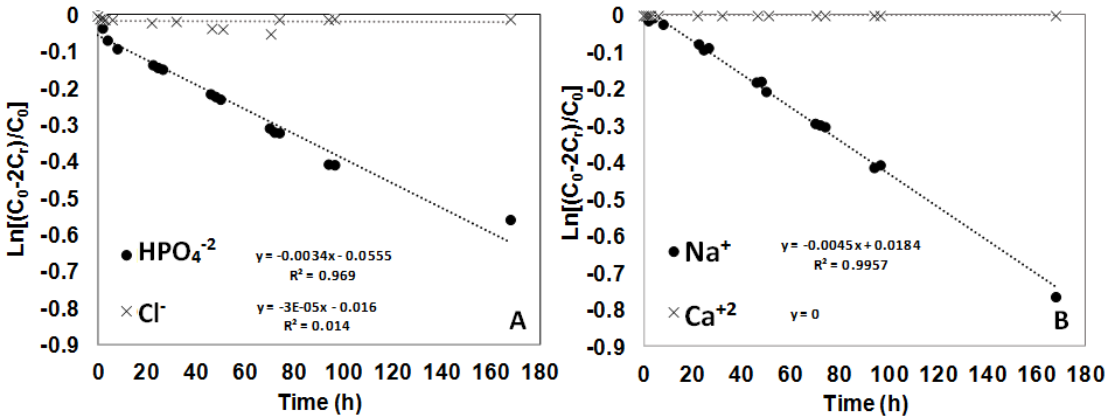


Figure 10. Plot of $ln[(C_0-2C_r)/C_0]$ *vs.* operation time for the transport of $CaCl_2$ and Na_2HPO_4through a PILIM based on 70 % $_{w/w}$of $[MTOA^+][Cl^-]$.

Table III-2. Permeability values of the polymer inclusion membranes based on the liquid $[MTOA^+][Cl^-]$ on the transport of $CaCl_2$ and Na_2HPO_4..

	PERMEABILITY ($x10^6$ cm.s^{-1})			
Membrane	**Na^+**	**HPO_4^{2-}**	**Ca^{+2}**	**Cl^-**
70% $_{w/w}$ $[MTOA^+][Cl^-]$	25.671	18.463	0.000	2.669
30% $_{w/w}$ $[MTOA^+][Cl^-]$	4.216	5.351	0.000	1.573

Table III-3. Permselectivity values of the polymer inclusion membranes based on the liquid [$MTOA^+$][Cl^-] on the transport of $CaCl_2$ and Na_2HPO_4.

	PERMSELECTIVITY (rP_i)			AVERAGE PERMSELECTIVITY ($r\bar{P}$)
Membrane	**Na^+/HPO_4^{-2}**	**Na^+/Cl^-**	**Cl^-/HPO_4^{-2}**	
70% w/w [$MTOA^+$][Cl^-]	1.39	9.62	6.92	5.98
	HPO_4^{-2}/Na^+	**Na^+/Cl^-**	**HPO_4^{-2}/Cl^-**	
30% w/w [$MTOA^+$][Cl^-]	1.27	2.68	3.4	2.45

Regarding the permeability values (see Table 2), all ions studied permeated through the membranes less Ca^{2+} in which non-permeation was found for both ionic liquid-based membranes (30 % w/w and 70 % w/w of IL). Significant differences in terms of permeation between ions and between the same ion through the different membranes (30 % w/w and 70 % w/w of IL) were found. As can be seen in Table 2, the permeation increases as the amount of IL in the membrane also increases. These results might confirm that the IL is the active phase of the membrane allowing the transport of a specific ion. By contrast, in the case of chloride anion low permeability values were obtained while non-permeability of Ca^{2+} was observed. For minimizing that difference, an assay without nutrient in the feeding phase was carried out. This assay was considered as "zero" when the permeation of chloride was calculated. Regarding the permselectivity of the membranes, the average permselectivity was higher in the case of membranes containing 70 % of IL. This result indicates that the higher amount of IL in the membrane structure, the better selective separation capacity (see Table 3). Comparing by pairs, higher values of selectivity were achieved for membranes containing 70 % of IL than for those prepared with 30 %. These results indicate the important role of the ionic liquid in the selective separation of the target ions. The selectivity which involves the ion Ca+2 has not been calculated since its permeability was near zero.

Some research works reported in literature study the mechanism of water transport through supported liquid membranes (SLM) and liquid membranes (LM) based on imidazolium ionic liquids ([CnMIM][PF6]). They report that the transport might be regulated by the mobility of water microenvironments inside the IL rather than by molecular diffusion through the bulk ionic

liquid. These results show a timeline in the transport through membranes caused by the time needed to reach a critical water concentration within the ionic liquid. Water content in ionic liquids was found to be necessary to facilitate the transport through the membrane of small ions, such as Na^+ and Cl^-, with a low affinity towards the ILs. So, transport through the SLM could be understanding as transport through water microenvironments in the supported liquid membrane [20][21].

In this work, non-permeation was observed for Ca^{2+} and a higher lag of time was observed for Cl^-. As commented above, the permeation value for chloride could be affected by the loss of chloride from the membrane. The low permeation value of Ca^{2+} and Cl^- might be due to their low solubility in the ionic liquid phase of the membrane. The water solubility in ionic liquids was studied by Freire et al. 2007 [22]. The authors found that the solubility of water follows the next sequence $[BIMIM^+][PF6^-]$>$[HIMIM^+][PF6^-]$ >[OIMIM+][$PF6^-$]>$[MTOA^+][Cl^-]$. So, in our case, the forming of a water microenvironment in the ionic liquid would be more difficult because of the low water solubility of $[MTOA^+][Cl^-]$. For that reason, low or negligible permeation of small ions such as Ca^{2+} and Cl^- was observed. In the case of Na_2HPO_4 the permeability could be explain by solvent ion-pair extraction and ion exchange as is it explained below.

It should also be considered that the permeability values depend on the method used for the immobilization of the ionic liquid on the supporting material. SILMs usually allow high permeability values however, their stability is lower compared with polymer inclusion membranes where the ionic liquids are trapped in the polymer matrix, as in this case [23]. The stability of the membranes is usually a critical factor for large-scale applications of membrane technology.

With regard to the transfer mechanism of ions through ionic liquid membranes, the ionic nature of the ionic liquid can result in a variety of extraction mechanisms, including solvent ion-pair extraction, ion exchange, transport through water microenvironment and simultaneous combinations of these. The ion-pair extraction mechanism depends on the solubilization of the salt on the ionic liquids whereas the ion exchange-based mechanism the second relies on the exchange capacity of the ionic liquid phase. Regarding the transport through water microenvironment, as previously commented, it depends on the water solubility on the ionic liquids. The main mechanism will depend on the importance of this over the rest of the mechanisms and should be studied for each pair of nutrient and ionic liquid phase. An in-depth

understanding of the transfer mechanism is of primary importance for establishing a predictive model that might be used in practical operations. According to the preliminary results obtained in the present work, it might be possible that the primary mechanism is by solvent ion-pair extraction however, other mechanisms could also be involved in the process. In order to a better understanding of the transfer mechanisms, further works are currently carrying out.

4. Conclusions

The aim of this work is to analyze the transport of nutrients such as $CaCl_2$ and Na_2HPO_4 through polymer inclusion membranes based on ammonium-based IL. To the best of the authors' knowledge, the existing literature focuses on this research field is limited or non-existent. The results show that polymer inclusion membranes containing $[MTOA^+][Cl^-]$ are stable towards an aqueous solution of the studied nutrients. In the case of $CaCl_2$, it showed the least permeation due to its low solubility in the ionic liquid $[MTOA^+][Cl^-]$. By contrast, Na_2HPO_4 was more permeable and its permeation increased as the amount of IL in the membrane also increased. The results reported in this work could be applied to different fields such as separation and purification of salt mixtures. This study would allow the efficient design of a two-chamber microbial fuel cell, which involves IL-based membrane technology and algae. The permeation values of different nutrients would also allow us to decide whether the nutrients should be added in the same chamber that microalgae or in the opposite chamber. Furthermore, the results obtained open new fields where polymer ionic liquid membrane might be applied.

References

1. Ortiz, I.; Bringas, E.; Samaniego, H.; San Román, M.F.; Urtiaga, A. Membrane processes for the effcient recovery of anionic pollutants. Desalination **2006**, 193, 375–380. [CrossRef]

2. Lozano, L.J.; Godínez, C.; de los Ríos, A.P.; Hernández-Fernández, F.J.; Sánchez-Segado, S.; Alguacil, F.J. Recent advances in supported ionic liquid membrane technology. J. Membr. Sci. **2011**, 376, 1–14. [CrossRef]

3. Plechkova, N.V.; Seddon, K.R. Applications of ionic liquids in the chemical industry. Chem. Soc. Rev. **2008**, 37, 123–150. [CrossRef] [PubMed]

4. de los Ríos, A.P.; Hernández-Fernández, F.J.; Lozano, L.J.; Sánchez, S.; Moreno, J.I.; Godínez, C. Removal of Metal Ions from Aqueous Solutions by Extraction with Ionic Liquids. J. Chem. Eng. Data **2010**, 55, 605–608. [CrossRef]

5. de los Ríos, A.P.; Hernández-Fernández, F.J.; Alguacil, F.J.; Lozano, L.J.; Ginestá, A.; García-Díaz, I.; Sánchez-Segado, S.; López, F.A.; Godínez, C. On the use of imidazolium and ammonium-based ionic liquids as green solvents for the selective recovery of Zn(II), Cd(II), Cu(II) and Fe(III) from hydrochloride aqueous solutions. Sep. Purif. Technol. **2012**, 97, 150–157. [CrossRef]

6. de los Ríos, A.P.; Hernández-Fernández, F.J.; Tomás-Alonso, F.; Rubio, M.; Gómez, D.; Víllora, G. On the importance of the nature of the ionic liquids in the selective simultaneous separation of the substrates and products of a transesterification reaction through supported ionic liquid membranes. J. Membr. Sci. **2008**, 307, 233–238. [CrossRef]

7. Soto, A.; Arce, A.; Khoshkbarchi, M.K. Partitioning of antibiotics in a two-liquid phase system formed by water and a room temperature ionic liquid. Sep. Purif. Technol. **2005**, 44, 242–246. [CrossRef]

8. de los Ríos, A.P.; Hernández-Fernández, F.J.; Presa, H.; Gómez, D.; Víllora, G. Tailoring supported ionic liquid membranes for the selective separation of transesterification reaction compounds. J. Membr. Sci. **2009**, 328, 81–85.

9. Noble, R.D.; Gin, D.L. Perspective on ionic liquids and ionic liquid membranes. J. Membr. Sci. **2011**, 369, 1–4. [CrossRef]

10. Dai, Z.; Noble, R.D.; Gin, D.L.; Zhang, X.; Deng, L. Combination of ionic liquids with membrane technology: A new approach for CO2 separation. J. Membr. Sci. **2016**, 497, 1–20. [CrossRef]

11. Deng, J.; Bai, L.; Zeng, S.; Zhang, X.; Nie, Y.; Deng, L.; Zhang, S. Ether-functionalized ionic liquid based composite membranes for carbon dioxide separation. RSC Adv. **2016**, 6, 45184–45192. [CrossRef]

12. Guo, L.; Zhang, J.; Zhang, D.; Liu, Y.; Deng, Y.; Chen, J. Preparation of poly (vinylidene fluoride-co-tetrafluoroethylene)-based polymer inclusion membrane using bifunctional ionic liquid extractant for Cr(VI) transport. Ind. Eng. Chem. Res. **2012**, 51, 2714–2722. [CrossRef]

13. Hernandez-Fernandez, F.J.; de Los Rios, A.P.; Salar-Garcia, M.J.; Ortiz-Martinez, V.M.; Lozano-Blanco, L.J.; Godinez, C.; Tomas-Alonso, F.; Quesada-Medina, J. Recent progress and perspectives in microbial fuel cells for bioenergy generation and wastewater treatment.Fuel Process. Technol. **2015**, 138, 284–297. [CrossRef]

14. Pandey, P.; Shinde, V.N.; Deopurkar, R.L.; Kale, S.P.; Patil, S.A.; Pant, D. Recent advances in the use of di_erent substrates in microbial fuel cells toward wastewater treatment and simultaneous energy recovery. Appl. Energy **2016**, 168, 706–723. [CrossRef]

15. Ortiz-Martínez, V.M.; Salar-García, M.J.; Hernández-Fernández, F.J.; de los Ríos, A.P. Development and characterization of a new embedded ionic liquid based membrane-cathode assembly for its application in single chamber microbial fuel cells. Energy **2015**, 93, 1748–1757.

16. Baicha, Z.; Salar-García, M.J.; Ortiz-Martínez, V.M.; Hernández-Fernández, F.J.; de los Ríos, A.P.; Labjar, N.; Lotfi, E.; Elmahi, M. A critical review on microalgae as an alternative source for bioenergy production: A promising low cost substrate for microbial fuel cells. Fuel Process. Technol. **2016**, 154, 104–116. [CrossRef]

17. Salar-García, M.J.; Ortiz-Martínez, V.M.; de los Ríos, A.P.; Hernández-Fernández, F.J. A method based on impedance spectroscopy for predicting the behavior of novel ionic liquid-polymer inclusion membranes in microbial fuel cells. Energy **2015**, 89, 648–654. [CrossRef]

18. Hernandez Fernandez, F.; de los Rios, A.P.; Rubio, M.; Tomas Alonso, F.; Gomez, D.; Villora, G. A novel application of supported liquid membranes based on ionic liquids to the selective simultaneous separation of the substrates and products of a transesterification reaction. J. Membr. Sci. **2007**, 293, 73–80. [CrossRef]

19. de los Ríos, A.P.; Hernández-Fernández, F.J.; Tomás-Alonso, F.; Palacios, J.M.; Gómez, D.; Rubio, M.; Víllora, G. A SEM–EDX study of highly stable supported liquid membranes based on ionic liquids. J. Membr. Sci. **2007**, 300, 88–94.

20. Fortunato, R.; González-Muñoz, M.J.; Kubasiewicz, M.; Luque, S.; Alvarez, J.R.; Afonso, C.A.M.; Coelhoso, I.M.; Crespo, J.G. Liquid membranes using ionic liquids: The influence of water on solute transport. J. Membr. Sci. **2005**, 249, 153–162. [CrossRef]

21. Fortunato, R.; Afonso, C.A.M.; Reis, M.A.M.; Crespo, J.G. Supported liquid membranes using ionic liquids: Study of stability and transport mechanisms. J. Membr. Sci. **2004**, 242, 197–209. [CrossRef]

22. Freire, M.G.; Santos, L.M.N.B.F.; Fernandes, A.M.; Coutinho, J.A.P.; Marrucho, I.M. An overview of the mutual solubilities of water–imidazolium-based ionic liquids systems.Fluid Phase Equilib.**2007**, 261, 449–454. [CrossRef]

23. Tomás-Alonso, F.; Rubio, A.M.; Giménez, A.; de los Ríos, A.P.; Salar-García, M.J.; Ortiz-Martínez, V.M.; Hernández-Fernández, F.J. Influence of ionic liquid composition on the stability of polyvinyl chloride-based ionic liquid inclusion membranes in aqueous solution. AIChE J. **2017**, 63, 770–780. [CrossRef]

Chapter IV: Use of microalgae as alternative in microbial fuel cells

1. INTRODUCTION

The energy sector worldwide has been forced to change rapidly due to global warming. The most widely used energy sources are based on fossil fuels even though it is well know that the reserves of oil, gas, coal or uranium will be exhausted in 55-75 years. The increase in the world population, estimated at around 9.7 billion people in 2050, along with a sharp economic growth of emerging countries as China and India, will increase the energy needs of the world [1, 2]. The continued use of fossil fuels has several negative environmental impacts due to greenhouse gas (GHG) emissions to the atmosphere, such as CO_2, which contributes to global warming and the acidification of the oceans [3, 4].

The long-term solution to such environmental problems is the development of renewable technologies to produce energy that will reduce CO_2 emissions to almost zero in 2100 [5]. To stimulate and promote research in the bioenergy field new policies have been adopted around the world. Thus, in July 2015, Denmark generated 140 % of its electricity needs from wind energy and shared its excess with other countries. This demonstrates that renewable energies are feasible for satisfying the world's energy needs. A recent report from the International Energy Agency (IEA) reveals that the energy produced from biofuels and waste have the highest potential. The energy obtained from biofuels and waste represented 10.0 % of the world`s total primary energy supply, compared with 2.4 % from water, and 1.1 % from other sources such as the wind or the sun. Therefore, it is expected that biofuels will play an important role in the future as sustainable energy source [6].

There are many options available to produce bioenergy and one of the most promising is the use of microorganisms. Microalgae are photosynthetic microorganisms that have the ability to accumulate lipids, hydrocarbons, etc. that are suitable for the production of biodiesel, methane, hydrogen or ethanol, among others.

Many studies demonstrate that the development of this biotechnology could supply 30 %of the global fuel demand in an environmentally friendly way, without having a negative impact on food production [6]. Some research works confirm that the production of biodiesel using microalgae is more sustainable than its production from agricultural crops since the process does not affect the supply of agricultural products [7]. In recent years, many advances have been made in the field of MFCs [8]. Microbial fuel cells are an alternative way to produce

electricity and microalgae can be grown in the cathode, capturing the small amount of CO_2 produced to be used as substrate in the anode [9].

This critical review provides an overview of the use of microalgae to produce a wide range of bioenergy, including bioelectricity and biodiesel. It focuses on their use in microbial fuel cells for electricity production, and the advantages of this technology and its limitations. However, the techniques involved still have many limitations, which hopefully, in the future will be overcome by genetic engineering.

2. MICROALGAE USED IN BIOTECHNOLOGY

2.1. Brief history of Microalgae

Microalgae were used in China 2000 years to survive famines but the first research works focusing on microalgae valorization were performed during the Second World War. Harder and von Witsch in 1942 proposed that some types of microalgae could be used as source of lipids, as a feasible food supplement or for producing oil [10, 11,12]. At the beginning of 1960s, the biology and physiology of microalgae were well known [12]. However, interest in this biomaterial increased rapidly with the oil crisis in the 1970s when microalgae started to be considered as a potential alternative to fossil fuels to produce energy [13].

Later, in 1980, the US Department of Energy started the Aquatic Species Programme (ASP), the main purpose of which was to promote algae as a source of green fuel which could compete with fossil fuels [14].

The great efforts made in recent years have led microalgae to be considered as a green and feasible alternative for bioenergy production based on an effective and low-cost technology [15]. Recent studies have demonstrated the positive evolution of microalgae for use as feedstock for producing biodiesel, bioethanol, bio-hydrogen, bio-oil and biogas [16, 17, 18, 19, 20, 21].

2.2. Definition of Microalgae

Microalgae, some of the oldest living organisms, are microscopic photosynthetic organisms that can be found in marine and freshwater environments. They are a diverse group of prokaryotic and eukaryotic photosynthetic microorganisms with a unicellular or simple multicellular

structure that allows them to grow rapidly and live in extreme conditions [16]. Due to their simple structure, they take advantage of solar energy quickly and efficiently. They usually grow in aquatic environments, which provide them with many nutrients in dissolved form, such as CO_2 [17].

Microalgae can be grouped into different categories based on the pigmentation of their biological structure: i) green algae *(Chlorophyta),* red algae *(Rhodophyta)* and diatoms *(Bacillariophyta)*[19]. They can also be classified into two groups: i) autotrophic, which only require inorganic compounds to grow, such as CO_2, salts (nitrate ion, phosphate) and light. This subcategory can be divided into photoautotrophs, which use solar light as a source of energy and chemoautotrophs, which require an external source of organic compounds as source of energy. ii) Heterotrophs can also grouped into photoheterotrophs, which use solar light as source of energy and chemoheterotroph, which oxidizes organic compounds to get energy. However, there are some types of microalgae that can use different sources of energy and carbon, which are called iii) mixotrophic [18,19, 22].

More than 100,000 different species of microalgae exist around the world, but no more than 30,000 have been investigated and classified, according to their color, size, pigments, cell wall constituencies or metabolism, as suitable for human needs. Microalgae also include the unicellular organisms (phytoplnakton) existing in natural water, which are an essential source of carbon for aquatic fauna [15, 23, 24].

Table IV-1 shows the most common species of microalgae used in biotechnology, their biomass production, the type of culture medium they require and the operation mode.

Table IV-1. Culture medium, operation mode and biomass production of different microalgae strains (*: data not shown).

Strains	Cultivation system	Biomass productivity	Culture medium	Culture mode	Optical density	Ref.
Chlorella vulgaris	Tubular photobioreactor	1.5 $g.m^{-2}.h^{-1}$	Artificial Wastewater	Semi-continuous	N.S*.	[25] [26] [27]
	Flat plate photobioreactor	1.0 $g.L^{-1}.day^{-1}$	Jaworski's medium	N.S.	N.S.	[28]
	Raceway ponds	40 $Mg.m^{-2}.day^{-1}$	Municipal Wastewater	N.S.	N.S.	[29] [30]
Chlorella sorokiniana	Flat plate photobioreactors	7.7 $g.m^{-2}.h^{-1}$	Swine manure	N.S.	N.S.	[31] [32]
	Tubular photobioreactor	1.47 $g.L^{-1}.day^{-1}$	Inorganic medium	Continuous	N.S.	[33]
Nannochloropsis sp.	Raceway ponds	0.35 $g.L^{-1}.day^{-1}$	N.S.	Continuous	N.S.	[34]
	Flat plat photobioreactors	2.7 $g.L^{-1}.day^{-1}$	N.S.	Continuous	N.S.	[34]
	Tubular photobioreactors	1.02 $g.L^{-1}.day^{-1}$	N.S.	Continuous	N.S.	[34]
Scenedesmus obliquus	Flat plate photobioreactors	100 $mg.^{m-2}.day^{-1}$	Synthetic wastewater	N.S.	N.S.	[31] [30]
Spirulina platensis	Raceway ponds	11. to 20.8 $g.m^{-2}dw^{-1}$	Zarouk's medium	Semi-continuous	0.5-0.6	[35] [36]

3. MICROALGAE CULTIVATION

Microalgae grow rapidly grow and, in adequate conditions, they can perform one or two cell divisions per day [37]. During the photosynthesis process, microalgae produce biomass, when they can be represented by the following equations:

$$\text{Lights} + CO_2 + H_2O + \text{Nutrients} \Rightarrow \text{Biomass} + O_2 \quad \textbf{\textit{(a)}}$$

Microalgae cultivation requires a culture medium rich in nutrients and salts, which will allows them to grow quickly. Many physicochemical and biological factors, such as light, temperature, pH or nutrient concentrations, affect algal growth. Of these, the most important are light and the amount of carbon available in the medium [15].

The growth of microalgae is strongly affected by the photosynthetic period. Indeed, when the photosynthetic period increases from 6 to 12 hours, the average concentration of biomass may reach 180% [38]. The photosynthetic process provides microalgae with a huge amount of inorganic carbon, which, combined with solar light, produces glucose that can be used as carbon source by the microalgae [39] according to the following equation:

$$6\,H_2O + 6\,CO_2 + \text{Light} \Rightarrow C_6H_{12}O_6 + 6\,O_2 \quad \textbf{\textit{(b)}}$$

As previously mentioned, temperature is another important factor that affects microalgae growth. The biomass produced increases with temperature, particularly in the first seven days [38]. The optimum temperature range for microalgal growth is usually 20-30 $^{\circ}$C. Outside of these values, the cells can be damaged [7]. High concentrations of CO_2 also increase biomass production, depending on the type of microalgae [23]. The CO_2 dissolved in water is inversely proportional to the pH value since the high values of pH are caused by inorganic carbon in carbonate form [39].

Microalgae cultivation also requires an aeration system, which provides them with the CO_2 necessary for the photosynthesis process and pH stabilization. To ensure that cells and nutrients are uniformly distributed, the reactor should be gently stirred [40].

As mentioned above, based on their metabolism, microalgae can be grouped into four categories: photoautrophic, (chemo) heterotrophic, mixotrophic and photoheterotrophic [41].

3.1. Photoautotrophic metabolism

Almost all microalgae species can grow in this mode since they can use inorganic carbon and light (sunlight or artificial) to obtain chemical energy via photosynthesis [41, 42]. Photoautotrophic cultivation is the most widely used microalgae cultivation mode. The lipid content of microalgae is one of the most important properties that enable their use for oil production. However, there are large differences in the lipid content between species, which may vary from 5 % to 68 %. Usually, the lipids retained in the cells depend on the nitrogen content, and if nitrogen is used in lipid production, the biomass of the culture is reduced. Hence, the lipid content is a key factor for potential industrial application. In the case of *Chlorella* sp., production can reach 179 mg. L^{-1}. D^{-1} of lipids in photoautotrophic mode, the maximum value described in the literature [42].

3.2. Heterotrophic metabolism

Heterotrophic metabolism does not involve photosynthesis for producing organic compounds but transforms organic carbon directly into other organic compounds useful for microalgal growth. In this case, organic carbon is used simultaneously as source of energy and carbon. The main advantage is that heterotrophic microalgae can grow quickly in the absence of light to reach high biomass concentrations.

Heterotrophic cultivation is more cost-effective and easier to scale-up than photoautotrophic mode since the cultivation conditions are simple. They even allow different industrial process such as wastewater treatment and biofuel production to be combined [17, 29].

The lipid content of many species of microalgae, such as *Chlorella protothecoides*, increases by up to 40 % when cultivated in heterotrophic rather than phototrophic conditions [19, 42]. Many research works have demonstrated the benefits of heterotrophic cultivation compared with photoautotrophic processes, in both open ponds and closed photobioreactors [19].

3.3. Mixotrophic metabolism

Mixotrophic metabolism, which can be phototrophic, heterotrophic or both, uses carbon dioxide and organic compounds as carbon source to produce microalgal biomass via photosynthesis. The growth of *Spirulina* sp. was investigated in photoautotrophic, heterotrophic and mixotrophic cultures. The highest growth rate was achieved under mixotrophic conditions (light intensity > 30 $W.m^{-2}_{carhode}$ and glucose concentration >0.5 $g.L^{-1}$) in a photobioreactor operating in batch mode [18]. The biomass produced was 5 times higher than in phototrophic cultivation and 2.3 times higher than in heterotrophic cultures [43]. Mixotrophic microalgae have many advantages over those with another type of metabolism since they do not present dark respiration-related problems, which have a negative effect on biomass production, and besides, the consumption of organic elements is lower.

3.4. Photoheterotrophic metabolism

Photoheterotrophy nutrition is the combination of heterotrophy and phototrophy. Microorganisms based on this mode use the light as energy source and organic matter as carbon source [41]. There are no notable differences between photoheterotrophic and mixotrophic nutrition modes, which are only different in the source of energy used [15]. Photoheterotrophic cultivation needs light as the energy source, whereas mixotrophic cultivation can use organic substances [42].

4. MICROALGAE CULTURE SYSTEMS

Microalgae cultivation systems can be divided into open and closed devices: open-air ponds or closed photobioreactors (PBRs). The selection of an appropriate cultivation system is crucial for growing microalgae and depends on the microalgae strain in question, the cost of land, the final product, the source of CO_2 and the nutrients required [44]. Most strains of microalgae can be grown in photobioreactors, whereas the culture in open ponds is suitable only for few types of strains [45].

4.1. Open systems

Raceway ponds are shallow ponds that consist of a circuit of parallel channels where microalgae, water and nutrients circulate propelled by a paddle wheel to prevent the

sedimentation [46]. As they must be exposed to sunlight, the ponds should have a depth of between 0.2 and 0.5 m to improve sunlight diffusion, which decreases as the depth increases. The CO_2 in the atmosphere is continuously in contact with pond surface, where it is captured by the microalgae and used as carbon source, enabling them to grow. The microalgae are harvested at the end of the circuit, as can be seen in Figure 1 [14,15, 17, 19].

Open systems have many advantages including simplicity; moreover, the construction, operating and production costs are lower compared with other systems. Another important advantage of open systems is that they can work with high amounts of CO_2 [17].However, they are exposed to weather fluctuations, which may cause evaporation when temperatures are high or dilute the existing biomass when it rains [38]. Furthermore, microalgae can be contaminated by external species. Cell density in these devices is low and the harvesting process expensive [15, 29]. Generally, raceway ponds vary from 10 to 100 m in length, 1 to 10 m in width and 0.25–0.30 m in depth. Their area can be approximately 0.5 ha or larger [47, 48]. Much progress has been made to reduce the energy consumed by the paddle wheel and to avoid dead zones [48].

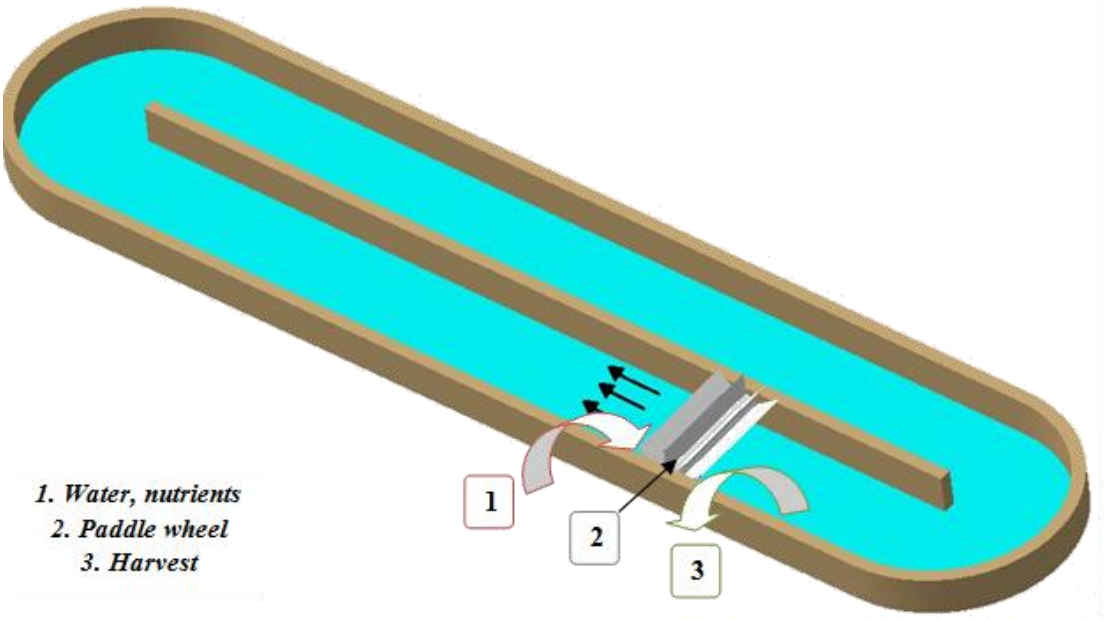


Figure 1: Scheme of a raceway pond for microalgae cultivation.

4.2. Closed systems: Photobioreactors

Photobioreactors are systems that allow an even distribution of light through the whole culture. They usually consist of transparent tubes made of glass or plastic [49]. Photobioreactors (Fig 2) were developed to overcome some of the problems related to open systems. They allow better control of important parameters such as nutrient concentration, temperature, dissolved CO_2 and pH, among others. Moreover, they avoid contamination by other species. These devices can be classified into different groups depending on their shape, the following being most commonly used: i) tubular; ii) flat plate; iii) vertical or horizontal column photobioreator [19, 49, 50, 51]. Although tubular and flat plate systems have been scaled-up, none has reached high production rates [31].

Most laboratory-scale photobioreactors use artificial sources of light such as fluorescent lamps to supply the light needed by microalgae. This obviously increases the operation costs of the process [42]. The use of artificial light reduces the sustainability of these devices since they still depend on fossil fuels. However, this drawback could be overcome using the bioenergy generated by the microalgae in other devices [52].

Some research works have compared different types of photobioreactors and have suggested improvements for classic tubular and flat plate designs. These include the development of novel designs such as hybrid systems, which combine open ponds and photobioreactors, or floating photobioreactors, which can operate in water rather than on land [46].

The main tool for optimizing microalgae production is the design of photobioreactors. They should maintain the ideal condition for microalgae growth avoiding the saturation of the system. The dimension of photobioreactors is a key factor for their design. The maximum length (L) of a continuous run tube is showed in following equation [53]:

$$L = \frac{U_L([O_2]_{out} - [O_2]}{R_{O2}} \qquad (c)$$

Where:

L:	Straight tube length (m)
U_L:	Superficial liquid velocity in the tube ($m.s^{-1}$)
RO_2:	Volumetric rate of oxygen generation (mol O_2 $m^{-3}.s^{-1}$)
$[O_2]in$:	Dissolved oxygen concentration at entrance of solar tube ($mol.l^{-1}$)
$[O_2]out$:	Dissolved oxygen concentration at the outlet of the solar tube ($mol.l^{-1}$)

During the design process, the amount of carbon dioxide should also be taken into consideration. Carbon dioxide capture in the photobioreactor and microalgae productivity can be estimated following the equation ***(d)*** [53, 54, 55].

$$w_{CO2\,in} \cdot F_{in} = w_{CO2out} \cdot F_{out} + W_{X/C} \cdot R_X V \quad \textbf{(d)}$$

Where:

W_{CO2in}	:	Mole fraction CO_2entrance (mol CO_2.mol $total^{-1}$)
W_{CO2out}	:	Mole fractionCO_2 outlet (mol CO_2 .mol $total^{-1}$)
F_{in}	:	Molar flow rate, entrance gas ($mol.h^{-1}$)
F_{out}	:	Molar flow rate, outlet gas ($mol.h^{-1}$)
$W_{X/C}$	:	Carbon content of biomass (mol . kg $biomass^{-1}$)
R_xV	:	Rate of algal biomass production kg $biomass.h^{-1}$

The use of photobioreactors has many advantages that have been reported in the literature [15, 17, 22, 37, 46, 56, 57]. They are suitable for outdoor cultivation, have a wide light exposure surface and produce high amount of biomass. They also reduce the contamination risk and

facilitate control. However, photobioreactors have several limitations such as the output capital required and the high operating costs. They are complex systems that need to avoid oxygen accumulation, biofouling and cell damage by shear stress. Moreover, the selection of bioreactor depends on the strain of microalgae, the place and the space available, and the nature of the final product required (see Table IV-1). All this demonstrates that a comparison between reactors based on the ratio: maximum productivity/minimum operation costs is not possible [46].

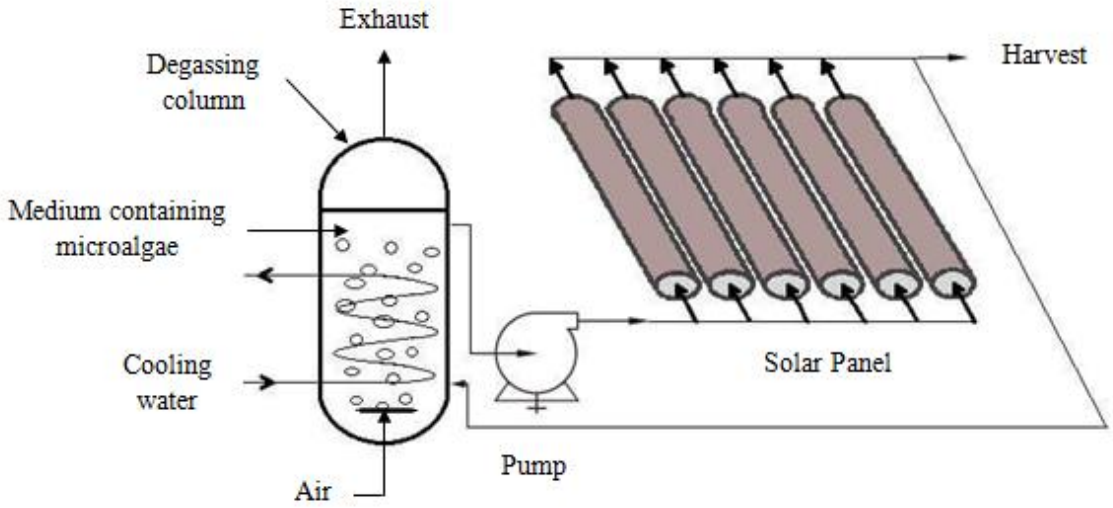


Figure 2: Scheme of a tubular photobioreactor for microalgae cultivation

4.3. Genetic engineering of microalgae

More than 200 microalgae species are used all over the world in many biotechnological processes. However, the most promising results have been obtained with strains of microalgae genetically modified for the production of pharmaceutical or cosmetic products, and bioenergy., For example, the genome of *Chlamydomonas reinhardtii* was successfully modified in 1989 [58]. The main purpose of the genetic transformation is to increase the content of lipids in microalgae. So far, many methods have been developed to perform the genetic modification. *Nannochloropsis sp.* has been transformed by homologous recombination, achieving a maximum efficiency of ~2,500 transformants/μg of DNA using a high electric field. The use of

homologous recombination to modify this strain allowed advances in algal functional genomics and biotechnology [59].

Another type of modification is that made to produce H_2. Some species naturally produce hydrogen, but others can be genetically altered to do so [60]. The genetic modification of microalgae can be performed for different purposes such as the phycoremediation of heavy metals. Some research papers have demonstrated that the capacity of genetically modified microalgae to fix metals is five times higher than that of the corresponding wild strain [61].Other research has focused on transforming phototrophic microalgae into their heterotrophic equivalent in order to help overcome some limitations in their cultivation [62]. However, some scientists do not expect genetically modified microalgae to survive in natural conditions. Moreover, this type of modification involves risks and it is necessary bear in mind many engineering and biological considerations to prevent their accidental release into the environment [61, 63].

5. MICROALGAE FOR ENERGY PRODUCTION

5.1. Biodiesel

Microalgae are increasingly considered as a promising alternative for biodiesel production due to their high lipid content, up to 70% [64]. Biodiesel is produced by transesterification of triglycerides with methanol in excess (see Fig 3). Other alcohols such as ethanol also can be used but they are more expensive than methanol. This reaction produces fatty acid methyl esters (FAMEs) and glycerol. To increase the conversion rate and reduce the reaction time, a catalyst is usually included in the reaction. The most commonly used are alkalis such as KOH or NaOH, in which case the reaction needs 90 min at 60 °C at atmospheric pressure to be completed. However, the presence of free fatty acids inhibits this reaction since they react with alkali catalysts and saponify, hindering the separation of biodiesel and glycerol. Other types of catalyst such as acids or enzymes are also used [52,65].

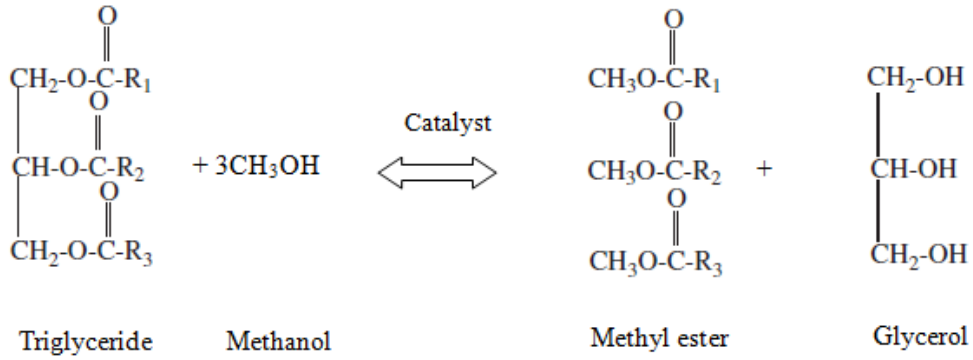


Fig 3: Transesterification reaction of triglycerides to produce biodiesel.

Depending on the origin of the triglycerides, biodiesel can be divided into first, second and third generation. First generation biodiesel is produced from edible oil such as soybean or palm oil, whereas second generation comes from non-edible oils, e.g. karanja or *jatropha* oil [66, 67]. Lastly, third generation biodiesel uses microalgae as feedstock. This last option seems to be the most promising alternative to fossil fuel for biofuel production. Microalgae offer many advantages over terrestrial crops: i) their biomass productivity is higher and faster; ii) their

accumulation of lipids is higher; iii) microalgae can grow on degraded land; iv) microalgae capture CO_2, which has many environmental benefits [68, 69].

According to their origin, microalgae can be divided in two types: freshwater and seawater microalgae, the latter being the most sustainable [70]. *Nannochloropsis* species are marine microalgae which grow very fast and their lipids content is very high, properties that make them suitable for biodiesel production. Within this species, *Nannochloropsis* gaditana have drawn the attention of many researchers since they have a high calorific capacity. Jazzar et al. investigated the production of biodiesel from *Nannochloropsis gaditana* by direct transesterification with methanol under supercritical conditions (no catalysts). They achieved the maximum yield of biodiesel (0.48 $g.g^{-1}$ of lipid) from wet unwashed microalgae at 255-265 °C and a 50 min reaction time [69].

Many research works have compared the oil content in biomass of different types of vegetable crops and microalgae, and the biodiesel production yield obtained when used as biodiesel source. The results demonstrate that even microalgae with low oil content (30 %) can produce more biodiesel than castor oil, whose seeds contain more oil (48 %). In the case of microalgae containing 70 % oil, they have been seen to produce as much as 121,104 kg biodiesel/ha year, almost 800 times higher than corn crops and require around 160 times less land space to grow [15]. There are some microalgae species, such as *Schizochytrium* sp., that contain up to 77 $\%_{dry\ wt}$ of oil, but they are not all suitable to produce biodiesel. However, as mentioned above, they could be genetically modified for this purpose [65].

5.2. Bioethanol

Another renewable biofuel is bioethanol, which can also be classified into three groups: i) First generation bioethanol, produced from sugar and starch crops; ii) second generation bioethanol, mainly produced from lignocellulosic biomass and iii) third generation bioethanol, based on microalgae. So far, the most commonly used feedstock has been sugar cane since the direct fermentation of the sugar contained in this type of biomass is the simpliest method for producing bioethanol. However, the price of this material is high. Lignocellulosic biomass and starch-based wastes have a lower price, but they need to be enzymatically hydrolyzed to produce fermentable sugars. An alternative low-cost substrate for bioethanol production are microalgae which can accumulate large amounts of carbohydrate biomass, making them ideal for generating this type of biofuel [71]. Some species of microalgae, such as *Spirulina*, *Chlamydomonas* or *Chlorella*, contain high levels of glycogen starch (more than 50 % dry weight), which can be enzymatically converted into glucose for transformation into bioethanol [71, 72]. However, other types of pre-treatment have also been used, including acids, alkalis or supercritical CO_2 [52]. The conversion of glucose into bioethanol and carbon dioxide follows the reaction:

$$C_6H_{12}O_6 \Rightarrow 2CH_3CH_2OH + 2CO_2 \quad \textbf{\textit{(e)}}$$

As long ago as 1997, Hirano et al. investigated the production of bioethanol from more than 250 microalgae strains. Among these, *Chlamydomonas reinhardtii* (UTEX2247) was seen to have a maximum starch content of 45 %, followed by *Chlorella vulgaris (IAM C-534)* with 37 % [73].

More recently, microalgae have been used to produce biodiesel and bioethanol simultaneously since lipids are extracted before performing the fermentation step. In the case of *Chlorococum* sp., lipids were extracted using supercritical CO_2 at 60 °C. After that, Sccharomyces bayanus ferment the sugars to bioethanol. In this case, supercritical CO_2 acts as double agent extractor of lipids and carbohydrates from the cell wall in only one step. This seems to be a promising method for large scale production [74].

Many research works have confirmed the suitability of microalgae starch for bioethanol production. The main advantage is its low consumption of energy compared with biodiesel

production, while the yields are similar to those obtained from sugary or lignocellulosic substrates. However, the efficiency of bioethanol extraction is low since bioethanol contains 5 % water, which can hinder the process [52, 62].

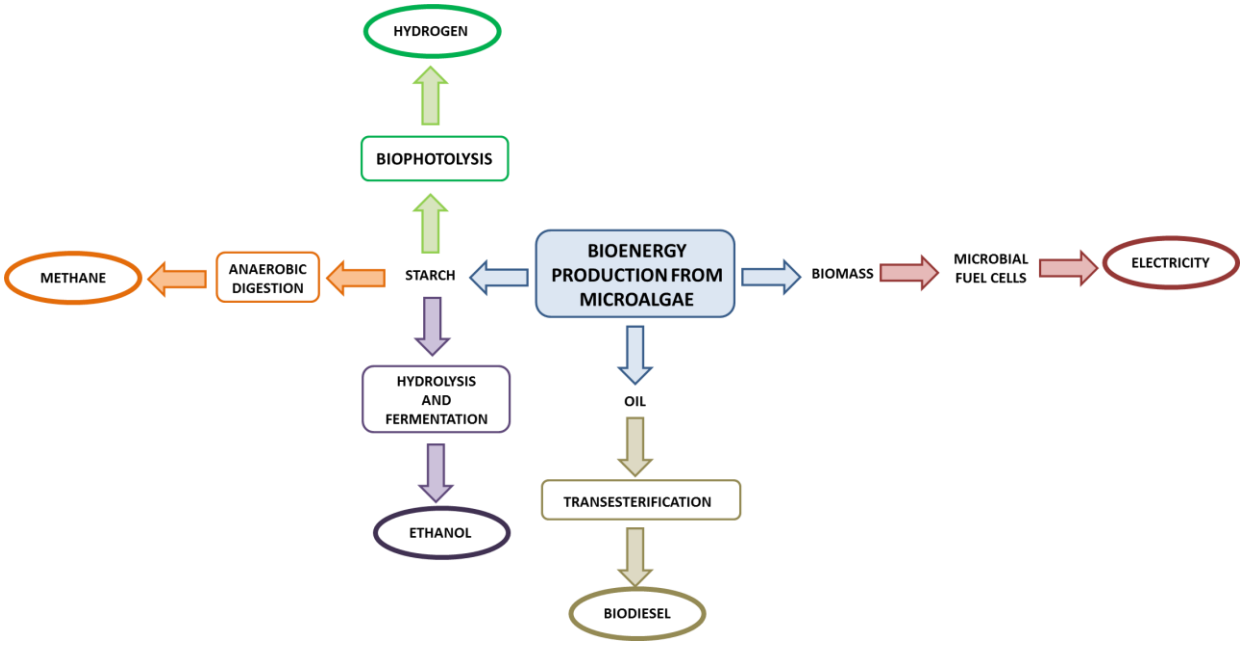


Fig 4: Scheme of bioenergy production from microalgae

5.3. Hydrogen

Another clean, renewable and sustainable for, of energy is hydrogen. Although the technology involved requires the development of new production, storage and utilization methods, it is expected that hydrogen will becomes a viable bioenergy source in the near future [76, 77].

Despite its limitations, the hydrogen produced by microalgae first became the subject of research works in the 1970s [78]. Their genetic, metabolic and enzymatic characteristics mean that microalgae can produce biohydrogen from water andsunlight as a photobiological process [19]. Depending on the culture conditions (aerobic darkness, anaerobic darkness, etc.), the oxidative reaction of starch will be different and the proportion of hydrogen, ethanol, carbon dioxide, etc. produced will vary for each species of microalgae [71].

Hydrogen can be produced in different ways but the most important are the following:

- Direct biophotolysis. This consists of breaking down the water molecule by microalgae photosynthesis. This reaction releases electrons, which are transferred by photosystem II (PSII) and photosystem I (PSI) to ferredoxin, directly reducing ito hydrogen. In general, this process does not need much time [78, 79, 80]. This method can be simplified in the following reaction:

Photosynthesis

$$H_2O + \text{Solar Energy} \Rightarrow H_2 + \tfrac{1}{2} O_2 \quad \textbf{\textit{(f)}}$$

- Indirect biophotolysis. In this case, hydrogen is produced by several steps. CO_2 is stored in the form of carbohydrates, which are used as chemical energy to break down the water molecule in a photosynthetic reaction. The aim of indirect biophotolysis is to temporally separate hydrogen production from oxygen production. This method is used by nitrogenase-based systems [81, 82] and it is represented by the following reactions:

$$12H_2O + 6CO_2 + \text{light energy} \Rightarrow C6H_{12}O_6 + O_2 \quad \textbf{\textit{(g)}}$$

$$C_6H_{12}O_6 + 12H_2O + \text{light energy} \Rightarrow 12H_2 + 6CO_2 \quad \textbf{\textit{(h)}}$$

Hydrogen can also be produced by the dark fermentation and photofermentation of organic compounds for use in a wide variety of applications such as fuel cells [83]. Photofermentation consists of the transformation of the acids contained in wastewater into H_2 and CO_2 under anaerobic conditions. During dark fermentation, hydrogen is produced from carbohydrates such as glucose, starch, arabinose or xylose, by strict anaerobes, e.g. *Clostridiaceae* family, as well as facultative anaerobes, e.g. *Enterobactericeae* family [84]. Heterocystous cyanobacteria can simultaneously produce hydrogen and oxygen. However, a gas separator is required, which means additional costs, among other drawbacks, so they are not recommended for hydrogen production [78]. Another pathway of hydrogen production from *Chlamydomonas* has been widely investigated, mainly from sulfur-deprived cultures of these green algae. In this case, the hydrogen production process is slightly different and also has some limitations to

simultaneously produce hydrogen and oxygen [80]. Despite this, *Chlamydomonas reinhardtii* is an excellent hydrogen producer compared to other microalgae. It can achieve, in direct biophotolysis mode, more than 22 % of light conversion [78, 85].

5.4. Methane

The idea of producing methane from microalgae was first investigated in the early 1950s. At the beginning of the energy crisis, the use of microalgae for methane production was seen as a green alternative to compete with fossil fuels [47, 50, 86].

Methane is produced by anaerobic digestion of microalgae waste after the oil is extracted. This residue can also be recycled to manufacture fertilizers (see Fig 4) [7, 17, 47]. Anaerobic digestion consists of the conversion of organic matter to methane (55-75 %) and carbon dioxide (25-45 %) by anaerobic microorganisms. Due to the low requirements of energy, this method seems to be an energetically feasible alternative [30]. The process is represented by the following reaction:

$$C_6H_{12}O_6\ (aq) \Rightarrow CH_4\ (g) + 3CO_2\ (g) \quad \textbf{\textit{(i)}}$$

Table IV-2 contains a selection of microalgal species which are used to produce methane. Some research has suggested that the strains *Chlamydomonas reinhardtii*, *Dunaliella salina*, *Arthrospira platensis* and *Euglena gracilis* give the best methane yield (0.58, 0.5, 0.48 and 0.48 $m^3.kg^{-1}$, respectively) (see Table IV-2).

Before using microalgae for methane production, a pre-treatment step is necessary. There are four principal types of pre-treatment methods: thermal, mechanical, chemical and biological [87]. More recently, in 2016 He et al. developed a new bio pretreatment method, whereby a facultative anaerobic bacterium, *Bacillus licheniformis*, is usedto pre-treat *Chlorella sp*. In this way, methane production was increased from 9.2 to 22.7 % by varying the content of *Bacillus licheniformis* in a range of 0 to $8\%_{v/v}$ [88].

The production of methane is affected by several factors such as temperature, pH, organic loading rate and hydraulic retention time. The optimization of these parameters allows the methane yield to be maximized. Usually a long solid retention time and high organic loading rate have a positive on methane yield (see Table IV-2) [75].

Table IV-2. Methane production from a selection of microalgal species.

Microalgal strains	Methane production ($m^3.Kg^{-1}$)	HRT* (days)	OLR** VS $.L^{-1}.d^{-1}$	References
Dunaliella salina	0.5	-	-	[89]
Ulva sp.	0.20	8	4.8	[90]
Euglena gracilis	0.48	-	-	[89]
Gracilaria sp.	0.28–0.4	12-16	-	[91]
Chlamydomonas reinhardtii	0.58	-	-	[89]
Chlorella vulgaris	0.24	33	0.97	[87]
	0.32	-	-	[92]
Laminaria sp.	0.26–0.28	-	-	[93]
Scenedesmus sp.	0.22	3.8	3.23	[94]
Arthrospira platensis	0.48	-	-	[89]
Chlorella sp.	0.41	4.4	-	[88]
Scenedesmus obliquus	0.28	-	-	[89]

* Hydraulic retention time; ** Organic loading rate

The methane produced by microalgae could be used to generate electricity to run an industrial plant (paddle wheels, pumps, etc.), while any excess of energy could be sold to cover the cost of biodiesel production. For example, a simultaneous wastewater treatment and methane production process is possible in a raceway pond [14, 17].

6. MICROALGAE USED IN MFC

Microbial fuel cells (MFCs) are devices that have the ability to produce energy and treat wastewater simultaneously. They use microorganisms to transform the chemical energy of organic matter contained in wastewater into electricity. The electrons and protons produced at the anode as a result of microbial metabolism flow to the cathode. The electrons travel through an external circuit and protons cross a separator, usually a proton exchange membrane. At the cathode, the electrons reduce atmospheric oxygen (a reaction usually catalyzed by platinum), which is combined with protons to form water [95, 96, 97].

The first report of electricity production by bacteria was made in 1991, although it was not until the beginning of the 1990s that MFCs focused researchers' attention [98, 99, 100, 101].In recent years, many advances have been made in terms of new materials, designs, low cost catalysts or substrates. These advances have reduced the final price of MFCs and increased their efficiency, which has expanded their application range [95, 102].

One of the most promising advances in MFCs is the use of microalgae. Microalgae can be use as substrate at the anode to remove nutrients or capture the CO_2 generated in the cathode, among other possibilities [103, 104, 105].

During the last ten years, many research works have focused on integrating microalgae and MFCs. In 2009, Powell et al. developed an MFC which contained *Saccharomyces cerevisiae* in the anodic chamber and *Chlorella vulgaris* in the cathode. The algal culture worked as a suitable electron acceptor and used the CO_2 generated in the cathode to grow. This configuration managed to produce 2.7 $mW.m^{-2}_{cathode}$ [106]. Furthermore, microalgae can be used as electrons donor, or acceptor and donor simultaneously, when they are in both the cathodic and anodic chambers. However, a distinction needs to be made between photo microbial fuel cells and microalgae-MFCs. Microalgae-MFC can work in dark and in light conditions, whereas photo microbial fuel cells can only work with light [107, 108].

Microalgae MFC configurations can be grouped into single chamber, double chambers or photosynthetic sediment (see Fig 5). Nevertheless, some of these configurations involve many difficulties, such as design and energy sustainability which are important if energy is to be produced in a low-cost manner [109].

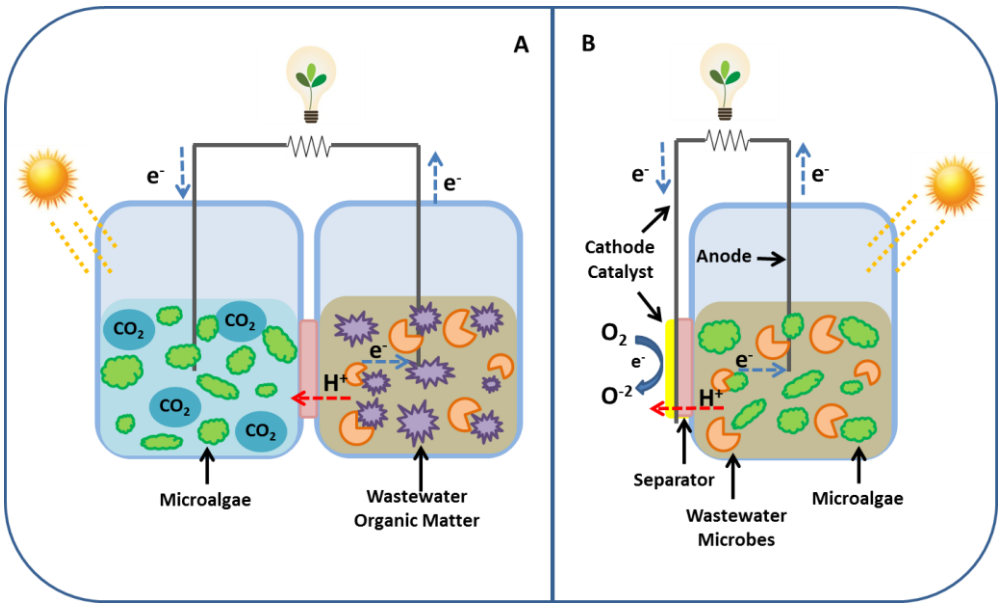


Figure 5. Scheme of two microalgae-MFC configurations.

6.1. Microalgae at the anode

Although microalgae can be used as substrate for exoelectrogenic bacteria in the anodic chamber of MFCs, fresh microalgal biomass does not always produce a sufficiently high-power density [36, 110, 111]. In 2009, Velasquez-Orta et al. compared the degradation of microalgae (*Chlorella vulgaris*) and macroalgae (*Ulva lactuca*) in a single chamber MFC. When *Chlorella vulgaris* was used as powder feedstock in MFCs it produced 980 $mW.m^{-2}_{cathode}$, whereas *Ulva lactuca* generated a power density of 760 $mW.m^{-2}_{cathode}$. Despite the power output, they concluded that macroalgae were more efficiently degraded [112]. Any byproducts created during the microalgae degradation process are almost totally consumed by microorganisms by the end of the cycle [110].

Spirulina platensis have also been used to produce electricity at the anode of a membrane-free MFC under both light and dark conditions. These blue-green microalgae, which have a high

content of proteins and vitamins, can undergo photosynthesis during the day and consume the glycogen stored during oxidation in darkness. Such devices can produce a power density of 1.64 $mW.m^{-2}_{cathode}$ in the dark (almost 10 times higher than during the day) [113].

In the case of phosynthetic microbial fuel cells (PMFCs), the source of the light and its intensity can affect the photosynthesis process and hence electricity production. Lan et al. investigated the influence of both parameters in PMFCs containing *Chlamydomonas reinhardtii transformation* F5, comparing various light intensities (100, 300, 600 and 900 lx) of monochromatic blue and red LED lights. They observed that red LED light allows PMFC to produce a higher power density (12.95 $mW.m^{-2}_{cathode}$) than with blue light. Moreover, the higher the light intensity the better the performance of the PMFC [114].

The materials used as electrode can also affect the PMFC energy performance. Li et al. investigated the influence of several electrode materials such as stainless steel, copper, gold and graphite carbon cloth in various sizes in a PMFC with *Spirulina platensis*. They observed that copper electrodes needed the shortest time to reach the maximum OCV value, although this material tended to oxidize. Stainless steel electrodes exhibited high internal resistance, which hindered electron transfer. The maximum power output (10 $mW.m^{-2}_{cathode}$) was obtained with graphite carbon cloth as cathode and a gold-coated mesh as anode [115].

In 2012, Lakaniemi et al. compared the use as feedstocks in MFCs of two types of microalgae: fresh (*Chlorella vulgaris*) and marine (*Dunaliella tertiolecta*). They used municipal sewage sludge as inoculum in the anode. The maximum power density achieved with *Chlorella vulgaris* was 3 times higher than that obtained using *Dunaliella tertiolecta* (15 and 5.3 $mW.m^{-2}_{cathode}$, respectively). The authors also analyzed the potential production of butane, observing that the anolyte produced when *Chlorella vulgaris* were used as substrate contained between 8.7 and 16 mM of butanol, which was higher than that obtained with *Dunaliella tertiolecta* [116].

More recently, Walter et al. used fresh algal biomass as substrate in a cascade of 9 MFCs, using activated sludge combined with *Synechococcus leopliensis* as anodic inoculum. They achieved 42 W using continuously algal biomass pre-processed with MFC effluent. However, this system was not very stable for longer than 2-4 days due to the limitations of the pre-digester process [103].

Finally, in 2016 Salar et al. developed a process to reuse the catholyte generated in terracotta-based MFCs for pre-digesting microalgae, which were used as substrate in the MFCs. The power output achieved was 6 times higher than when using untreated microalgae. This method allows the catholyte produced in terracotta-based MFCs to be reused and increases the performance of the system when microalgae are used as feedstocks [104].

Despite all the research work reported, further work is needed for a better understanding that will allow the bioenergy generated by these devices to be increased.

6.2. Microalgae at the cathode

In recent years, interest in using phototrophic microorganisms in the cathode of an MFC has increased because of their numerous advantages such as oxygen production and ability to capture the CO_2 generated. Photosynthesis allows phototrophic microorganisms to produce oxygen, which is consumed at the cathode, while carbon dioxide is used as carbon source during the process [117].

Kakarla et al investigated the performance of MFC with a biocathode based on *Scenedismus obliquus*. Aeration of the oxygen produced by these photoautotrophic algae allows them to generate a maximum power density 32% higher (153 $mW.m^{-2}_{cathode}$) than that possible with mechanical aeration [118].

Chlorella vulgaris has already been suggested as a suitable electron acceptor in the cathode of MFCs and for simultaneously fixing the CO_2 [106]. Wang et al. studied the performance of a sediment MFC with biocathode based on *Chlorella vulgaris,* managing to produce 21 $mW.m^{-2}_{cathode}$ with this assembly [119]. Other more complex designs have also been developed. For example, Wu et al. built a MFC with a tubular photobioreactor assembly as cathodic chamber, again using *Chlorella vulgaris* to produce oxygen. They reached 21.4 $mW.m^{-2}_{cathode}$ of maximum power output by combining the algae with a catalyst as biocathode, a value 2.8 times higher than that obtained by an abiotic cathode [129]. The use of *Chlorella vulgaris* suspended or immobilized in the cathode of an MFC was investigated by Zhou et al., who observed that energy production was 88% higher when microalgae were immobilized. The maximum power density obtained was 2.5 $W.m^{-3}_{anode}$ and the coulombic efficiency was 9.4% [120]. Wang et al. designed a novel MFC assembly that discharges the off-gases including CO_2 into the cathode

chamber for microalgae growth. Such devices are called microbial carbon capture cells (MCCs) and have been seen produced 5.6 $W.m^{-3}_{anode}$. The results showed that all the CO_2 produced in the anode was totally removed in the cathode, while the system treated wastewater and produced electricity [121]. In 2014, Cui et al. investigated the use of dead microalgal biomass as substrate in the anode and the reuse of the CO_2 produced in the anode to grow microalgae in the cathode. This assembly managed to generate a maximum power density of 1.9 $W.m^{-2}$ with a Coulombic efficiency of 6.3 % [108].

Other authors have focused on the study of the influence of cathode materials on MFC performance in devices that include algae-assisted cathodes. Kakarla et al. compared the use of a carbon fiber brush and plain carbon paper as material for the biocathode. The results showed that a cathode based on carbon fiber brush maximizes both power density (30 $mW.m^{-2}_{cathode}$) and biomass production [122].

Gadja et al. developed the first stack-biotic-MFCs with phosynthetic algae in the cathode. The oxygen produced by microalgae supplies enough gas for oxygen reduction [123].

Another important factor that affects algae-assisted cathodes is the intensity of the light. Wu et al. investigated the influence of different light intensities on photo-microbial fuel cells (photo-MFCs) with a biocathode containing *Desmodesmus* sp. A8. They observed that the anode and cathode resistances were strongly affected by changes in light intensity, and hence the voltage. The results confirmed than when the light intensity is around 3000 lx, the voltage generated reaches a plateau [9]. Other researchers have investigated the effect of continuous flow mode on the performance of *Chlorella vulgaris* - assisted cathode MFCs. Gonzalez del Campo et al. achieved a higher power output in continuous mode than in sequencing-batch mode [124].

Finally, in 2015 Gadja et al. described a full operation cycle including a biotic MFC fed with microalgae grown in the cathode, achieving 128 μW. This assembly simultaneously produces electricity and biomass (to be used in the process) and can be considered a promising alternative for producing electricity from biomass generated in the cathode of MFCs [105].

6.3. Integrated systems

These advances have enabled the development of integrated systems for simultaneous microalgae growth and energy production. They include the use of algae in both the cathodic and anodic chambers of MFCs. In 2015 Gajda et al. [108] presented a full operation cycle including a biotic MFC fed with microalgae grown in the cathode, achieving 128 μW of power output. This assembly simultaneously produces electricity and biomass (to be employed in the process), and is considered as a promising alternative to generate electricity from the biomass produced in the cathode of MFCs.

Other authors have combined the production of different types of biofuel. De Schamphelaire and Verstraete [139] developed a closed loop system to transform the sunlight into biogas. The assembly consisted of feeding algae contained in a photobioreactor with the CO_2 produced by a microbial fuel cell. Simultaneously, the algal biomass generated is employed as feedstock in an anaerobic tank to produce biogas. The effluent of the anaerobic digestion that still contains nutrients is recirculated to the anode of the MFC where they are totally oxidized. Under these conditions, the authors reached an algal biomass of 24– 30-ton VS ha^{-1} $year^{-1}$ and a biogas production of 0.5 N m^3 kg^{-1} algae.

Powell and Hill [140] integrated the production of biodiesel, bioethanol and bioelectricity in a single system. They coupled a photobioreactor to a MFC to grow C. vulgaris (yeast) that captures the CO_2 produced in the cathode. Simultaneously, the algal biomass obtained is used as a feedstock in the anode, which allows the system to produce electricity. On the other hand, the high oil content of C. vulgarism makes them suitable for biodiesel production.

All these integrated systems prove the suitability of the simultaneous production of different types of biofuel at low cost and with very low environmental impact. These processes represent a significant advance to produce green energy at large scale.

	Microalgae strains	MFC type	Anode	Cathode	Max. power density ($mW.m^{-2}$)	COD Removal (%)	OCV (V)	Operating time (h)	Ref.
Microalgae used at Anode	*Scenedesmus obliquus*	Double chamber	Toray carbon paper	Toray carbon paper	102	74	-	150	[110]
	Chlorella pyrenoidosa	Double chamber	Graphite Rod	Graphite Rod	30.15	-	-	-	[107]
	Spirulina platensis	Double chamber	Platinum electrodes	Platinum electrodes	6.5	-	0.24	12	[36]
		Duran bottle + beaker	Gold mesh	Carbon cloth	10	-	0.49	168	[115]
	Chlamydomonas reinhardtii	Single Chamber	Graphite felt	Carbon cloth-coated Pt	78	-	-	96	[125]
	Arthrospira axima (Spirulina maxima)	Double chamber	Graphite	Graphite	20.5	67	-	1920	[126]
	Chlamydomonas reinhardtii	Double chamber	Graphite electrodes	Graphite electrodes	12.947	-	0.215	24	[114]

	transformation F5								
	Chlorella vulgaris	Double chamber	Graphite brushes	Graphite brushes	13	73 ± 3	-	336	[127]
		Double chamber	Graphite plate electrodes	Graphite plate electrodes	15	18.4	-	480	[116]
		Single chamber	Graphite fiber Brush	Carbon cloth-coated Pt	980	79.6	-	144	[112]
	Dunaliella tertiolecta	Double chamber	Graphite plate electrodes	Graphite plate electrodes	5.3	16.8	-	480	[116]
Microalgae used at Cathode	*Chlorella vulgaris*	Double chamber	Glassy graphite rods	Glassy graphite rods	2.7	-	-	130	[106]
		Double chamber	Carbon felt	Carbon fiber cloth	8.79	84.8	-	400	[120]
		Single chamber + Sediment	Graphite felt	Multi-walled carbon nanotubes	38	-	-	288	[119]
		Double chamber	Toray carbon cloth	Toray carbon cloth	13.5	80	0.49	600	[128]

		Modified MFC + Tubular photobioreactor	Carbon felt	Carbon paper-coated Pt	27.5	-	-	432	[129]
		Double chamber	Carbon fiber brush	Carbon cloth	19.45	-	0.8	200	[121]
		Double chamber	Carbon fiber brush	Carbon cloth	1926 ± 21.4	90	-	480	[108]
		Double chamber	Plain graphite	Plain graphite	62.7	-	-	264	[130]
		Double chamber	Toray carbon cloth	Toray carbon cloth	23.97	75	0.43	-	[124]
	Scenedesmus obliquus	Double chamber	Plain carbon paper	Carbon fiber brush	30	-	0.52	1008	[122]
		Double chamber	Plain carbon paper	Carbon paper-coated Pt	154	-	-	192	[118]
	Desmodesmus sp. *A8*	Double chamber	Plain graphite felt	Plain graphite felt	99.09	-	-	140	[9]

Table IV-3: Energy production and COD removal by different microalgae strains

7. CONCLUSIONS AND REMARKS

The present review describes the current technologies based on microalgae used to produce bioenergy and discusses their advantages, limitations and future prospects. Microalgae appear to be a solution for many environmental problems. For example, microalgae have been seen to be a low-cost biomaterial suitable for producing several types of bioenergy sources such as biodiesel, bioethanol, methane or hydrogen. However, the processes to generate these green energies still present some limitations such as the need for pre-treatments, which increases their price and hinders scaling up, or complex oil extraction methods, which complicates the process.

On the other hand, microalgae can fix CO_2, which will help to reduce greenhouse gas emissions and can be used to treat wastewater and generate many types of high value-added products, their use in MFCs being one of the most promising applications. This allows us to produce electricity and capture CO_2 simultaneously. As previously mentioned, microalgae can be used in the anode, as substrate, or in the cathode to produce oxygen and fix carbon dioxide. The results show that much remains to be done to maximize MFC performance when microalgae are used as feedstock, since the levels of power output are still low. However, in the case of microalgae-assisted cathodes, the results are very promising. Moreover, by fixing the CO_2 produced, they can generate oxygen to be employed for the oxygen reduction reaction (ORR). In this case, energy production rate is higher and besides the algal biomass can be reused to produce other types of biofuels.

Many factors must be considered if microalgae are to be used to produce biofuels. The correct selection of the strain is crucial since, for instance, not all of them contain sufficiently high amounts of lipids or carbohydrates to generate biofuels. However, many advances have been made in microalgae genetic engineering, leading to increased lipid production rates, and some strains previously unable to produce lipids now can do so. Certain species can be used to produce different types of biofuels, but the production processes still need to be optimized. The future use of microalgae for bioenergy production will depend on advances in genetic engineering, which will allow the genes of microalgae species to produce higher yields of biofuel, increase the biomass production, reduce the growth time and simplify extraction processes.

References

[1] E. Stephens, I.L. Ross, J.H. Mussgnug, L.D. Wagner, M.A. Borowitzka, Posten, O. Kruse, B. Hankamer, Future prospects of microalgal biofuel production systems, Trends Plant Sci. 15 (2010) 554–564.

[2] United Nations, Department of Economic and Social Affairs, Population Division, World Population Prospects: The 2015 Revision, Key Findings and Advance Tables, Working Paper No. ESA/P/WP.241, 2015.

[3] N. Pragya, K.K. Pandey, P.K. Sahoo, A review on harvesting, oil extraction and biofuels production technologies from microalgae, Renew.Sust.Energ. Rev. 24 (2013) 159–171.

[4] C.A. Logan, A review of ocean acidification and America's response, Bioscience 60 (10) (2010) 819–828. [5] IPCC, Climate change 2014: synthesis report, in: CoreWriting Team, R.K. Pachauri, L.A. Meyer (Eds.), Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, IPCC, Geneva, Switzerland, 2014 (151 pp.).

[6] International Energy Agency, Key World Energy Statistics(https://www.iea.org/ publications/freepublications/publication/KeyWorld_Statistics_2015.pdf) 2015 81.

[7] Y. Chisti, Biodiesel from microalgae beats bioethanol, Trends Biotechnol. 26 (2008) 126–131.

[8] M. Zhou, H. Wang, D.J. Hassett, T. Gu, Recent advances in microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) for wastewater treatment, bioenergy and bioproducts, J. Chem. Technol. Biotechnol. 88 (2013) 508–518.

[9] D.J. Lee, J.S. Chang, J.Y. Lai, Microalgae-microbial fuel cell: a mini review, Bioresour. Technol. 198 (2015) 891–895.

[10] P. Spolaore, C. Joannis-Cassan, E. Duran, A. Isambert, Commercial applications of microalgae, J. Biosci. Bioeng. 101 (2006) 87–96.

[11] R. Harder, H. Witsch, Die Massenkultur von Diatomeen, Ber. Deut. Bot. Ges. 60 (1942) 146–152.

[12] M.A. Borowitzka, Algae for biofuels and energy, Dev. Appl. Phycol. 5 (2012) 1–15.

[13] W.C.W. Barkley, R.A. Lewin, L. Cheng, in: T. Sadler (Ed.), Development of Microalgal Systems for the Production of Liquid Fuels. Villeneuve d'Ascq, France, Elsevier Applied Science, 1987.

[14] J. Sheehan, T. Dunahay, J. Benemann, P. Roessler, A Look Back at the U.S Department of Energy's Aquatic Species Program: Biodiesel From Algae, National Renewable Energy Laboratory, USA, 1998.

[15] T.M. Mata, A.A. Martins, N.S. Caetano, Microalgae for biodiesel production and other applications: a review, Renew. Sust.Energ. Rev. 14 (2010) 217–232.

[16] M. Hannon, J. Gimpel, M. Tran, B. Rasala, S. Mayfield, Biofuels from algae: challenges and potential, Biofuels. 1 (5) (2010) 763–784.

[17] C.U. Ugwu, H. Aoyagi, H. Uchiyama, Photobioreactors for mass cultivation of algae, Bioresource Technol. 99 (10) (2008) 4021–4028.

[18] R.E. Lee, Phycology, 4th ed. Cambridge University Press, Cambridge, 2008.

[19] L. Brennan, P. Owende, Biofuels from microalgae-a review of technologies for production, processing, and extractions of biofuels and co-products, Renew. Sust.Energ. Rev. 14 (2010) 557–577.

[20] Y. Li, B.Wang, N.Wu, C.Q. Lan, Effects of nitrogen sources on cell growth and lipid production of Neochloris oleoabundans, Appl. Microbiol. Biotechnol. 81 (4) (2008) 629–636.

[21] J.R. Benemann, D.M. Tillett, J.C. Weissman, Microalgae biotechnology, Trends Biotechnol. 5 (1987) 47–53.

[22] G. Dragone, B. Fernandes, A.A. Vicente, J.A. Teixeira, Third generation biofuels from microalgae, in: A. Mendez-Vilas (Ed.), Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, Formatex, Madrid 2010, pp. 1355–1366.

[23] S.P. Singh, P. Singh, Effect of CO_2 concentration on algal growth: a review, Renew. Sust.Energ. Rev. 38 (2014) 172–179.

[24] C. Enzing, M. Ploeg, M. Barbosa, L. Sijtsma, Microalgae-based products for the food and feed sector: an outlook for Europe, JRC Scientific and Policy Reports, European commission, 2014

[25] K.R. Mandalam, B.Ø. Palsson, Elemental balancing of biomass and medium composition

enhances growth capacity in high-density Chlorella vulgaris cultures, Biotechnol. Bioeng. 59 (5) (1998) 605–611.

[26] M.A. Chia, A.T. Lombardi, M.G. MELÃO, Growth and biochemical composition of Chlorella vulgaris in different growth media, An. Acad. Bras. Cienc. 85 (4) (2013) 1427–1438.

[27] O. Pulz, Photobioreactors: production systems for phototrophic microorganisms, Appl. Microbiol. Biotechnol. 57 (2001) 287–293.

[28] A.M. Lakaniemi, V.M. Intihar, O.H. Tuovinen, J.A. Puhakka, Growth of Chlorella vulgaris and associated bacteria in photobioreactors, Microb. Biotechnol. 5 (1) (2012) 69–78.

[29] O. Perez-Garcia, F.M.E. Escalante, L.E. de-Bashan, Y. Bashan, Heterotrophic cultures of microalgae, metabolism and potential products. Water Res. 45 (2011) 11-36.

[30] D. Aitken, B. Antizar-Ladislao, Achieving a green solution: limitations and focus points for sustainable algal fuels, Energies 5 (2012) 1613–1647.

[31] J. Li, M. Stamato, E. Velliou, C. Jeffryes, S.N. Agathos, Design and characterization of a scalable airlift flat panel photobioreactor for microalgae cultivation, J. Appl. Phycol. 27 (2015) 75–86.

[32] M. Cuaresma, M. Janssen, C. Vilchez, R.H. Wijffels, Productivity of Chlorella sorokiniana in a short light-path (SLP) panel photobioreactor under high irradiance, Biotechnol.Bioeng. 104 (2) (2009) 352–359.

[33] C.U. Ugwu, J.C. Ogbonna, H. Tanaka, Improvement of mass transfer characteristics and productivities of inclined tubular photobioreactors by installation of internal static mixers, Appl. Microbiol. Biotechnol. 58 (2002) 600–607.

[34] O. Jorquera, A. Kiperstok, E.A. Sales, M. Embiruçu, M.L. Ghirardi, Comparative energy lifecycle analyses of microalgal biomass production in open ponds and photobioreactors, Bioresour. Technol. 101 (2010) 1406–1413.

[35] A. Vonshak, R. Guy, Photoadaptation, photoinhibition and productivity in the bluegreen alga, Spirulina platensis grown outdoors, Plant Cell Environ. 15 (1992) 613–616.

[36] C.C. Fu, T.C. Hung,W.T. Wu, T.C.Wen, C.H. Su, Current and voltage responses in instant photosynthetic microbial cells with Spirulina platensis, Biochem. Eng. J. 52 (2010) 175–180.

[37] Y.C. Sharma, B. Singh, J. Korstad, A critical review on recent methods used for economically

viable and eco-friendly development of microalgae as a potential feedstock for synthesis of biodiesel, Green Chem. 13 (2011) 2993–3006.

[38] S.Y. Ip, J.S. Bridger, C.T. Chin, W.R.B. Martin,W.G.C. Raper, Algal growth in primary settled sewage: the effects of five key variables,Water Res. 16 (5) (1982) 621–632.

[39] K. Larsdotter, Wastewater treatment with microalgae – a literature review, Waste Manag. Res. 62 (2006) 31–38.

[40] L. Creswell, Phytoplankton Culture for Aquaculture Feed, Southern Regional Aquaculture Center (SRAC), Department of Agriculture, US, 2010 (Pub.No. 5004).

[41] K. Chojnacka, F.J. Marquez-Rocha, Kinetic and stoichiometric relationships of the energy and carbon metabolism in the culture of microalgae, Biotechnology 3 (1) (2004) 21–34.

[42] C.Y. Chen, K.L. Yeh, R. Aisyah, D.J. Lee, J.S. Chang, Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review, Bioresour. Technol. 102 (2011) 71–81.

[43] H. Yu, S. Jia, Y. Dai, Growth characteristics of the cyanobacterium Nostoc flagelliforme in photoautotrophic, mixotrophic and heterotrophic cultivation, J. Appl. Phycol. 21 (1) (2009) 127–133.

[44] S.A. Razzak, M.M. Hossain, R.A. Lucky, A.S. Bassi, H. Lasa, Integrated CO2 capture, wastewater treatment and biofuel production by microalgae culturing-a review, Renew. Sust.Energ. Rev. 27 (2013) 622–653.

[45] A. Bahadar, M.B. Khan, Progress in energy from microalgae: a review, Renew. Sust.Energ. Rev. 27 (2013) 128–148.

[46] C.G. Zittelli, N. Biondi, L. Rodolfi,M.R. Tredici, Photobioreactors formass production of microalgae, in: A. Richmond, Q. Hu (Eds.),Handbook of Microalgal Culture: Applied Phycology and Biotechnology, Second edition 2013, pp. 225–266.

[47] A. Darzins, P. Pienkos, L. Edye, Current status and potential for algal biofuels production, A Report to IEA Bioenergy T39-T2, 2010.

[48] K. Sompech, Y. Chisti, T. Srinophakun, Design of raceway ponds for producing microalgae, Biofuels 3 (4) (2012) 387–397.

[49] [A].W.J. Oswald, C.G. Golueke, Biological transformation of solar energy, Adv. Appl. Microbiol. 11 (1960) 223–242.

[50] [B]. J.B.K. Park, R.J. Craggs, A.N. Shilton,Wastewater treatment high rate algal ponds for biofuel production, Bioresour. Technol. Biofuels-II: Algal Biofuels Microb. Fuel Cells 102 (1) (2011) 35–42.

[51] M.R. Tredici, Mass production of microalgae: photobioreactors, in: A. Richmond (Ed.), Handbook of Microalgal Culture: Biotechnology and Applied Phycology 2004, pp. 178–214.

[52] D. Chaumont, Biotechnology of algal biomass production: a review of systems for outdoor mass culture, J. Appl. Phycol. 5 (1993) 593–604.

[53] I.A.J. Ratchford, H.J. Fallowfield, Performance of a flat plate air-lift reactor for the growth of high biomass algal culture, J. Appl. Phycol. 4 (1992) 1–9.

[54] M.K. Lam, K.T. Lee, Microalgae biofuels: a critical review of issues, problems and the way forward, Biotechnol. Adv. 30 (2012) 673–690.

[55] E. Molina, J. Fernández, F.G. Acién, Y. Chisti, Tubular photobioreactor design for algal cultures, J. Biotechnol. 92 (2001) 113–131.

[56] A.P. Carvalho, L.A. Meireles, F.X. Malcata, Microalgal reactors: a review of enclosed system designs and performances, Biotechnol. Prog. 22 (2006) 1490–1506.

[57] I. Akkerman, M. Janssen, J. Rocha, R.H. Wijffels, Photobiological hydrogen production: photochemical efficiency and bioreactor design, Int. J. Hydrog. Energy 27 (2002) 1195–1208.

[58] A.S. Carlsson, J.B. van Beilen, R. Moller, D. Clayton, Micro- and Macro-algae: Utility for Industrial Applications, 1st ed. University of York, CPL Press, Newbury, 2007.

[59] K. Kumar, C.N. Dasgupta, B. Nayak, P. Lindblad, D. Das, Development of suitable photobioreactors for CO2 sequestration addressing global warming using green algae and cyanobacteria, Bioresour. Technol. 102 (2011) 4945–4953, http://dx.doi.org/10.1016/j.biortech.2011.01.054.

[60] E.H. Harris, G. Witman, D. Stern, Second ed., The Chlamydomonas Sourcebook, vol. 1-3, Academic Press, 2009.

[61] O. Kilian, C.S.E. Benemann, K.K. Niyogi, B. Vick, High-efficiency homologous recombination

in the oil-producing alga Nannochloropsis sp. Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 21265–21269.

[62] C.H. Tan, P.L. Show, J.S. Chang, T.C. Ling, G.C.W. Lan, Novel approaches of producing bioenergies from microalgae: a recent review, Biotechnol. Adv. 33 (2015) 1219–1227.

[63] S. Rajamani, S. Siripornadulsil, V. Falcao, M. Torres, P. Colepicolo, R. Sayre, in: R. Leon, A. Galván, E. Fernández (Eds.), Phycoremediation of Heavy Metals Using Transgenic Microalgae, in: Transgenic Microalgae as Green Cell Factories, Landes Bioscience and Springer Science+Business Media, LLC 2007, pp. 99–109.

[64] P.W. Behrens, Photobioreactors and fermentors: the light and dark sides of growing algae, in: R.A. Andersen (Ed.), Algal Culturing Techniques 2005, pp. 189–203.

[65] A.A. Snow, V.H. Smith, Genetically engineered algae for biofuels: a key role for ecologists, Bioscience 62 (8) (2012) 765–768.

[66] S.A. Scot, M.P. Davey, J.S. Dennis, I. Horst, C.J. Howe, D.J. Lea-Smith, A.G. Smith, Biodiesel

from algae: challenges and prospects, Curr. Opin.Biotechnol. 21 (2010) 277–286.

[67] Y. Chisti, Biodiesel from microalgae, Biotechnol. Adv. 25 (3) (2007) 294–306.

[68] M.J. Salar-García, V.M. Ortiz-Martínez, P. Olivares-Carrillo, J. Quesada-Medina, A.P. de los Ríos, F.J. Hernández-Fernández, Analysis of optimal conditions for biodiesel production from Jatropha oil in supercritical methanol: quantification of thermal decomposition degree and analysis of FAMEs, J. Supercrit. Fluids 112 (2016) 1–6.

[69] V.M. Ortiz-Martínez, M.J. Salar-García, F.J. Palacios-Nereo, P. Olivares-Carrillo, J. Quesada-Medina, A.P. de los Ríos, F.J. Hernández-Fernández, In-depth study of the transesterification reaction of Pongamia pinnata oil for biodiesel production using catalyst-free supercritical methanol process, J. Supercrit. Fluids 113 (2016) 23–30.

[70] A. Singh, S.I. Olsen, A critical review of biochemical conversion, sustainability and life cycle assessment of algal biofuels, Appl. Energy 88 (2011) 3548–3555.

[71] S. Jazzar, P. Olivares-Carrillo, A.P. de los Ríos, M.N. Marzouki, F.G. Acién-Fernández, J.M. Fernández-Sevilla, E.Molina-Grima, I. Smaali, J. Quesada-Medina, Direct supercritical methanolysis of wet and dry unwashed marine microalgae (Nannochloropsis gaditana) to biodiesel, Appl. Energy 148 (2015) 210–219.

[72] S.B. Velasquez-Orta, J.G.M. Lee, A.P. Harvey, Evaluation of FAME production from wet marine and freshwater microalgae by in situ transesterification, Biochem. Eng. J. 76 (2013) 83–89.

[73] R.P. John, G.S. Anisha, K.M. Nampoothiri, A. Pandey,Micro andmacroalgal biomass: a renewable source for bioethanol, Bioresour. Technol. 102 (1) (2011) 186–193.

[74] F.M. Demirbas, Biofuels from algae for sustainable development, Appl. Energy 88 (2011) 3473–3480.

[75] A. Hirano, R. Ueda, S. Hirayama, Y. Ogushi, CO2 fixation and ethanol production with microalgal photosynthesis and intracellular anaerobic fermentation, Energy 22 (1997) 137–142.

[76] R. Harun, M.K. Danquah, G.M. Forde, Microalgal biomass as a fermentation feedstock for bioethanol production, J. Chem. Technol. Biotechnol. 85 (2010) 199–203.

[77] B.E. Barton, M.T. Olsen, T.B. Rauchfuss, Artificial hydrogenases, Curr. Opin.Biotechnol. 21 (2010) 292–297.

[78] C.L. Cheng, J.S. Chang, Hydrolysis of lignocellulosic feedstock by novel cellulases originating from Pseudomonas sp. CL3 for fermentative hydrogen production, Bioresour. Technol. 102 (2011) 8628–8634.

[79] J.R. Benemann, Hydrogen production by microalgae, J. Appl. Phycol. 12 (2000) 291–300.

[80] A. Melis, L. Zhang,M. Forestier,M.L. Ghirardi, M. Seibert, Sustained photobiological hydrogen gas production upon reversible inactivation of oxygen evolution in the green alga Chlamydomonas reinhardtii, Plant Physiol. 122 (2000) 127–135.

[81] P.C. Hallenbeck, Microbial paths to renewable hydrogen production, Biofuels 2 (3) (2011) 285–302.

[82] W. Klinthong, Y.H. Yang, C.H. Huang, C.S. Tan, A review:microalgae and their applications in CO2 capture and renewable energy, Aerosol Air Qual. Res. 15 (2015) 712–742.

[83] R.C. Prince, H.S. Kheshgi, The photobiological production of hydrogen: potential efficiency and effectiveness as a renewable fuel, Crit. Rev. Microbiol. 31 (2005) 19–31.

[84] Y. Li, M. Horsman, N. Wu, C.Q. Lan, N. Dubois-Calero, Biofuels from microalgae, Biotechnol. Prog. 24 (4) (2008) 815–820.

[85] M.Waligórska, Fermentative hydrogen production-process design and bioreactors, Chem. Process. Eng. 33 (4) (2012) 585–594.

[86] O. Kruse, B. Hankamer,Microalgal hydrogen production, Curr. Opin.Biotechnol. 21 (2010) 238–243.

[87] Q. Hu, M. Sommerfeld, E. Jarvis, M. Ghirardi, M. Posewite, M. Seibert, A. Darzins, Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances, Plant J. 54 (2008) 621–639.

[88] C. Gonzalez-Fernandez, B. Sialve, B. Molinuevo-Salces, Anaerobic digestion of microalgal biomass: challenges, opportunities and research needs, Bioresour. Technol. 198 (2015) 896–906.

[89] S. He, X. Fan, N.R. Katukuri, X. Yuan, F.Wang, R.B. Guo, Enhanced methane production from microalgal biomass by anaerobic bio-pretreatment, Bioresour. Technol. 204 (2016) 145–151..

[90] R. Harun, M. Singh, G.M. Forde, K.M. Danquah, Bioprocess engineering of microalgae to produce a variety of consumer products, Renew. Sust.Energ. Rev. 14 (2010) 1037–1047.

[91] J.H. Mussgnug, V. Klassen, A. Schlüter, O. Kruse, Microalgae as substrates for fermentative biogas production in a combined biorefinery concept, J. Biotechnol. 150 (2010) 51–56.

[92] P. Morand, X. Briand, Anaerobic digestion of Ulva sp. 2. Study of Ulva degradation and methanisation of liquefaction juices, J. Appl. Phycol. 11 (1999) 165–177.

[93] K.T. Bird, D.P. Chynoweth, D.E. Jerger, Effects of marine algal proximate composition on methane yields, J. Appl. Phycol. 2 (1990) 207–213.

[94] F. Lü, J. Ji, L. Shao, P. He, Bacterial bioaugmentation for improving methane and hydrogen production from microalgae, Biotechnol.Biofuels 6 (2013) 92.

[95] D.P. Chynoweth, Renewable biomethane fromland and ocean energy crops and organic wastes, HortSci. 40 (2) (2005) 283–286.

[96] B. Tartakovsky, F.M. Lebrun, S.R. Guiot, High-rate biomethane production from microalgal biomass in a UASB reactor, Algal Res. 7 (2015) 86–91.

[97] F.J. Hernández-Fernández, A.P. de los Ríos, M.J. Salar-García, V.M. Ortiz-Martínez, L.J. Lozano-Blanco, C. Godínez, F. Tomás-Alonso, J. Quesada-Medina, Recent progress and perspectives in microbial fuel cells for bioenergy generation and wastewater treatment, Fuel Process. Technol. 138 (2015) 284–297.

[98] B.E. Logan, Exoelectrogenic bacteria that power microbial fuel cells, Nat. Rev. Microbiol. 7 (2009) 375–381.

[99] B.E. Logan, B. Hamelers, R. Rozendal, U. Schroder, J. Keller, S. Freguia, P. Aelterman, W. Verstraete, K. Rabaey, Microbial fuel cells: methodology and technology, Environ. Sci. Technol. 40 (17) (2006).

[100] Z. Du, H. Li, T. Gu, A state of the art reviewonmicrobial fuel cells: a promising technology for wastewater treatment and bioenergy, Biotechnol. Adv. 25 (2007) 464–482.

[101] M.C. Potter, Electrical effects accompanying the decomposition of organic compounds, Proc. R. Soc. Lond. Ser. B 84 (1911) 260–276.

[102] H.P. Bennetto, Electricity generation by microorganisms, Biotechnol. Educ. 1 (4) (1990) 163–168.

[103] R.M. Allen, H.P. Bennetto,Microbial fuel cells: electricity production from carbohydrates, Appl. Biochem. Biotechnol. 39 (2) (1993) 27–40.

[104] V.M. Ortiz-Martínez, M.J. Salar-García, F.J. Hernández-Fernández, A.P. de los Ríos, Development and characterization of a new embedded ionic liquid based membrane- cathode assembly for its application in single chamber microbial fuel cells, Energy 93 (2015) 1748–1757.

[105] G. Gnana Kumar, V.G. Sathiya Sarathi, K. Suk Nahm, Recent advances and challenges in the anode architecture and their modifications for the applications ofmicrobial fuel cells, Biosens. Bioelectron. 43 (2013) 461–475.

[106] X.A. Walter, J. Greenman, B. Taylor, I. Ieropoulos, Microbial fuel cells continuously fuelled by untreated fresh algal biomass, Algal Res. 11 (2015) 103–107.

[107] M.J. Salar-García, I. Gajda, V.M. Ortiz-Martínez, J. Greenman, M.M. Hanczyc, A.P. de los Ríos, I. Ieropoulos, Microalgae as substrate in low cost terracotta-based microbial fuel cells: novel application of the catholyte produced, Bioresour. Technol. 209 (2016) 380–385.

[108] I. Gajda, J. Greenman, C.Melhuish, I. Ieropoulos, Self-sustainable electricity production fromalgae grown in amicrobial fuel cell system, Biomass Bioenergy 82 (2015) 87–93.

[109] I. Ieropoulos, J. Greenman, M. Sauer, Microbial fuel cells and algae, ECS Trans. 28 (9) (2010) 23–30.

[110] S. Kondaveeti, K.S. Choi, R. Kakarla, B. Min, Microalgae Scenedesmus obliquus as renewable biomass feedstock for electricity generation inmicrobial fuel cells (MFCs), Front. Environ. Sci. Eng. 8 (5) (2014) 784–791.

[111] C. Xu, K. Poon, M.M.F. Choi, R. Wang, Using live algae at the anode of a microbial fuel cell to generate electricity, Environ. Sci. Pollut. Res. Int. 22 (20) (2015) 15621–15635.

[112] C.C. Lin, C.H.Wei, C.I. Chen, C.J. Shieh, Y.C. Liu, Characteristics of the photosynthesis microbial fuel cell with a Spirulina platensis biofilm, Bioresour. Technol. 135 (2013) 640–643.

[113] K. Nishio, K. Hashimoto, K.Watanabe, Digestion of algal biomass for electricity generation in microbial fuel cells, Biosci.Biotechnol.Biochem. 77 (3) (2013) 670–672.

[114] A.E. Inglesby, A.C. Fisher, Downstream application of amicrobial fuel cell for energy recovery from an Arthrospira maxima fed anaerobic digester effluent, RSC Adv. 3 (2013) 17387–17394.

[115] J.C.W. Lan, K. Raman, C.M. Huang, C.M. Chang, The impact of monochromatic blue and red LED light upon performance of photo microbial fuel cells (PMFCs) using Chlamydomonas reinhardtii transformation F5 as biocatalyst, Biochem. Eng. J. 78 (2013) 39–43.

[116] H. Wang, D. Liu, L. Lu, Z. Zhao, Y. Xu, F. Cui, Degradation of algal organic matter using microbial fuel cells and its association with trihalomethane precursor removal, Bioresour. Technol. 116 (2012) 80–85.

[117] A.M. Lakaniemi, O.H. Tuovinen, J.A. Puhakka, Production of electricity and butanol from microalgal biomass in microbial fuel cells, Bioenergy Res. 5 (2012) 481–491.

[118] S.B. Velasquez-Orta, T.P. Curtis, B.E. Logan, Energy from algae using microbial fuel cells, Biotechnol.Bioeng. 103 (2009) 1068–1076.

[119] E.E. Powell,M.L.Mapiour, R.W. Evitts, G.A. Hill, Growth kinetics of Chlorella vulgaris and its use as a cathodic half-cell, Bioresour. Technol. 100 (2009) 269–274.

[120] M. Zhou, H. He, T. Jin, H.Wang, Power generation enhancement in novel microbial carbon capture cells with immobilized Chlorella vulgaris, J. Power Sources 214 (2012) 216–219.

[121] D.B. Wang, T.S. Song, T. Guo, Q. Zeng, J. Xie, Electricity generation from sediment microbial fuel cells with algae-assisted cathodes, Int. J. Hydrog. Energy 39 (2014) 13224–13230.

[122] A. González del Campo, P. Cañizares, M.A. Rodrigo, F.J. Fernández, J. Lobato, Microbial fuel cell with an algae-assisted cathode: a preliminary assessment, J. Power Sources 242 (2013) 638–645.

[123] X.Y.Wu, T.S. Song, X.J. Zhu, P.Wei, C.C. Zhou, Construction and operation ofmicrobial fuel cell with Chlorella vulgaris biocathode for electricity generation, Appl. Biochem. Biotechnol. 171 (2013) 2082–2092.

[124] X. Wang, Y. Feng, J. Liu, H. Lee, C. Li, N. Li, N. Ren, Sequestration of CO2 discharged from anode by algal cathode in microbial carbon capture cells (MCCs), Biosens. Bioelectron. 25 (2010) 2639–2643.

[125] Y. Cui, N. Rashid, N. Hu, M.S.U. Rehman, J.I. Han, Electricity generation and microalgae cultivation in microbial fuel cell using microalgae-enriched anode and bio-cathode, Energy Convers. Manag. 79 (2014) 674–680.

[126] L. Gouveia, C. Neves, D. Sebastião, B.P. Nobre, C.T. Matos, Effect of light on the production of bioelectricity and added-value microalgae biomass in a Photosynthetic Alga Microbial Fuel Cell, Bioresour. Technol. 154 (2014) 171–177.

[127] A. González del Campo, J.F. Perez, P. Cañizares, M.A. Rodrigo, F.J. Fernández, J. Lobato, Study of a photosynthetic MFC for energy recovery from synthetic industrial fruit juice wastewater, Int. J. Hydrog. Energy 39 (2014) 21828–21836.

[128] R. Kakarla, B. Min, Evaluation of microbial fuel cell operation using algae as an oxygen supplier: carbon paper cathode vs. carbon brush cathode, Bioprocess Biosyst. Eng. 37 (12) (2014) 2453–2461.

[129] R. Kakarla, B. Min, Photoautotrophic microalgae Scenedesmus obliquus attached on a cathode as oxygen producers for microbial fuel cell (MFC) operation, Int. J. Hydrog. Energy 39 (2014) 10275–10283.

[130] A. ElMekawy, H.M. Hegab, K. Vanbroekhoven, D. Pant, Techno-productive potential of photosyn-thetic microbial fuel cells through different configurations, Renew. Sust.Energ. Rev. 39 (2014) 617–627.

[131] A.M. Lakaniemi, O.H. Tuovinen, J.A. Puhakka, Anaerobic conversion of microalgal biomass to sustainable energy carriers-a review, Bioresour.Technol. 135 (2013) 222–231.

[132] S. Mateo, A. Gonzalez del Campo, P. Cañizares, J. Lobato, M.A. Rodrigo, F.J. Fernandez, Bioelectricity generation in a self-sustainable Microbial Solar Cell, Bioresour. Technol. 159 (2014) 451–454.

[133] C.C. Fu, C.H. Su, T.C. Hung, C.H. Hsieh, D. Suryani, W.T. Wu, Effects of biomass weight and light intensity on the performance of photosynthetic microbial fuel cells with Spirulina platensis, Bioresour. Technol. 100 (2009) 4183–4186.

[134] L. Xiao, Z. He, Applications and perspectives of phototrophic microorganisms for electricity generation from organic compounds in microbial fuel cells, Renew. Sust.Energ. Rev. 37 (2014) 550–559.

[135] I. Gajda, J. Greenman, C.Melhuish, I. Ieropoulos, Photosynthetic cathodes forMicrobial Fuel Cells, Int. J. Hydrog. Energy 38 (2013) 11559–11564.

[136] E.E. Powell, R.W. Evitts, G.A. Hill, J.C. Bolster, A microbial fuel cell with a photosynthetic microalgae cathodic half cell coupled to a yeast anodic half cell, Energy Sources Part A Recover. Util. Environ. Effects 33 (2011) 440–448.

[137] I. Gajda, A. Stinchcombe, J. Greenman, C. Melhuish, I. Ieropoulos, Algal 'lagoon' effect for oxygenating MFC cathodes, Int. J. Hydrog. Energ. 39 (2014) 21857–21863.

[138] Y.C. Wu, Z.J. Wang, Y. Zheng, Y. Xiao, Z.H. Yang, F. Zhao, Light intensity affects the performance of photo microbial fuel cells with Desmodesmus sp. A8 as cathodic microorganism, Appl. Energy 116 (2014) 86–90.

[139] L. de Schamphelaire,W. Verstraete, Revival of the biological sunlight-to-biogas energy conversion system, Biotechnol. Bioeng. 103 (2009) 296–304.

[140] E.E. Powell, G.A. Hill, Economic assessment of an integrated bioethanol-biodieselmicrobial fuel cell facility utilizing yeast and photosynthetic algae, Chem. Eng. Res. Des. 87 (2009) 1340–1348.

Chapter V: General Conclusions and Recommendation for Future Research

6.1. General Conclusions

The environmental and health challenges posed by the population growth, water stress and high energy demand has considerably increased the interest of researcher in developing effective, efficient, economical and environmentally compatible technologies for bioenergy production, and wastewater treatment.

The goal of the present thesis was to design a microbial fuel cells (MFCs) working in a countinous flow that allow effluents to be treated while simultaneously generating electricity, offering a two-fold benefit.

In order to facilitate the commercialization of this technology, reducing the overall cost of the devices as well as improving the energy harvesting was one of the challenges of this thesis. Thus far, Ion-Liquid-based membranes has shown promising results in gas separation and metal extraction. The use of IL-based membranes addresses both challenges by replacing the expensive and sometimes low-efficiency commercial membranes.

MFCs technology was combined with algae as the oxygen supplier. In this case, bacteria degrade the organic matter in the anodic chamber while algae grow in the cathodic compartment, providing the oxygen necessary for completing the redox reaction on the cathode. This novel approach improves the power performance and the wastewater treatment efficiency of MFCs.

Moreover, the transport of specific compounds from the anode to the cathode might also help algae growth, reducing the nutrients added to the cathode chamber. The selective transport of nutrients through the membrane allows us to design a more efficient system for both bioenergy production and wastewater treatment. The mainoutcomesofthisworkarepresentedbelow:

The first chapter is a recent bibliographyto the subject ofelectricity production, and wastewater treatment. It explains the background information needed to understand the chemistry behind microbial fuel cells as new sources of energy with smaller carbon footprints as the subject of interest.

Principle of MFC Electricity Generation and their capacity of Wastewater treatment and COD removalare discussed.

The basis of this first chapter is to provide a general background to separation processes, components and material, reactor design investigating the double chamber MFC and single-

chamber MFC, a more detailed introduction was given at the beginning of each individual chapter, concerning the purpose of the research that has been conducted there.

The second part was dedicated to the operational Factors Affecting MFC Performance, electron Transfer Mechanisms; investigation of IL based membrane in transport, Practical Applications and Scale-up, and finally resuming the opportunities and Challenges for MFCs in Wastewater Treatment.

The last part entails the study of Microlagae, including its definition, ability to generate bioenergy, and treat wastewater, Microlagae for MFCs, and capacity of organic matter removal was also investigated in detail.

In the second chapter, Ionic-liquid based PIMs were tested asseparator in a new horizontal and vertical configurations of MFCs fed with wastewater.

It has been concluded that the effectiveness of treatment is directly dependent to the HRT, pH, T° operated in continuous mode, and the highest performance of wastewater treatment was reached for the flow of 0.25 $ml.min^{-1}$.

For horizontal configuration of MFCs, COD value was found to be higher than the initial value after one day of treatment, which proves that an important part of the membrane was dissolved in the wastewater. This membrane degradationis mainly attributed to the small distance between the tube containing carbon granules and carbon graphite bar, in that case, carbon is precipitated in the bottom of reactor result of gravity in direct contact with the membrane.Wastewater is then charged by carbon particles due to its contact with carbon granules and PIM.Althoughthe membrane was degraded partially, the percentage of many pollutants and heavy metals have decrease considerably after only 48 h of treatment.

In contrary to the horizontal reactor, the vertical one has performed in good conditions in different conditions.

the efficiency of wastewater treatment through the MFC system, the effluent from the reactor was examined with regard to, COD (Chemical Oxygen Demand), total P (Phosphorus), TSS (Total Suspended Solids), SO_4^{2-} (sulfate), Cl^- (Chloride), NO_3^- (Nitrate), HPO_4^{2-} (Hydrogen phosphate), Fe^{2+} (Iron) and pH, Total suspended solids, hardness, alkalinity and ion analysis.The following parameters were examined for both flows (0.25 $ml.min^{-1}$and 0.35 $ml.min^{-1}$).

It has been concluded that the wastewater treatment reached the highest performance for the flow of 0.25 $ml.min^{-1}$

The aim of third chapter is to analyze the transport of nutrients such as $CaCl_2$ and Na_2HPO_4 through polymer inclusion membranes based on ammonium-based IL. To the best of the authors' knowledge, the existing literature focuses on this research field is limited or non-existent. The results show that polymer inclusion membranes containing $[MTOA^+][Cl^-]$ are stable towards an aqueous solution of the studied nutrients. In the case of $CaCl_2$, it showed the least permeation due to its low solubility in the ionic liquid $[MTOA^+][Cl^-]$. By contrast, Na_2HPO_4 was more permeable and its permeation increased as the amount of IL in the membrane also increased. The results reported in this work could be applied to different fields such as separation and purification of salt mixtures. This study would allow the efficient design of a two-chamber microbial fuel cell, which involves IL-based membrane technology and algae. The permeation values of different nutrients would also allow us to decide whether the nutrients should be added in the same chamber that microalgae or in the opposite chamber. Furthermore, the results obtained open new fields where polymer ionic liquid membrane might be applied.

The fourth chapter describes the current technologies based on microalgae used to produce bioenergy and discusses their advantages, limitations and future prospects. Microalgae appear to be a solution for many environmental problems. For example, microalgae have been seen to be a low-cost biomaterial suitable for producing several types of bioenergy sources such as biodiesel, bioethanol, methane or hydrogen. However, the processes to generate these green energies still present some limitations such as the need for pre-treatments, which increases their price and hinders scaling up, or complex oil extraction methods, which complicates the process.

On the other hand, microalgae can fix CO_2, which will help to reduce greenhouse gas emissions and can be used to treat wastewater and generate many types of high value-added products, their use in MFCs being one of the most promising applications. This allows us to produce electricity and capture CO_2 simultaneously. As previously mentioned, microalgae can be used in the anode, as substrate, or in the cathode to produce oxygen and fix carbon dioxide. The results show that much remains to be done to maximize MFC performance when microalgae are used as feedstock, since the levels of power output are still low. However, in the case of microalgae-assisted cathodes, the results are very promising. Moreover, by fixing the CO_2 produced, they

can generate oxygen to be employed for the oxygen reduction reaction (ORR). In this case, energy production rate is higher and besides the algal biomass can be reused to produce other types of biofuels.

Many factors must be considered if microalgae are to be used to produce biofuels. The correct selection of the strain is crucial since, for instance, not all of them contain sufficiently high amounts of lipids or carbohydrates to generate biofuels. However, many advances have been made in microalgae genetic engineering, leading to increased lipid production rates, and some strains previously unable to produce lipids now can do so. Certain species can be used to produce different types of biofuels, but the production processes still need to be optimized. The future use of microalgae for bioenergy production will depend on advances in genetic engineering, which will allow the genes of microalgae species to produce higher yields of biofuel, increase the biomass production, reduce the growth time and simplify extraction processes.

6.2. Recommendations and future direction

Based on the results obtained, the following recommendations are made in order to continue with this line of research:

- In order to be able to use MFC for the treatment of wastewater with a high organic load, it would be necessary to investigate another way to increase the energy production and wastewater treatment performance in the up-flow reactor. To this end, it is proposed to control the pH of the anodic compartment by using buffer substances. Another approach for the treatment of wastewater with high COD concentration could be to perform the start-up and adaptation of the micro-organisms by using wastewater with higher COD concentration values.
- Investigate the treatment of other types of wastewater from the oil industry and the agri-food sector, where large volumes of wastewater are generated, which is essential and increases plant costs.
- Increase the surface/volume ratio of the microbial fuel cell reactor in order to promote the growth of microorganisms by forming a biofilm on the anode electrode. In this way, energy losses would be reduced since the electron transfer mechanism in the biofilm is direct, while the electron transfer mechanism of the microorganisms in suspension is by means of mediators, which means high energy losses.
- Investigate of new microalgae stains present in the Mediterranean (Moroccan side) in MFCs in both cathode and anode chambers
- Study the membrane degradation in MFCs, and design new types of stable membranes to be employed in MFCs.
- Design and build a Microalgae Microbial Fuel Cell pilot plant in order to study electricity production and wastewater treatment on a larger scale. Based on these results, a study of the profitability and economic viability of this system can be carried out.

List of publications

This thesis is based on the following papers, which are referred throughout the text by their Roman numerals:

I. **Z. Baicha**, M.J. Salar-García, V.M. Ortiz-Martínez, F.J. Hernández-Fernández, A.P. de los Ríos, D.P. Maqueda Marín , J.A. Collado, F. Tomás-Alonso and M. El Mahi. On the Selective Transport of Nutrients through Polymer Inclusion Membranes Based on Ionic Liquids. Processes **2019**, 7, 544; doi:10.3390/pr7080544

II. **Z. Baicha**, M.J. Salar-García, V.M. Ortiz-Martínez, F.J. Hernández- Fernández, A.P. de los Ríos, N. Labjar, E. Lotfi, M. Elmahi. A critical review on microalgae as an alternative source for bioenergy production: A promising lowcost substrate for microbial fuel cells. Fuel Processing Technology 2016;154: 104-116.

III. M.J. Salar-García, V.M. Ortiz-Martínez, **Z. Baicha**, A.P. de los Ríos, F.J. Hernández-Fernández. Scaled-up continuous up- flow microbial fuel cell based on novel embedded ionic liquid-type membrane-cathode assembly. Energy 2016; 101: 113-20.

Participation in Conferences

I. M.J. Salar-García, V.M. Ortiz-Martínez, **Z. Baicha**, A.P. de los Ríos, F.J. Hernández Fernández. Novel embedded ionic liquid type membrane-cathode assembly for a continuous up-flow microbial fuel cell. SOC – 77091 presented at the 10 the World Congress of Chemical Engineering, Barcelona, Spain, October 1 st -5 th 2017.

II. M.J. Salar-García, V.M. Ortiz-Martínez, **Z. Baicha**, A.P. de los Ríos, F.J. Hernández Fernández, N. Labjar, E. Lotfi, M. Elmahi. Microalgae as alternative source for bioenergy production. SOC – 77091 presented at the 10 the World Congress of Chemical Engineering, Barcelona, Spain, October 1 st -5 th 2017.

III. **Z. Baicha**, N. Labjar, E. Lotfi, M. Elmahi. Waste to energy: Comparative study of concrete digesters and prefabricated digesters. Presented at the 5th International symposium on environment and sustainable development: Climate Change: Causes, Impacts, Mitigation and Adaptation; Rabat, Morocco, October 10th-15th 2016.

IV. **Z. Baicha**, N. Labjar, E. Lotfi, M. Elmahi. Traitement des eaux usées industrielle et production d'énergie par les piles à combustibles microbienne: Intégration d'une nouvelle membrane basé sur les liquides ionique. Presented at les Journées Scientifiques Maghrébines: La Réutilisation des Eaux Usées Traitées dans les Pays du Maghreb (Algérie, Maroc et Tunisie), Hammamet, Tunisia, September 27th-30th 2016.

V. **Z. Baicha**, M.J. Salar-García, V.M. Ortiz-Martínez, F.J. Hernández- Fernández, L. Latrach, N. Labjar, E. Lotfi, M. Elmahi. A single chamber microbial fuel cell for energy production and wastewater treatment using a new membrane. Presented at the International Conference on Water, Energy & Climate change WECC‟2016, Cadi Ayyad University, Marrakesh, Morocco, June 01st-04th 2016.

VI. **Z. Baicha**, M.J. Salar-García, V.M. Ortiz-Martínez, F.J. Hernández- Fernández, L. Latrach, N. Labjar, E. Lotfi, M. Elmahi. Energy production by continuous microbial fuel cell using a new membrane based on ionic liquid. Presented at the International Conference on Water, Energy & Climate change WECC‟2016, Cadi Ayyad University, Marrakesh, Morocco, June 01st-04th 2016.

VII. VM. Ortiz-Martínez, MJ. Salar-García, **Z. Baicha**, A. Pérez de los Ríos, FJ. Hernández Fernández. Membranas poliméricas de inclusión basadas en líquidos iónicos: Aplicación a una pila de combustible microbiana que opera en continuo. Presented at the XXXV Biennial Meeting of the Spanish Royal Chemistry Society, Coruña, Spain, July 19th – 23rd 2015.

VIII. **Z. Baicha**, K. Touati, M.J. Salar-García, V.M. Ortiz-Martínez, F.J. Hernández-Fernández, E. Lotfi, N. Labjar, M. Elmahi. Mechanism of the microbial oxidation and energy production in the microbial fuel cell. Presented at the 1st International Symposium of Applied Biology «CIBA 2015», University of Science and Technology - Mohamed Boudiaf, Oran, Algeria, November 29th - December 1st, 2015.

IX. K. Touati, A. P. de los Ríos, F.J. Hernández- Fernández, L. J. L. Blanco, C. Godínez, C. Mekadim, **Z. Baicha**. Pile à combustible microbienne : un nouveau concept biotechnologique pour la dépollution. Presented at the 1st International Symposium of Applied Biology «CIBA 2015», University of Science and Technology - Mohamed Boudiaf, Oran- Algeria, November 29th - December 1st, 2015.