## PROJECTE O TESINA D'ESPECIALITAT

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<td>Autor/a</td>
<td>Albert Latorre Lladonosa</td>
</tr>
<tr>
<td>Tutor/a</td>
<td>Ignacio Carol Vilarasau – Zhiyong &quot;Jason&quot; Ren</td>
</tr>
<tr>
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<td>Enginyeria del Terreny, Cartogràfica i Geofísica – Environmental and Sustainability Engineering</td>
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NOVEL REACTOR DESIGN AS AN INTEGRAL
SOLUTION:
MICROBIAL FUEL CELL AND WASTEWATER
TREATMENT SYSTEM
by
ALBERT LATORRE LLADONOSA
Department of Environmental and Sustainability Engineering
University of Colorado at Boulder
USA
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Microbial Fuel Cells (MFCs) are reactors engineered to convert organic and inorganic matter to electricity. There exists a wide variety of substrates that can be consumed by these systems, ranging from acetate, lactate or glucose until domestic or industrial wastewater. During the last decade, MFCs powered by wastewater have specially attracted the attention of scientists and engineers because of its potential to treat wastewater and generate power at the same time. Thus, the theoretical application of this technology in replacement of current sewage treatment plants would mean not only huge monetary and energy savings, but also a sustainable process of net energy recovery. Although important technology advances have been achieved so far, reactor performances still have to be significantly improved and the cost of the materials and methods used for reactor configurations are also to be reduced in order to make its application feasible.

In this study, a new MFC reactor design is presented with the peculiarity of its novel working principle and its enhanced wastewater treatment potential. The system is first tested using synthetic wastewater (acetate) and later, under theoretical real conditions using brewery industry wastewater. In the first case, over 90% chemical oxygen demand (COD) reduction is reached after two days of treatment and a maximum power density of 11 W/m³ is extracted at a current density of 40 A/m³, although it is to say that results could have been much better if some faults had been avoided in time. In the second case, COD removal is around 90% after two days of operation. Minerals’ concentrations reduction is relevant, as well, eliminating phosphate and ammonia traces respectively up to 90% and 100% after 4-5 days of treatment. However, a nitrification process is observed, increasing nitrate concentration up to around 10 mg/l after the same treatment length.
The maximum power density extracted is of 4.5 W/m$^3$ at a current density of 16.5 A/m$^3$.

RESUM

Les cel·les de combustible microbià (MFCs) són reactors dissenyats per convertir материя orgànica i inorgànica en electricitat. Existeix una àmplia varietat de substrats que poden ser consumits per aquests sistemes, desde acetat, lactat o glucosa fins a aigües residuals domèstiques o industrials. Durant l’última dècada, les MFCs alimentades a base d’aigües residuals han atret especialment l’atenció de científics i enginyers, degut al seu potencial per a la depuració d’aigües residuals i la generació d’energia a la vegada. Doncs, l’aplicació d’aquesta tecnologia en substitució a les plantes de tractament d’aigües residuals actuals significaria no solament un gran estalvi econòmic i energètic, sinó que també un procés sostenible de recuperació d’energia neta. Encara que força avanços tecnològics s’han aconseguit fins al moment, el rendiment d’aquest tipus de reactors encara ha de millorar de manera significativa i el cost dels materials i mètodes utilitzats per a la seva configuració també ha de ser reduït, per a què la seva aplicació es consideri viable.

En aquest estudi, es presenta un nou disseny de cel·la de combustible microbià amb la peculiaritat de tenir un "modus operandi" novedós i un gran potencial per tractar aigües d’origen residual. El sistema es posa a prova primer utilitzant aigua residual sintètica (acetat) i posteriorment, en condicions reals utilitzant aigües residuals procedents d’una indústria ceresera. En el primer cas, s’aconsegueix una reducció de la demanda química d’oxigen (DQO) superior al 90% després de dos dies de tractament i una densitat de potència màxima de 11 W/m$^3$ es obtinguda a una densitat de corrent de 40 A/m$^3$, encara que val a dir que els resultats podrien haver estat molt millor si alguns errors haguéssin estat evitats a temps. En el segon cas, l’eliminació de DQO és al voltant del 90% després de dos dies d’operació. A més, la reducció de concentracions de minerals és rellevant, eliminant traces de fosfat i amoni, respectivament fins al 90% i 100% després de 4-5 dies de tractament. No obstant, també s’observa un procés de nitrificació on la concentració de nitrat augmenta fins al voltant de 10 mg/l després de la mateixa duració de tractament. La màxima densitat de potència extreta és de 4.5 W/m$^3$ a una densitat de corrent de 16.5 A/m$^3$. 
The present work is dedicated to my family: Júlia, Ramona and Ramon.
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Chapter 1

Introduction

1.1 Motivation

The earth represents a whole ecosystem with its own animal and vegetal species, its atmosphere and climate conditions and a great variety of resources. During the history, this global ecosystem has not remained the same, but it has fluctuated in terms of biodiversity, population share of the different species and so, extinction and development of new species. Following the principle of natural selection, popularized by Charles Darwin, living organisms that we find in today’s environment are the result of their recurrent survival to the different threats they have been exposed to, which can emerge from a wide range of distinct nature, such as natural disasters, changes in climate and atmosphere conditions, species’ unbalance, habitat fragmentation, degradation or destruction, over-hunting, invasion of non-native species, pollution and lack of resources, among many others.

Over the last 50 years, the world’s human being population has risen up to 7 billion, twice as many people as just before this period and over six times the human population at the beginning of the industrial revolution, which was a determining factor in this aspect. Furthermore, it has been basically due to the improvement in food production and distribution, the progress in human health and the conquest of disease. This growth has gone by the hand with a huge resources consumption striving for the satisfaction of human needs, often without taking into account the effects that it can have over the environment and other organisms and ecosystems.

In this direction, human beings have become not only a relevant threat for many living
species, but probably also their own threat, in a world running out of their indispensable but non-renewable resources, such as oil, natural gas, coal and fresh water, and with an atmosphere affected by the human activity, as the climate change claims.

Unfortunately, it was not till the early 70’s that some scientists started to become aware of the impact their actions had over the nature and natural systems, leading to a loss of biodiversity. In 1987 the term “sustainable development” was first used by the Brundtland Commission in the report Our Common Future, and as of this moment the concept, often broken out into environmental, social and economic sustainability, gained every day more and more understanding, importance and support until today.

Nowadays we are conscious that the world’s population will keep on growing exponentially and the developing countries will be willing to have the same comfort and quality of life as the already developed ones. This situation will probably lead to conflicts and a competition for energy
and resources among different countries because, for sure, we live in a finite earth and resources will come to an end, unless we start changing our route for a sustainable one before it is too late.

That is why, during the next years, engineering and scientific communities are to exert themselves to increase processes’ efficiency, make them less energy consuming, empower recycling and the processing of our waste, boost renewable energies and try to find new sustainable, renewable and feasible worth energy sources.

As the need for water is expected to rise along with population growth, urbanization and consequently, also household and industrial uses, an outstanding management of water will become a must so that it can be distributed and delivered in proper conditions. For this reason and considering also the consequences of the climate change and the every time more frequent droughts, the treatment of used and wastewater, and its recycling, will be essential in the upcoming future.

1.2 Current situation

Water and specially fresh water is a scarce good that is vital for living organisms. Humans though, need it not only as drinking water but they also use it for cooking, personal hygiene, crop watering in agriculture, cattle watering in stockbreeding, for a great variety of production processes in industry, energy production and well-being, as well.

Many regions in the world suffer a relevant lack of water, being maybe more affected those in developing countries and outlining the African continent, where over 345 million people do not have available safe water. Nevertheless, even some places in developed countries cannot get away and occasionally go through serious water problems, mainly due to droughts in these cases.

Despite much water treatment experience has been collected during centuries, there is still a lot to be learnt, processes and efficiencies to be improved, chemical interactions and microbiology to be studied and understood, and new feasible systems to be discovered.

In fact, current wastewater treatment requires nowadays enormous amounts of energy for its operation, ranging from 100 to 16000 kWh per million gallons [12] and represents 3-5% of a developed-country total electricity consumption [66], and so it results in huge costs for the admin-
istration. Moreover, the global cost of energy is expected to rise due to depletion of carbon-based resources and as a consequence, within the next decades energy price will reach such an amount that will probably not be affordable by plenty of industrial activities, or even household budgets.

When considering efficiency, it is also referred to the ratio of the useful and expected output to the total input in a system and this, applied to waste water treatment, is translated to the output water quality obtained, considering the thresholds established by the respective administrations, and compared to the raw and fresh non-treated wastewater. In this aspect, it is to mention that the water remediation from micro-pollutants released by chemical industries, domestic personal care products, fertilizers, pesticides, herbicides, stockbreeding farms and pharmaceutical industries, among many others, it is getting more difficult because of its every time more common and higher presence. Unfortunately, contents and sorts of trace organic pollutants in drinking water are increasing, which will threaten its safety. Thus, in the next years wastewater treatment processes will have to be more energy and water-quality efficient.

In addition, some regions in developing countries can hardly afford the energy cost of waste water treatment plants and in some cases, the problem is ignored just by doing without them, which is unacceptable and can lead to multiple diseases and health problems, as well as, a huge threat for the protection and preservation of species living in freshwater ecosystems. This is why developing countries will be ones in most need of cheap, simple and effective waste water treatment systems.

For this reason, society has to not only find alternative, sustainable and renewable sources of energy, but also improve energy efficiencies of its processes so that less energy is used to satisfy the same needs. In these directions, much should be done in wastewater treatment in order to turn it into a less energy consuming activity.

In this sense, Microbial Fuel Cells (MFCs) have potential to play an important role and during the last decade, reactors powered by wastewater have specially attracted the attention of scientists and engineers because of its potential to treat wastewater and generate power at the same time. Thus, the theoretical application of this technology in replacement of current sewage
treatment plants could mean not only huge monetary and energy savings, but also a sustainable process of net energy recovery. Although important technology advances have been achieved so far, reactor performances still have to be significantly improved and the cost of the materials and methods used for reactor configurations are also to be reduced in order to make its application feasible.

1.3 Objectives

This work has the main purpose of contributing to the research on microbial fuel cells and wastewater treatment, by the study of the conception, building and performance of a new reactor design that embraces these two bioengineering fields. Any possible deduction extracted from this work, that helped to turn this world into a more sustainable ecosystem, would be objective achieved.
Chapter 2

Background

2.1 Microbial fuel cells

Microbial fuel cells (MFCs) embrace knowledge and topics from a wide range of scientific and engineering fields, such as microbiology, electrochemistry, mechanics, fluid mechanics or materials and environmental engineering.

Microbial fuel cell research is a developing field that has evolved fast within the last decade and its relative novelty has occasionally lead to a lack of standardized terminology and analysis methods in order to test the performance of the different systems and devices. That is why it has been sometimes difficult to compare them and get solid conclusions but nowadays, after a lot of experience gained, we have a better understanding and we have the appropriate tools to carry out reliable and comparable device analysis.

2.1.1 What is an MFC?

Microbial fuel cells are engineered bio-electrochemical reactors designed to use bacteria, which creates, lives and grows in an exoelectrogenic biofilms, as the biocatalysts to transform organic and inorganic matter, substrates present in a solution, through oxidation-reduction reaction and generate electric power at the same time. Hence, chemical energy is converted to electrical energy with the aid of microorganisms.
2.1.2 How does it work?

The standardized microbial fuel cell typically comprises two chambers: an anaerobic anode chamber or anode and an aerobic cathode chamber or cathode. This chambers are separated by an ion exchange membrane (PEM), which is impermeable but allows proton migration between the two chambers. An electrode is set in each chamber, both connected by means of a circuit so that the collection, transfer and release of electrons is possible.

The oxidation reduction occurs in the anode (negative pole), where the anaerobic living bacteria can respire with the solid electrode, while conserving energy, by oxidizing organic and/or inorganic matter from the anolyte solution, and produce protons, electrons and off-gas containing mainly carbon dioxide. Next, the bacteria release these fresh electrons first to the anode electrode and then, they are transferred to the cathode electrode through the external circuit. The electron transfer to the anode electrode can be either direct or indirect, as it will be described and explained in the next pages. Meanwhile, the protons resulting from the oxidation reaction migrate to the cathode, through the solution and across the proton exchange membrane.

Once the electrons reach the cathode electrode, they are ready to take part in the reduction reaction occurring in the aerated cathode (positive pole). Thus, in aerobic conditions the, in most cases, dissolved oxygen plays the role of the terminal electron acceptor of the overall fuel cell reaction, and so it is reduced in combination with the electrons and the received protons, already present in the catholyte solution, to form water. Catalysts are often used in the cathode to facilitate the reactions.

In order to create a current through the external circuit, according to Ohm’s law:

$$ I = \frac{V}{R} $$

where \( I \) is the current through the conductor in units of amperes, \( V \) is the potential difference measured across the conductor in units of volts, and \( R \) is the resistance of the conductor in units of ohms, a resistor is needed between the positive and the negative pole.

MFC cathodes can have different configurations and catholyte fluid options [79, 38, 37]. In
figure 2.1 a general layout of a MFC is shown: Bacteria metabolizes substrate and transfer the gained electrons to the anode electrode.

Figure 2.1: The working principle of a microbial fuel cell. MED represent the redox mediators; Red ovals are the terminal electron shuttles in or on the bacterium.

2.1.3 Evolution of reactor design

The idea of producing electricity by means of a microbial cell was first conceived at the beginning of the twentieth century by M. Potter, who started working on the subject [54]. A bit later, in the early thirties Barnet Cohen connected in series a number of half microbial fuel cells, which apparently produced over 35 volts with a little current of 2 milliamps [13].

However, it was not until the sixties that deeper research was developed and clear reactor designs were achieved. In 1962 J. B. Davis and H. F. Yarbrough created a 3-chamber MFC, with anode and cathode separated by an additional buffer-zone chamber. Platinum sheets and glucose
carbon source were used as electrodes and substrate, respectively. A common dialysis membrane was used as a proton exchange membrane and with 1.2 litres of total anolyte volume, this configuration led to a voltage of 625 mV at a resistor of 1000Ω [32].

In the late 1970s the current standard design concept of a microbial fuel cell was resolved by Suzuki (1977) [31]. It consisted of an immobilized whole cell-platinum black electrode at the cathode, a carbon electrode at the anode and a salt bridge to allow the proton exchange as we can see in figure 2.3.

In 1989 the two chamber microbial fuel cell is devised by S. Tanisho. This device used glucose as the substrate and potassium ferricyanide as the catholyte. A fairly large current density (ca. 60
$\mu A/cm^2$ was obtained by using a stainless-steel net electrode plated with platinum black and the maximum open-circuit voltage of cell was 1.04 V [64]. In the figure 2.4 the schema of this first two chamber MFC is represented.

The same year, the first air cathode MFC was published, which was also characterized by two chambers, separated by a cation exchange membrane (CEM) but the main difference was that it used an oxygen gas diffusion cathode and a three-dimensional packed bed graphite electrode employed as an anode [14]. Related to the cross-sectional area, a value of 1.3 mA/cm$^2$ was achieved. Most MFCs use aqueous cathodes where water is bubbled with air to provide dissolved oxygen to electrode.
The stack or currently also called flat plate MFC was conceived in 1993 by R.M. Allen and H.P. Bennetto. It consisted of chemically immobilized bacteria onto the surface of graphite felt electrodes, which supported production of continuous electric current and could be reused after storage. This MFC incorporated a computer-controlled carbohydrate feed system that enabled the cell to generate a constant output with improved efficiency compared to the performance obtained with single large additions of fuel. The response to additions of substrate when immobilized bacteria were used was faster than that achieved with freely suspended organisms. This was attributed to the advantageous mass-transfer kinetics resulting from the proximity of the immobilized bacteria and the electrode surface [57]. The maximum open-circuit reached voltage was 0.8 volts.

In the early 21st century, the sediment microbial fuel cell was developed by Leonard M. Tender and Clare E. Reimers (2002). In many marine environments, a voltage gradient exists across the water–sediment interface resulting from sedimentary microbial activity. A fuel cell consisting of an anode embedded in marine sediment and a cathode in overlying seawater can use this voltage gradient to generate electrical power in situ. The power generation results from at least two anode reactions: oxidation of sediment sulfide (a by-product of microbial oxidation of sedimentary organic carbon) and oxidation of sedimentary organic carbon catalyzed by microorganisms colonizing the anode [40]. This system is characterized by a low voltage and power production.

In order to increase energy output and reduce the cost of MFCs, in 2004 Hong Liu and Bruce
E. Logan examined power generation in a single-chamber air-cathode MFC containing carbon electrodes in the presence and absence of a polymeric proton exchange membrane (PEM). Bacteria present in domestic wastewater were used as the biocatalyst, and glucose and wastewater were tested as substrates. Power density was found to be much greater than typically reported for aqueous-cathode MFCs, reaching a maximum of $262 \pm 10 \text{ mW/m}^2$ ($6.6 \pm 0.3 \text{ mW/L; liquid volume}$) using glucose. Removing the PEM increased the maximum power density to $494 \pm 21 \text{ mW/m}^2$ ($12.5 \pm 0.5 \text{ mW/L}$). Coulombic efficiency was 40-55% with the PEM and 9-12% with the PEM removed, indicating substantial oxygen diffusion into the anode chamber in the absence of the PEM. Similar results on the effect of the PEM on power density were found using wastewater. The increase in power output when a PEM was removed was attributed to a higher cathode potential as shown by an increase in the open circuit potential [25].
Also in 2004, an improved version of the stack MFC, called flat plate MFC (FPMFC) was published by Booki Min and Bruce E. Logan. In their study, a flat plate MFC (FPMFC) was designed to operate as a plug flow reactor (no mixing) using a combined electrode/proton exchange membrane (PEM) system. The reactor consisted of a single channel formed between two non-conductive plates that were separated into two halves by the electrode/PEM assembly. Each electrode was placed on an opposite side of the PEM, with the anode facing the chamber containing the liquid phase and the cathode facing a chamber containing only air. Electricity generation using the FPMFC was examined by continuously feeding a solution containing wastewater, or a specific substrate, into the anode chamber. Average power density using only domestic wastewater was 72 ± 1 mW/m² at a liquid flow rate of 0.39 mL/min [7].

![Figure 2.9: Schematic (a) (upper, side view; lower, top view) and laboratory-scale prototype (b) of the FPMFC.](image)

In 2006 an increased performance of single-chamber microbial fuel cells was developed using a multiple diffusion layer in the air cathode. Shaoan Cheng, Hong Liu and Bruce E. Logan showed that the application of successive polytetrafluoroethylene (PTFE) layers (DLs), on a carbon/PTFE base layer, to the air-side of the cathode in a single chamber MFC significantly improved coulombic efficiencies (CEs), maximum power densities, and reduced water loss (through the cathode). Electrochemical tests using carbon cloth electrodes coated with different numbers of DLs indicated
an optimum increase in the cathode potential of 117 mV with four-DLs, compared to a 10 mV increase due to the carbon base layer alone. In MFC tests, four-DLs was also found to be the optimum number of coatings, resulting in a 171% increase in the CE (from 19.1% to 32%), a 42% increase in the maximum power density (from 538 to 766 mW m\(^{-2}\)), and measurable water loss was prevented. The increase in CE due was believed to result from the increased power output and the increased operation time (due to a reduction in aerobic degradation of substrate sustained by oxygen diffusion through the cathode) [62].

Figure 2.10: (a) Schematic of a single chamber MFC showing relative location of DL and catalyst layers on the cathode; (b) Power density as a function of current density for MFCs with different cathodes.

The same year, increased power generation in a continuous flow MFC with advective flow through the porous anode and reduced electrode spacing was published. The maximum power generated in a single-chamber air-cathode microbial fuel cell (MFC) had previously been shown to increase when the spacing between the electrodes was decreased from 4 to 2 cm. However, the maximum power from a MFC with glucose (500 mg/L) decreased from 811 mW/m\(^2\) (\(R_{\text{ex}} = 200 \Omega\), Coulombic efficiency of CE=28%) to 423 mW/m\(^2\) (\(R_{\text{ex}} = 500 \Omega\), CE=18%) when the electrode spacing was decreased from 2 to 1 cm (batch mode operation, power normalized by cathode projected area). This decrease in power was unexpected as the internal resistance decreased from
35 Ω (2-cm spacing) to 16 Ω (1-cm spacing). However, providing advective flow through the porous anode toward the cathode substantially increased power, resulting in the highest maximum power densities yet achieved in an air-cathode system using glucose or domestic wastewater as substrates. For glucose, with a 1-cm electrode spacing and flow through the anode with continuous flow operation of the MFC, the maximum power increased to 1540 mW/m² (51 W/m³) and the CE increased to 60%. Using domestic wastewater (255 ± 10 mg of COD/L), the maximum power density was 464 mW/m² (15.5 W/m³; CE=27%) [63].

Finally, the upflow microbial fuel cell (UMFC) was also conceived in 2006 by Zhen He, Norbert Wagner, Shelley D. Minteer and Largus T. Angenent. The system consisted of an U-shaped cathode inside the anode chamber and produced a maximum volumetric power of 29.2 W/m³ at a volumetric loading rate of 3.40 kg COD/(m³/day) and an operating temperature of 35 °C while feeding sucrose continuously. The Coulombic efficiency decreased from 51.0% to 10.6% with the increase in the volumetric loading rate from 0.57 to 4.29 kg COD/(m3 day). In addition, the lab-scale UMFC maintained soluble chemical oxygen demand (COD) removal efficiencies exceeding 90% and volatile fatty acid concentrations of ~40 mg/L, indicating efficient wastewater treatment [80]. In figure 2.12 a schema of the UMFC can be observed.
2.1.4 Bacteria diversity in anode chamber and biofilm formation

Unfortunately, our lack of understanding of the ecology of the microbial communities responsible for electricity production is still notorious. This is probably where the greatest uncertainty in MFC design lies. It is known that the inoculation of an MFC and the subsequent process of acclimatisation or enrichment is fundamental, but in the majority of MFCs it is not known what species were in the inoculum and how that influences the species that dominate the fuel cell during optimal performance. Therefore, we have yet no rational means of control in shaping the community composition.

Although, it may be possible to evolve and optimise the functioning of microbial communities in an MFC by an informed process of trial and error, this paradigm for design is hardly surprising given that until recently scientists relied on culture-based methods for investigating microbial communities but it is widely accepted that less than 1% of naturally occurring microorganisms are culturable [66].

The biofilm communities have been shown to be phylogenetically diverse and able to degrade a range of compounds synergistically [78]. In addition, there appears to be no single electrogen species that dominates and the community composition depends on MFC configuration, operating
conditions, inoculum sources and substrates regardless of phylum and inoculum [41]. It has been also reported that mixed cultures show higher performance than isolated pure cultures in MFCs [68].

Moreover, mixed populations are more likely to survive in the face of environmental changes, such as temperature or organic loading rate, among other parameters, with the possibility of shifts in metabolic pathways and/or populations which can facilitate their adaptation to the new environment.

Possible inocula to be used are: Anaerobic digestion sludge, Activated sludge, Membrane bioreactor treating domestic wastewater, Marine plankton, Rice paddy field, Marine sediment, Salt marsh sediment and Freshwater sediment.

Substrates commonly used are: Glucose, Sodium acetate, Glutamate, Lactate, Cellulose, Pyruvate, Formate, Wastewater and Seawater. Finally, some of the most dominant bacteria species to be mentioned are: Schewanella putrefaciens, Geobacteraceae, Escherichia coli, Clostridium butyricum and Rhodoferax ferrireducens.

2.1.5 Cathode performance

The cathode performance is very relevant regarding the global performance of an MFC and that is why it is often assisted and improved. Thus, the cathode reduction reaction can be accelerated through 3 different methods.

The first method consists of an electrode modification, due mainly to the poor oxygen-reducing catalytic activity of the electrode used as the cathode (e.g. carbon electrode), with metals, surfactants, or organic materials [53]. Secondly, a catalyst can be used to facilitate the reduction of the dissolved oxygen, such as Platinum (Pt), Iron(II) phthalocyanine (FePc), Cobalt tetramethoxyphenylporphyrin (CoTMPP) [34] and Manganese dioxide [42]. The third option is the use of cathode mediators or ”oxygen equivalents for reduction” such as ferricyanides, methylene blue, viologens, thionines and quinoid compounds [53].
2.1.6 Electrode materials used in MFCs

Electrode materials also play an important role in the performance and specially cost of microbial fuel cells. Different electrode materials present distinct physical and chemical properties (e.g., surface area, electric conductivity, and chemical stability), thus, they also vary in their impact on microbial attachment, electron transfer, electrode resistance and the rate of electrode surface reaction. Therefore, it is of great significance to select and develop suitable electrode materials to optimize and promote the performance of MFCs. Moreover, as a main component, the electrode materials determine the price of MFCs [48].

Electrode materials can be basically divided into three categories: anode, cathode, and filling materials as three-dimensional electrodes.

2.1.6.1 Anode materials

A proper anode material should have: good electrical conductivity and low resistance; strong biocompatibility; chemical stability and anti-corrosion; large surface area; and appropriate mechanical strength and toughness.

Carbon materials are the most widely used anodes in the present MFCs studies; they traditionally embrace graphite rod, graphite fiber brush, carbon cloth, carbon paper, carbon felt, and reticulated vitreous carbon (RVC), as is shown in table 2.1, where their characteristics are listed [48].

<table>
<thead>
<tr>
<th>Anode materials</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graphite rod</td>
<td>Good electrical conductivity and chemical stability, relatively cheap, and easy to get</td>
<td>Difficult to increase the surface area</td>
</tr>
<tr>
<td>Graphite fiber brush</td>
<td>Higher specific surface areas, easy to produce</td>
<td>Clogging</td>
</tr>
<tr>
<td>Carbon cloth</td>
<td>Large relative porosity</td>
<td>Expensive</td>
</tr>
<tr>
<td>Carbon paper</td>
<td>Easy to connect wiring</td>
<td>Lack of durability, fragile</td>
</tr>
<tr>
<td>Carbon felt</td>
<td>Large aperture</td>
<td>Large resistance</td>
</tr>
<tr>
<td>RVC</td>
<td>Good electrical conductivity and plasticity</td>
<td>Large resistance, fragile</td>
</tr>
</tbody>
</table>

Table 2.1: Comparison of the characteristics of traditional anode materials in MFCs.

Since their discovery, Carbon nanotubes (CNTs) and conductive polymer have become one of the electrode materials with the most potential because of their large specific surface area, high
mechanical strength and ductility, and excellent stability and conductivity.

In the study of MFCs, carbon-based materials are generally regarded as the most versatile anodes although several reports have attempted to use non-carbon anode materials, such as stainless steel [11], highly conductive gold [23] or titanium [4].

2.1.6.2 Cathode materials

The cathode materials are highly influential regarding cathode performance and thus, impact on the power capacity of MFCs. They should have a high redox potential and ease to capture protons. So far, the most common cathode materials are graphite, carbon cloth and carbon paper.

![Graphite sheet; Carbon cloth; Carbon paper](image)

Figure 2.13: (a) Graphite sheet; (b) Carbon cloth; (c) Carbon paper.

2.1.6.3 Three-dimensional electrode materials

Increasing the electrode surface area is an effective way to improve the performance of MFCs because it enhances the microbe attachment and the bio-electron transfer area. However, in a conventional two-dimensional electrode system, the increase in the electrode size is accompanied by an increase in the reactor volume and the infrastructure costs. The use of inexpensive three-dimensional electrodes, e.g., certain small particle conductive materials, to fill into the chamber may offer one solution [48].

The graphite particle is perhaps the most commonly used filling material in the anode chamber in a three-dimensional cell. Granular activated carbon (GAC), a commonly used packing
material in wastewater treatment processes, is an inexpensive and durable material with a high surface area that could greatly improve bacterial adhesion and might be used as a suitable anode material in MFCs.

![Graphite particles](image1.png) ![Granular activated carbon](image2.png)

Figure 2.14: (a) Graphite particles; (b) Granular activated carbon.

### 2.1.7 Mechanisms of electron transfer

Electrodes are solid materials that cannot penetrate bacterial cells, thus the electrons produced by the oxidized bacteria are to be transferred from the inside of the microbial cells membrane to its outside. This can be possible either via the physical transfer of reduced compounds, or via electron hopping across the membrane using membrane bound redox enzymes [60]. In order to enable electron transfers, bacterial cells must be electronically linked to the electrode by means of different methods. Such methods can be of distinct nature as discussed as follows:

#### 2.1.7.1 Direct electron transfer (DET)

The direct electron transfer occurs when exists physical contact between a bacterial cell membrane or a membrane organelle with the fuel cell anode, with no diffusional redox species being involved. Since living cells are generally assumed to be electronically non-conducting, the direct electron transfer requires that the microorganisms possess membrane bound electron transport protein relays that transfer electrons from the inside of the bacterial cell to its outside, terminating in an outermembrane (OM) redox protein that allows the electron transfer to an external, solid electron acceptor (a metal oxide or an MFC anode) [60]. It has also been demonstrated that, e.g.,
some Geobacter and the Shewanella strains can evolve electronically conducting molecular pili, also called nanowires, that allow the microorganism to reach and utilize more distant solid electron acceptors[21, 22]. These pili also allow the organisms to use an electrode that is not in direct cell contact as its sole electron acceptor (figure 2.15). The pili are connected to the membranebound cytochromes, via which the electron transfer to the outside of the cell is accomplished [60].

![Figure 2.15: Illustration of the DET via (A) membrane bound cytochromes, (B) electronically conducting nanowires.](image)

### 2.1.7.2 Mediated electron transfer (MET)

Although it is believed for some scientists that DET is the only choice for an efficient current generation, MET mechanisms have to be considered. In function of the nature of the redox mediating species, MET mechanisms can be divided into three groups:

**MET via exogenous (artificial) redox mediators**

These mediators seem to have the ability of enhancing the electric current generation, but a big disadvantage of its use is the low current density and the need for repeated addition, which is costly, because they are usually unstable. Moreover these artificial mediators can generate environmental concerns due to their toxicity. Thus, the approach of using these kind of mediators is for now abandoned. Some compounds were investigated, such as phenazines, phenothiazines, phenoxazines and quinones [60].

**MET via secondary metabolites**

Often microorganisms grow under conditions in which neither soluble electron acceptors are
available nor solid electron acceptors are in direct reach (for DET). Then, the microorganism can either (i) use externally available (exogenous) electron shuttling compounds like humic acids or metal chelates, or (ii) can itself even produce low-molecular, electron shuttling compounds via secondary metabolic pathways [46]. For MFC applications, the secondary metabolites (endogenous redox mediators) are especially of great interest, as their synthesis makes the electron transfer independent of the presence of exogenous redox shuttles. The mediator serves as a reversible terminal electron acceptor, transferring electrons from the bacterial cell either to a solid oxidant (the MFC anode) or into aerobic layers of the biofilm, where it becomes re-oxidized and is again available for subsequent redox processes, as it can be seen in figure 2.16

![Figure 2.16: Schematic illustration of MET via microbial secondary metabolites.](image)

**MET via primary metabolites**

In contrast to the secondary metabolites the production of reduced primary metabolites is closely associated with the oxidative substrate degradation. Naturally, the total amount of reduction equivalents produced matches the amount of oxidized metabolites. To be utilizable as a reductant for anodic oxidation the metabolite has to fulfil certain requirements. Its redox potential should be as negative as possible and it must be accessible for electrochemical oxidation under MFC conditions. In principle, two major anaerobic metabolic pathways can lead to the formation of reduced metabolites suitable for MFC utilization: anaerobic respiration and fermentation [60].
2.1.8 Optimizing biofilm parameters

The activity and performance of biofilms formed on the electrodes of bio-electrochemical systems like microbial fuel cells, is regulated by physical, chemical, biological and electrochemical parameters. However, three parameters in particular contribute directly to the efficiency of biofilms: (1) operating parameters, (2) system design parameters, and (3) biological parameters. Thus, achieving good condition in these three categories, would result in fertile ground for achieving ideal system performance [1].

2.1.8.1 System design parameters

The properties of the electrode used, affect both the propagation and electron transfer characteristics of electro-active biofilms. The electrode material, for example, affects biofilm formation under open circuit conditions (absence of an electron sink) yet has little or no effect when the circuit is closed [3]. Multiple electrode materials like, e.g., paper, sponge, cloth, felt, fiber, and foam made from graphite or carbon, can produce similar voltage outputs [3] but the electrochemical performance can vary [76], as well as the microbial biomass grown on the electrodes [51]. Moreover, electrode materials with higher microbi ally accessible surface area can result in higher current density [76].
2.1.8.2 Biological parameters

The most important biological parameters affecting the activity of electro-active biofilms are the source of inoculum, nature of pre-enrichment, type of microbial catalyst (consortia or pure culture), and type of microorganisms (Gram positive or Gram negative). The genotype (at the species and strain level) of the microorganisms present in electro-active biofilms depends on the source of inoculum. Acetate-fed systems maintained under strict anaerobic conditions, often select for Geobacter spp., which become the dominant members of the biofilm [1]. Gram-negative microorganisms generally result in higher current than Gram-positive bacteria, and mixtures of the two often result in higher current densities than the individual species.

2.1.8.3 Operating parameters

In batch systems, external resistance, redox potential, pH, substrate concentration, temperature, presence of oxygen, and ionic strength have been shown to affect current production. Operation of the anode in a continuous system introduces further complexity, and additional parameters for consideration include flow rate, rate of substrate loading, space velocity, shear rate, and related hydrodynamic changes. Below, the effect of each of these parameters on the microbiology and electrochemical performance of biofilms is considered [1]:

**External resistance and redox potential**

Electro-active biofilms can respond to external electrochemical stimuli such as resistance (to electron flow) by optimizing biofilm growth and structure and balancing carbon source utilization and electrode-based respiration [1].

**pH**

At the micro-level, the formation of pH gradients across the biofilm depth can lead to reduced performance of microorganisms close to the electrode surface and affect their growth [5]. Low pH in an inner section of the biofilm has also been linked to higher stress levels [6].

At the macro-level, use of a low bulk pH can be advantageous for proton transfer to the
cathode as well as minimize proton gradients; however, not many acidophilic microorganisms are known that can form electro-active biofilms [1].

**Temperature**

The effect of temperature is important because commercial-scale bioelectrochemical systems might be exposed to a variety of environmental conditions and the operation of these systems should be assessed under psychrophilic and thermophilic conditions. Patil et al. reported that temperature affected not only the growth but also the bioelectrocatalytic performance of electro-active biofilms [67]. Biofilms grown at lower temperatures outperformed those previously grown at higher temperatures and operated at low temperatures, suggesting that different microorganisms were enriched at low temperature.

**Oxygen**

For MFCs that operate with dissolved oxygen present in the anode chamber and/or in close proximity to the cathode, biofilms may function as an active barrier to oxygen diffusion. Experimental evidence suggests that facultative aerobes preferentially form biofilms on the anode when dissolved oxygen is present. However, in all cases oxygen diffusion to an MFC anode has a negative effect on power production, as electrons are diverted to oxygen and away from the surface of the anode [1].

**Shear rate**

Biofilms formed under high shear conditions are denser, which can improve electron transfer because cell–cell contact is better [1].

**Substrate concentration and loading**

Since electron transfer to the anode by electrochemically active microorganisms (EAMs) is a result of primary metabolism, the relationship between substrate concentration and current generation follows Monod’s equation under conditions where EAMs can function without any limitations. Increases in substrate concentration or loading therefore lead to increased current [47, 59].

The presence of excess substrate and the absence of a low-resistance path to an electron sink can divert the electron flow from current production to other processes such as methanogenic
metabolisms [58].

Figure 2.18: Parameters important for optimization of electroactive biofilms in bioelectrochemical systems.

### 2.1.9 Electricity production performance

In the field of MFCs, it is adequate to evaluate the reaction in terms of the overall cell electromotive force (emf), \( E_{emf} \) (V), defined as the potential difference between the cathode and anode.

The cell emf is calculated as:

\[
E_{emf} = E_{cat} - E_{an}
\]

where the minus sign is a result of the definition of the anode potential as reduction reaction (although an oxidation reaction is occurring).

The cell emf is a thermodynamic value that does not take into account internal losses. The open circuit voltage (OCV) is the cell voltage that can be measured after some time in the absence of current. Theoretically, the OCV should approach the cell emf. In practice, however, the OCV
is substantially lower than the cell emf, due to various potential losses. This energy loss is often referred to as overpotential, or the difference between the potential under equilibrium conditions and the actual potential. This illustrates that the main application of thermodynamic calculations is to identify the size and nature of energy losses, which will be discussed in the next section.

When comparing electricity generation performance of different reactors, one has to be cautious because there are several factors to take into account and for sure, better results in one factor doesn’t mean better global performance. Among these factors to be considered, we can mention voltage, current, resistor used, power output, power desity, electrochemical losses, convective transport limitations, coulombic efficiencies and evolution of the polarization curves of the individual MFCs. However, there is the tendency of reporting the power density as an evaluation of the electricity production performance of each reactor.

In this direction, the best ever published power density delivered by an MFC, as far as I am aware, was produced by a double cloth-electrode-assembly (CEA) microbial fuel cell (CEA-MFC) and its maximum power density was of 4.3 \( \text{W m}^{-2} \) at a current density of 16.4 \( \text{A m}^{-2} \), corresponding to a volumetric power density of 2.87 \( \text{KW m}^{-3} \) at 10.9 \( \text{kA m}^{-3} \) [75]. Unfortunately, this outstanding performance is not very common in MFCs’ field yet.

Other more common and humbler examples are: 170 mW \( \text{m}^{-2} \) reached by an artificial-wastewater-fed upflow MFC [79]; 258 W \( \text{m}^{-3} \) produced by six individual continuous MFC units in a stacked configuration (Stacked MFC) [50]; 26 mW \( \text{m}^{-2} \) generated by a wastewater-fed single chamber MFC [26].

2.1.10 Voltage and Power output limiting factors

The maximum attainable MFC voltage (emf) is theoretically on the order of 1.1 V [10]. However, the measured MFC voltage is considerably lower due to a number of losses. In general, the difference between the measured cell voltage and the cell emf is referred to as overvoltage and
is the sum of the overpotentials of the anode and the cathode, and the ohmic loss of the system:

$$E_{cell} = E_{emf} - \left( \sum \eta_a + |\sum \eta_c| + IR_\Omega \right)$$

where $\sum \eta_a$ and $\sum \eta_c$ are the overpotentials of the anode and the cathode respectively, and $IR_\Omega$ is the sum of all ohmic losses which are proportional to the generated current (I) and ohmic resistance of the system ($R_\Omega$). The overpotentials of the electrodes are generally current dependent and in an MFC, they can roughly be categorized as follows: (i) activation losses; (ii) bacterial metabolic losses; and (iii) mass transport or concentration losses.

**Ohmic losses:** The ohmic losses (or ohmic polarization) in an MFC include both the resistance to the flow of electrons through the electrodes and interconnections, and the resistance to the flow of ions through the cation exchange membrane (if present) and the anodic and cathodic electrolytes [28, 2]. Ohmic losses can be reduced by minimizing the electrode spacing, using a membrane with a low resistivity, checking thoroughly all contacts, and (if practical) increasing solution conductivity to the maximum tolerated by the bacteria [10].

**Activation losses:** Due to the activation energy needed for an oxidation/reduction reaction, activation losses (or activation polarization) occur during the transfer of electrons from or to a compound reacting at the electrode surface. This compound can be present at the bacterial surface, as a mediator in the solution, or as the final electron acceptor reacting at the cathode. Activation losses often show a strong increase at low currents and steadily increase when current density increases. Low activation losses can be achieved by increasing the electrode surface area, improving electrode catalysis, increasing the operating temperature, and through the establishment of an enriched biofilm on the electrode(s) [10].

**Bacterial metabolic losses:** In an MFC, the anode is the final electron acceptor and its potential determines the energy gain for the bacteria. The higher the difference between the redox potential of the substrate and the anode potential, the higher the possible metabolic energy gain for the bacteria, but the lower the maximum attainable MFC voltage. To maximize the MFC voltage, therefore, the potential of the anode should be kept as low (negative) as possible. However,
if the anode potential becomes too low, electron transport will be inhibited and fermentation of the substrate (if possible) may provide greater energy for the microorganisms [10].

**Concentration losses:**  Concentration losses (or concentration polarization) occur when the rate of mass transport of a species to or from the electrode limits current production [28, 2]. Concentration losses occur mainly at high current densities due to limited mass transfer of chemical species by diffusion to the electrode surface. At the anode concentration losses are caused by either a limited discharge of oxidized species from the electrode surface or a limited supply of reduced species toward the electrode. This increases the ratio between the oxidized and the reduced species at the electrode surface which can produce an increase in the electrode potential. At the cathode side the reverse may occur, causing a drop in cathode potential. By recording polarization curves, the onset of concentration losses can be determined [10].

In MFCs the measured cell voltage is usually a linear function of the current, and can be described simply as:

$$E_{cell} = OCV - IR_{int}$$

where $IR_{int}$ is the sum of all internal losses of the MFC, which are proportional to the generated current ($I$) and internal resistance of the system ($R_{int}$). MFC systems that are well described by this last equation, show a maximum power output when the internal resistance, $R_{int}$, is equal to external resistance, $R_{ext}$ [63].

Thus, MFC performance can be assessed in terms of both overpotentials and ohmic losses or in terms of OCV and internal losses.

### 2.2 Wastewater treatment by means of MFCs

Microbial fuel cells were first conceived and later developed with the main purpose of either capturing energy in the form of electricity or hydrogen gas. At the beginning, all kinds of different inocula were tested and distinct buffer solutions were used as substrates. However, in the late 1990s, Kim and coworkers demonstrated that electricity generation in an MFC could be sustained
by starch using an industrial wastewater [30], despite the power production was low and it was not clear whether the technology would have much impact on reducing wastewater strength. In 2004, this changed and the link between electricity using MFCs and wastewater treatment was clearly forged when it was demonstrated that domestic wastewater could be treated to practical levels while simultaneously generating electricity [26]. Then, as it was discovered that wastewater could function well as both inoculum and substrate for MFCs and in addition, there were evidences that the reduction of chemical oxygen demand (COD) seemed to be accelerated and when using wastewater-fed devices, scientists realized that MFCs’ research field should be complemented with wastewater treatment process applications. Thus, great efforts were made to develop MFCs in order to create integral devices capable of not only producing electrical energy, but also treating wastewater efficiently at the same time. As a consequence the number of published research papers related to wastewater-fed MFC reactors increased significantly.

2.2.1 Traditional biological wastewater treatment technologies

Nowadays, the most successful and widely used biological treatment technology is the activated sludge process in which aerobic microorganisms metabolise the organic waste. This process represents the secondary treatment (out of three) of any conventional wastewater treatment plant. In this process, though, the main issue is the huge amount of energy required, so pumping and aeration are the main energy consuming stages, which respectively account for 21% and 30–55% of the total treatment energy demand, according to the US Environmental Protection Agency [19]. Similarly, in the UK 3–5% of the national electricity consumption goes for wastewater treatments [66]. Another important con of this technology is the big quantity of sludge produced as a by-product, which has to be adequately managed. Only treatment and disposal of sludge could count up to 60% of the wastewater treatment total operation cost [71]. Fortunately, bonded to this technology there are also a few remarkable established methods for recovering energy such as sludge incineration [49], sludge gasification [17], sludge pyrolysis [45], fermentation [18] or anaerobic digestion [52], all with their own advantages and disadvantages, which are considered out of the scope of this thesis.
and will not be further discussed.

Figure 2.19: Activated sludge wastewater treatment flow diagram.

It is estimated that municipal wastewater contains approximately 9.3 times more energy than currently needed for its treatment in a modern municipal wastewater treatment plant [20]. The value is even higher for carbohydrate-rich wastewaters such as those from food-processing and brewery industries. Thus, the idea of the possibility of turning wastewater treatment into a self-sufficient or even a net energy-producing process arises, although energy loss is unavoidable and could be considerable in a practical conversion process [73].

2.2.2 MFCs’ chances to replace conventional sewage treatment systems

Microbial fuel cell technology provides a low cost alternative to conventional aerated wastewater treatment. MFCs have been shown effective in treating almost all kinds of waste streams, including municipal, brewery, agricultural, refinery, paper cycling wastewater, and even landfill leachate [16]. The advantages of MFCs in wastewater treatment mainly come from the energy saving and production and sludge minimization. The functional bacteria in MFCs are generally anaerobic or facultative microorganisms, so the operation of MFCs may not use any active aeration [9], which means important savings up to over half of the total treatment energy demand. In an experiment carried out by Tyler Huggins et al. a wastewater treatment efficiency and energy consumption and generation comparison was studied among three reactor systems - a traditional
aeration process, a simple submerged MFC configuration, and a control reactor acting similar as natural lagoons [71]. Results showed that all three systems were able to remove >90% of COD, but the aeration used shorter time (8 days) than the MFC (10 days) and control reactor (25 days). Compared to aeration, the MFC showed lower removal efficiency in high COD concentration, but much higher efficiency when the COD is low. Suspended solid measurements showed that MFC reduced sludge production by 52-82% as compared to aeration, and it also saved 100% of aeration energy. Furthermore, though not designed for high power generation, the MFC reactor showed a 0.3 Wh/g COD/L or 24 Wh/m3 (wastewater treated) net energy gain in electricity generation. These results demonstrate that MFC technology could be integrated into wastewater infrastructure to meet effluent quality and save operational cost. However, Only the aeration system showed complete nitrification during the operation, reflected by completed ammonia removal and nitrate accumulation. This means there is still much to be investigated and improved if MFCs pretend to be a feasible alternative to current sewage treatment systems.

2.2.3 Adsorption technique

Given the nature of the new MFC reactor later presented in this thesis, it is considered necessary to give some brief notions of adsorption technique regarding the possible effects that can have on wastewater treatment improvement.

Toxic organic pollutants present in sewage cause several environmental problems to our environment. The most common organic pollutants named persistent organic pollutants (POPs), are compounds of great concern due to their toxicity, persistence, long-range transport ability [24] and bioaccumulation in animals [39], travel long distances and persist in living organisms. POPs are carbon-based chemical compounds and mixtures (twelve pollutants) that include industrial chemicals such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), and some organochlorine pesticides (OCPs), such as hexachlorobenzene (HCB) or dichloro-diphenyl-trichloroethane (DDT), dibenzo-p-dioxins (dioxins) and dibenzo-p-furans (furans) [69]. Many of these compounds have been or continue to be used in large quantities and due
to their environmental persistence, have the ability to bioaccumulate and biomagnify [55]. Thus, they must be removed from our water sources and properly disposed of.

Efficient techniques for the removal of highly toxic organic compounds from water have drawn significant interest. A number of methods such as coagulation, filtration with coagulation, precipitation, ozonation, adsorption, ion exchange, reverse osmosis and advanced oxidation processes have been used for the removal of organic pollutants from polluted water and wastewater. Most of these methods have been found to be limited, since they often involve high capital and operational costs. However, the adsorption process by solid adsorbents shows potential as one of the most efficient methods for the treatment and removal of organic contaminants in wastewater treatment. Adsorption has advantages over the other methods because of simple design and can involve low investment in term of both initial cost and land required. The adsorption process is already widely used for treatment of industrial wastewater from organic and inorganic pollutants and meet the great attention from the researchers [55].

Adsorption is a surface phenomenon with common mechanism for organic and inorganic pollutants removal. When a solution containing absorbable solute comes into contact with a solid with a highly porous surface structure, liquid–solid intermolecular forces of attraction cause some of the solute molecules from the solution to be concentrated or deposited at the solid surface. The solute retained (on the solid surface) in adsorption processes is called adsorbate, whereas, the solid on which it is retained is called as an adsorbent. This surface accumulation of adsorbate on adsorbent is called adsorption. This creation of an adsorbed phase having a composition different from that of the bulk fluid phase forms the basis of separation by adsorption technology.

In a bulk material, all the bonding requirements (be they ionic, covalent, or metallic) of the constituent atoms of the material are filled by other atoms in the material. However, atoms on the surface of the adsorbent are not wholly surrounded by other adsorbent atoms and therefore can attract adsorbates. The exact nature of the bonding depends on the details of the species involved, but the adsorption process is generally classified as physisorption (characteristic of weak Van Der Waals forces) or chemisorption (characteristic of covalent bonding). It may also occur due
to electrostatic attraction. Adsorption depends on several factors such as surface area, nature of adsorbate, molecular sizes, charge (ionic species), pH, temperature, nature of the adsorbent and mixed solute conditions.

The adsorption amount \((qe, \text{mmol} \, g^{-1})\) of the molecules at the equilibrium step are determined according to the following equation [55]:

\[
qe = V \frac{(Co - Ce)}{M}
\]

where \(V\) is the solution volume (L); \(M\) is the mass of monolithic adsorbents (g); and \(Co\) and \(Ce\) are the initial and equilibrium adsorbate concentrations, respectively.

### 2.2.4 Types of adsorbents

Different types of adsorbents are classified into natural adsorbents and synthetic adsorbents. Natural adsorbents include charcoal, clays, clay minerals, zeolites, and ores. These natural materials, in many instances are relatively cheap, abundant in supply and have significant potential for modification and ultimately enhancement of their adsorption capabilities. Synthetic adsorbents are adsorbents prepared from agricultural products and wastes, household wastes, industrial wastes, sewage sludge and polymeric adsorbents. Each adsorbent has its own characteristics such as porosity, pore structure and nature of its adsorbing surfaces [55]. Many waste materials used include fruit wastes, coconut shell, scrap tyres, bark and other tannin-rich materials, sawdust and other wood type materials, rice husk, petroleum wastes, fertilizer wastes, fly ash, sugar industry wastes, blast furnace slag, chitosan and seafood processing wastes, seaweed and algae, peat moss, clays, red mud, zeolites, sediment and soil and ore minerals, among others [29] as we can see in table 2.2.

Activated carbons (AC) (both granular activated carbon (GAC) and powdered activated carbons (PAC)) are lately common adsorbents used for the removal of undesirable odor, color, taste, and other organic and inorganic impurities from domestic and industrial wastewater owing to their large porosity and surface area, micro porous structure non-polar character and due to its economic viability. The major constituent of activated carbon is the carbon that accounts up
Table 2.2: Waste products used for generating low cost adsorbents.

<table>
<thead>
<tr>
<th>A. House hold wastes</th>
<th>D. Sea materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Fruit waste</td>
<td>a. Chitosan and seafood</td>
</tr>
<tr>
<td>b. Coconut shell</td>
<td>processing wastes.</td>
</tr>
<tr>
<td>c. Scrap tyres</td>
<td>b. Sea weed and algae</td>
</tr>
<tr>
<td></td>
<td>c. Peat moss</td>
</tr>
<tr>
<td></td>
<td>d. Miscellaneous waste</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Agricultural products</th>
<th>E. Soil and ore materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Bark and other tannin-rich</td>
<td>a. Clays</td>
</tr>
<tr>
<td>materials</td>
<td>b. Red mud</td>
</tr>
<tr>
<td>b. Saw dust and other wood</td>
<td>c. Zeolites</td>
</tr>
<tr>
<td>type materials</td>
<td>d. Sediment and soil</td>
</tr>
<tr>
<td>c. Rice husk</td>
<td>e. Ore minerals</td>
</tr>
<tr>
<td>d. Other agricultural waste.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>C. Industrial waste</th>
<th>F. Metal oxides and hydroxides</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Petroleum wastes</td>
<td></td>
</tr>
<tr>
<td>b. Fertilizer wastes</td>
<td></td>
</tr>
<tr>
<td>c. Fly ash</td>
<td></td>
</tr>
<tr>
<td>d. Sugar industry wastes</td>
<td></td>
</tr>
<tr>
<td>e. Blast furnace slag</td>
<td></td>
</tr>
</tbody>
</table>

to 95% of the mass weight. Active carbons also contain other hetero atoms such as hydrogen, nitrogen, sulfur, and oxygen.

2.3 Scaling-up difficulties

Most of the MFC prototypes developed and studied so far, are laboratory small-size scale devices that have shown promising results for both renewable energy production and wastewater treatment and remediation. However, when it comes to apply this technology to real scale cases, difficulties and unexpected issues come up.

Innovation in the design of new MFC reactors has been driven by the desire to increase the power output and decrease capital costs. So materials that minimise the internal electrical resistance, designs that maximise the surface area that electrogenic bacteria can attach to and the removal of expensive materials such as noble metal catalysts on the cathode have been and will be the focus of much of the research activity. Material costs have decreased but need to decrease more to make MFCs attractive alternatives to other forms of wastewater treatment and pilot plants are emerging [33, 35].
Microbial communities have been used in conventional wastewater treatment technologies without necessarily having a deep knowledge of the dynamics of the populations, so perhaps our lack of knowledge is not a barrier to the adoption of MFCs, but a good understanding of the acclimatisation of the communities in MFCs and their response to environmental perturbations, would reduce the perceived risks and accelerate the adoption of MFCs.

Reported power outputs in laboratory scale MFCs have increased three orders of magnitude in less than a decade. Maintaining or even increasing total power and current output performance during scale-up of MFCs is essential and in this direction, relatively low power density is the greatest challenge for practical application of MFC technology in wastewater treatment. A major reason for decreased performance during scale-up is the enlarged anode–cathode spacing [75].

Air-cathode MFCs seem to hold a greater promise for practical applications due to the fact that oxygen is the only ubiquitous and virtually free electron acceptor.

It is inevitable that much of the scaling-up progress will be made through a process of trial and error.
Chapter 3

Materials and methods

In this chapter, the reactor design, materials used for the construction of its distinct parts, electrodes’ material, type of proton exchange membrane and complementary used devices are described. An explanation of the different testing procedures and tools used for collecting all data is also presented.

3.1 Reactor design

The reactor design has a slight physical similitude with the upflow microbial fuel cell but as it will be later explained, its working principle is different.

The device consists of an inner vertical cylinder that constitutes the anode chamber and an outer wall circumventing this inner cylinder, forming the cathode chamber in the space bounded by the inner and the outer cylinder. Both cylindrical chamber walls share the same vertical longitudinal axis and are supported by a basement that holds them from the bottom.

Thus, the anode and cathode chambers are separated by the inner cylinder wall delimiting the anode chamber, in which anaerobic conditions are desired for the proper grow and behaviour of bacterial biofilm. As it is also expected that cations can be freely transferred from the anode to the cathode without allowing liquid to permeate through the anode fouling-resistant boundary wall in any of the two directions, a proton exchange membrane (PEM) is the tool chosen to perform best this purpose. On the other hand, aerobic conditions are preferred for the cathode chamber, which means that the material forming the outer and external cylindrical wall must be permeable
in order to let oxygen dissolve itself into the liquid flowing through the cathode chamber and must be resistant to operating conditions, i.e. oxidation-resistant. In this case, a stainless steel mesh will cover properly this sought characteristics.

The basement consists of a T-shaped PVC pipe fitting, in which one of the two outlets that follow the principal axis’ direction, is covered with a cap and acts as the bottom of the basement. The shape and sizes of this basement are presented in figure 3.1. The opposite outlet offers a pipe-fitting cavity that is adequate to support and give a circular shape to the outer stainless steel mesh reactor wall, whose diameter will be determined by the size of this cavity, which is 3.5 inches and corresponds to an extended mesh of 21x12.5 inches (53.34x31.75 cm).

At this point, it is time to assemble the most complex part of the reactor design and construction we have to cope with: the anode inner cylinder support. A 90-degree elbow-shaped PVC pipe is used to perform this function. It has a smaller diameter so that it can be attached into the T-shaped PVC pipe fitting offering a support for the anode chamber, so its longitudinal axis stays concentric with the outer cathode chamber wall. The elbow is fixed to the T-shaped PVC pipe
using epoxy resin and reinforced with generous amounts of silicone. As it can be observed in figure 3.2, this elbow pipe will also define the diameter of the PEM wall and anode chamber, 1.9 inches.

![Image of 90-degree elbow-shaped PVC pipe as the support of the anode chamber: (a) 3D view; (b) Profile view and dimensions.](image)

Figure 3.2: 90-degree elbow-shaped PVC pipe as the support of the anode chamber: (a) 3D view; (b) Profile view and dimensions.

In order to give a first cylindrical shape to the proton exchange membrane (PEM), two PVC male fittings are used in its both opposite ends (top and bottom of the cylinder). The PEM cloth is rolled up so that its diameter is delimited by the PVC male fittings cavities and once it perfectly fits them, epoxy first and silicone later, are used to affix all three parts and seal the PEM longitudinal closing joint. The size of the PEM to be cut will then be a rectangle of 21x6 inches (53.34x15.24 cm). Moreover, PVC female fitting will be needed to attach and connect the anode chamber (PEM) with its support (the elbow-shaped PVC pipe). These latter PVC parts will be joined using PVC Primer and Solvent Cement and the connection between the respective male and female fittings will be previously sealed with teflon plumber’s tape and then screwed. All used fittings’ sizes are indicated in figure 3.3.

After delimiting anode and cathode chambers, it is necessary to provide them with their corresponding inlets and outlets. In this reactor, due to its own flow circuit, the liquid coming out of the anode pours into the cathode by gravity force. The anode outlet and the cathode inlet are the same and consequently, no additional object is used to accomplish its function.
The anode chamber will be closed by placing a PVC adapter on the open side of the 90-degree elbow-shaped PVC pipe with the help of a connector between them, and using again PVC Primer and Solvent Cement. The adapter will end up connecting with another smaller barbed nozzle adapter able to fit a hose, which will be the one injecting the substrate to the anode. Both adapters will be also first sealed using teflon plumber’s tape and later screwed to be attached. In figure 3.4, the relevant sizes of the previously mentioned adapters and connector are presented.

The space left between the T-shaped PVC pipe fitting and the 90-degree elbow-shaped PVC pipe will be sealed with great amounts of silicone in order to close the cathode chamber, which will be also provided with an outlet at the bottom of the T-shaped PVC pipe fitting, so that the liquid can exit the chamber by gravity. This outlet will consist of a straight coupling PVC fitting (figure 3.5) through an inch-diameter drilled hole. This nozzle will be first affixed with epoxy and then reinforced with a bit of silicone, so no leaks are present when running the reactor.

The stainless steel mesh delimiting the cathode chamber will be reinforced with three adjustable clamp rings distributed along the vertical wall, and with a 3,5-inch-diameter PVC pipe adapter fitting that will also help shape the cylindrical cathode chamber thanks to its cavity (figure 3.5). Two more nozzles will be set attached through opposite holes on this PVC pipe adapter
Figure 3.4: Anode inlet PVC assembly: (a) Anode closing PVC adapter; (b) PVC connector; (c) Barbed nozzle adapter.

Figure 3.5: (a) Cathode outlet PVC fitting; (b) Adjustable clamp ring; (c) PVC pipe adapter fitting.

fitting’s wall in order to carry out a recirculation process of the treated liquid, explained later on next pages, where these will re-inject the treated liquid to the cathode chamber without passing through the anode chamber.

The bottom of both anode and cathode chamber are padded out with stainless steel scrubber
sponge to prevent the smaller particles of electrode from flushing out of the respective chambers. A fine and narrow highly conductive stainless steel strip (25x1 inches) will be set into and along the anode chamber, which will serve us as the connection between the anode electrode and the external circuit. No stainless steel strip will be needed for the cathode, as the stainless steel external wall is already highly conductive and can be used as a connector with the external circuit. Anode and cathode cylinders will be filled up with the three-dimensional electrode particles, whose characteristics will be explained in next section 3.2. Finally, as the anode liquid will be pumped upwards, a plastic mesh will be affixed on top of the anode cylinder with a zip tie, so that no anode electrode particles are poured off with the liquid. Of course, the pores of all stainless steel and plastic meshes used are significantly smaller than the smallest electrode particle size, around 2 millimeters.

![Figure 3.6: (a) Stainless steel scrubber sponge; (b) Fine highly conductive stainless steel mesh; (c) Plastic mesh.](image)

In figure 3.7, the reactor with its main parts taken apart can be observed, as well as the full assembled reactor.
3.2 Reactor electrodes and biochar production

The same nature of electrode is used for both anode and cathode chambers. It is a novel highly conductive three-dimensional type of electrode with similar physical characteristics to active carbon but with a significant lower cost and carbon footprint, known as biomass-derived black carbon or also called biochar.

Previous studies like the one carried out by Tyler Huggins et al., compared biochar made using forestry residue (BCc) and compressed milling residue (BCp), side-by-side with granular activated carbon (GAC) and granular graphite (GG). The results showed that the specific area of BCp ($469.9 \text{ m}^2/\text{g}$) and BCc ($428.6 \text{ m}^2/\text{g}$) is lower than GAC ($1247.8 \text{ m}^2/\text{g}$) but higher than GG
Moreover, when tested as MFC electrodes, both biochars showed power outputs of 532 ± 18 mW/m² (BCp) and 457 ± 20 mW/m² (BCc), comparable with GAC (674 ± 10 mW/m²) and GG (566 ± 5 mW/m²). However, lower material expenses made their power output cost 17–35 US$/W, 90% cheaper than GAC (402 US$/W) or GG (392 US$/W). Biochar from waste also reduced the energy and carbon footprint associated with electrode manufacturing and the disposal of which could have additional agronomic benefits [70].

Biochar is normally obtained as a by-product of thermal decomposition of waste biomass and so far, it has been widely employed as an agricultural fertilizer to improve soil quality. As biochar is mostly produced from locally available bio-waste, such as agricultural and forestry residues, the costs from raw material purchasing, extraction and transportation are greatly reduced. Consequently, biochar becomes a very competitive option with prices ranging from 51 to 381 US$/ton [61], which means almost ten times cheaper than granular activated carbon or granular graphite.

For the present reactor, biochar is produced using pine wood excess sticks, which after being cut for construction industry purposes are no longer useful and its price sinks 75% off, but any other type of biological waste could have been used. Its production consists of a high temperature gasification process and little external energy is used: The pine wood sticks are carbonized in absence of oxygen by means of a custom made top-lit up-draft biomass gasifier with external fan, as described by Kearns (2012) [36]. Biomass is carbonized using a highest heating temperature (HHT) of 1000 °C, residence time of 1 hour, and a ramp rate of 16 °C/min and temperature is measured using a programmable thermocouple.

The carbonization process results in biochar blocks that will be first crushed into smaller pieces, secondly screened out through a sieve of 8 millimetres and lately washed with deionized (DI) water over a 2-millimetre sieve, so that all powder and other minuscule particles resulting from the previous crushing process are released. The result is clean biochar particles with high specific surface area and a powerful adsorption capacity, ready to be used and set into the respective anode and cathode chambers.

Unlike the common electrodes used in microbial fuel cells, the biochar particles will take up
all the space in both anode and cathode chambers and in this sense, they will not only perform as an electrode but will also behave as a filter and adsorbent helping clean the treated liquid. Furthermore, the spaces left among particles will be pretty favourable for the creation and grow of biofilm.

In next figure 3.8, pictures from the pine wood carbonization process by means of the gasifier and the following biochar crush are shown.

![Figure 3.8: (a) Screening out crushed biochar through a 8-millimeter sieve; (b) Biochar production using a custom made top-lit up-draft biomass gasifier.](image)

### 3.3 Acetate and industrial wastewater

The reactor performance in both power output and water treatment potential will be tested first using acetate and later using industrial wastewater as a real application case.

The chemical composition of the substrate solution that will be prepared and used to run the reactor consists of three main ingredients: Acetate solution, vitamins solution and minerals
solution.

The acetate solution will be prepared every time a new batch of substrate solution is needed and its ingredients are shown in table 3.1.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Concentration (grams/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium acetate anhydrous</td>
<td>1.30</td>
</tr>
<tr>
<td>Sodium phosphate monobasic monohydrate</td>
<td>2.00</td>
</tr>
<tr>
<td>Sodium phosphate dibasic heptahydrate</td>
<td>5.26</td>
</tr>
<tr>
<td>Ammonium chloride</td>
<td>0.25</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Table 3.1: Acetate solution formula.

Unlike the acetate solution, vitamins and minerals solutions will be prepared only once, before starting the experiment, in concentrated solutions and they will be stocked in the fridge at 4°C. The ingredients used for their making, as well as their concentrations in the final substrate solution are respectively shown in tables 3.2 and 3.3.

Then, every time a new batch of substrate solution is needed, the concentrated vitamins and minerals solutions are mixed and diluted with the acetate solution, which will be freshly prepared and all is let sit for at least four hours so that none or very low dissolved oxygen concentration can be guaranteed before it is run through the reactor. During the acclimation phase, a bit of activated sludge, obtained from a local wastewater treatment plant and kept permanently in the fridge, is also poured into the substrate solution to help accelerate this process.

3.4 Other tools and machinery used

The main objects that configure the reactor have been already described but there are some other tools that have facilitated the experiment development, which are described as follows:

Multimeter:

A common multimeter (figure 3.10) is used to measure different parameters, such as voltage, resistance, current and conductivity.
Resistor box and axial-lead resistors:

A resistor box (figure 3.10) with a wide range of applicable resistors is used to change resistors quickly and easily, without having to rearrange the external electrical circuit every time they have to be switched to change cell conditions. At some point, common axial-lead resistors will also be

<table>
<thead>
<tr>
<th>Vitamins solution ingredient</th>
<th>Concentration (milligrams/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotin</td>
<td>0.04</td>
</tr>
<tr>
<td>Folic acid</td>
<td>0.04</td>
</tr>
<tr>
<td>Pyridoxine HCL</td>
<td>0.2</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.1</td>
</tr>
<tr>
<td>Thiamin</td>
<td>0.1</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>0.1</td>
</tr>
<tr>
<td>Pantothenic acid</td>
<td>0.1</td>
</tr>
<tr>
<td>B-12</td>
<td>0.002</td>
</tr>
<tr>
<td>P-aminobenzoic acid</td>
<td>0.1</td>
</tr>
<tr>
<td>Thiocitic acid</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Table 3.2: Vitamins concentration in final substrate solution.

<table>
<thead>
<tr>
<th>Minerals solution ingredient</th>
<th>Concentration (milligrams/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTA</td>
<td>15</td>
</tr>
<tr>
<td>MgSO4</td>
<td>30</td>
</tr>
<tr>
<td>MnSO4·H2O</td>
<td>5</td>
</tr>
<tr>
<td>NaCl</td>
<td>10</td>
</tr>
<tr>
<td>FeSO4·7H2O</td>
<td>1</td>
</tr>
<tr>
<td>CaCl2·2H2O</td>
<td>1</td>
</tr>
<tr>
<td>CoCl2·6H2O</td>
<td>1</td>
</tr>
<tr>
<td>ZnCl2</td>
<td>1.3</td>
</tr>
<tr>
<td>CuSO4·5H2O</td>
<td>0.1</td>
</tr>
<tr>
<td>AlK(SO4)2·12H2O</td>
<td>0.1</td>
</tr>
<tr>
<td>H3BO3</td>
<td>0.1</td>
</tr>
<tr>
<td>Na2MoO4</td>
<td>0.25</td>
</tr>
<tr>
<td>NiCl2·6H2O</td>
<td>0.24</td>
</tr>
<tr>
<td>Na2WO4·2H2O</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Table 3.3: Minerals concentration in final substrate solution.
Figure 3.9: (a) Chemicals used for the acetate solution; (b) Vitamins and minerals concentrated stock solutions, and activated sludge sample.

used, especially during cell acclimation stage, due to its comfortable and reduced size.

Figure 3.10: (a) Multimeter used to control electrical parameters; (b) Resistor box; (c) Axial-lead resistor.

**Pumps:**

A couple of pumps (figure 3.11), whose model is Masterflex S/L from Cole-Parmer are used
when gravity force is lacking or it is not strong enough to create a natural flow of the liquid to be treated.

**Chemical oxygen demand digestor and spectrophotometer:**

When preparing the samples taken and analysing them, a chemical oxygen demand (COD) digestor (DRB 200 Dual Block reactor, Hach) and a spectrophotometer with RFID Technology (DR 3900 Benchtop Spectrophotometer, Hach) are respectively used (figure 3.11). This latter system, gives us the sample concentrations for COD, phosphate, ammonia and nitrate.

![Figure 3.11](image)

Figure 3.11: (a) Pumps used for the experiments; (b) COD digestor; (c) Spectrophotometer.

**Data logger:**

Thanks to my colleague Alex Haeguer, a custom made data logger (figure 3.12) is used to collect full cell voltage evolution over time, data which is stored in a SD card.

**Reference electrode:**

A reference electrode (figure 3.12) is used to daily measure anode and cathode half cell potentials, control their performance and check or prove their working conditions, such as aerobic climate for the cathode and anaerobic climate for the anode. Its type is Silver-Chloride (AgCl).

**pH and dissolved oxygen concentration meter**

A pH and dissolved oxygen concentration meter (Orion Star™ A216 pH/Dissolved Oxygen
Benchtop Multiparameter Meter) is used to measure the pH evolution that the liquid (different samples) suffers as the reactor keeps on working and treating over time, and to measure and prove a very low dissolved oxygen concentration of fresh acetate/wastewater batches before starting pumping them to the delicate anaerobic anode (figure 3.12).

Figure 3.12: (a) Data logger; (b) Reference electrode; (c) pH and dissolved oxygen meter.

**Plastic hose, hose adapters, buckets, wires, and crocodile clips:**

Several meters of hose are used to set the flow circuit, from the buckets containing the liquid to be treated to the reactor, passing through the pumps. As the hose section which is in physical contact with the pump is not a regular one, but thinner and with special precision pump tubing mechanical characteristics, some adapters are also needed to connect hoses of distinct diameter. Plenty of electric wire and some crocodile clips are also used, no only for the external circuit through the resistor box, but also to allow logger voltage data collection.

### 3.5 Reactor working principle

The reactor is conceived and designed to achieve a synergistic performance for generating electricity and even improving the acetate/wastewater treatment potential that other common
MFC designs show. As follows, the flow circuit will be explained in detail, as well as the different reactions and occurrences expected in each section.

An oxygen-free substrate is first pumped at a very low pumping rate into the anode chamber through its inlet nozzle, where it flows upwards very slowly, and fills up and keeps filled all the anode chamber, without letting any oxygen in so as to maintain anaerobic conditions. In this process, the substrate is expected to create and grow a biofilm over time, either attached to the biochar particles or floating into the liquid among them, and to take part into the oxidation reaction that will make the electron transfer possible, which at the same time, will be facilitated by the biofilm-created cell linkage.

Moreover, apart from the MFC-common oxidation reaction that takes place in the anode, the reactor is also designed so that, in this first anode section the acetate/wastewater suffers a first filtration and adsorption process to help improve the reactor contribution to water purification. Thus, the biochar particles function as a sieve that retains the big and medium size water pollutants, meanwhile the smallest particles are held in the smallest pours by mainly physical but also chemical adsorption. All these processes contribute to an easier and accelerated biofilm formation.

As it occurs in other MFC designs, the oxidation reaction in the anode chamber is expected to result in both electron transfer to the anode electrode (biochar particles) and proton transfer to the cathode chamber through its separation wall, the proton exchange membrane (PEM). The electron transfer to the conductive electrode is possible thanks to several methods already mentioned in the previous background section, such as direct or mediated electron transfer. The fine stainless steel mesh set into the anode is in charge of collecting the electrodes through its contact points with biochar particles, and connect them to the external circuit, which links anode and cathode chambers by means of the resistor box.

Once the acetate/wastewater has filled all the anode volume, it pours off the anode’s top and automatically enters the aerobic cathode chamber, through which it will trickle down by gravity. The acetate/wastewater is self-oxygenated by its bare contact with air and the mixing effect produced by the irregular biochar particles and their cavities. The significant amount of
dissolved oxygen in water leads then to favourable conditions for the reduction reaction, where oxygen combines with the incoming protons from the anode and takes the electrodes from the cathode electrode to form water. Unlike in the anode, no fine stainless steel mesh is placed in the cathode because in fact, the external wall mesh already does this function and is able to transfer the electrons from the external circuit to the biochar electrode, through their contact points. Of course, the cathode biochar particles also have a great contribution to water treatment by means of the same methods present in the anode chamber. Despite that the cathode external wall is permeable, the water stays inside the chamber because it is not under pressure and flows embracing the particles due to its surface tension. The water trickles down until it reaches the bottom of the basement, gets out of the chamber through its outlet and is collected into a bucket, thanks to gravity, by a hose.

Finally, the water already treated and collected into the bucket is again pumped up to the top of the cathode chamber for recirculation, without passing through the anode to avoid altering its anaerobic conditions. In fact, this water is mixed with the one pouring from the anode chamber and both, merged together, go through the cathode following the same process recently described. The recirculation has the object of keeping the cathode chamber wet to facilitate the proton exchange through the PEM and of course, it has also the goal of keeping on treating the water by increasing its hydraulic contact time (HCT) with the biochar particles.

In figure 3.13, a schematic sketch of the reactor and the water treatment process with its different flow circuits are represented, which may help to better understand how it works.
Figure 3.13: Reactor and flow circuit sketch.
Chapter 4

Methodology

In this chapter, the different research experiments carried out in the lab will be thoroughly described, including all the procedures followed for their development.

4.1 First experiment: Comparison between an MFC-working-like and an open-circuit reactor

In the first part of the research that this thesis deals with, two parallelly-built identical reactors will be tested using only acetate, synthetic wastewater, as a substrate. One reactor will be equipped with a closed circuit through a 100-Ohm resistor and consequently will be expected to work simultaneously as a filter and an MFC, whereas the other one will only be expected to perform as a filter, since it will be under open circuit conditions and thus, cannot be considered an MFC. The objective sought in this case is to compare behaviours of the two reactors and see the effects that a closed circuit reactor (MFC) can have on water treatment performance, compared to the one of the non-MFC reactor. For this purpose, both performances regarding water treatment potential will be evaluated and the MFC reactor cell voltage will be also controlled over time.

Before anything else, it is necessary to get rid of the powder and other fine biochar particles that may still remain stuck within the chambers of both reactors and which could later cause alterations in the water chemical composition, that will be sampled and tested along the experiment. For this reason, although deionized (DI) water, ultra-purified water, should be ideally run through the reactor so that all the non-desired excess minuscule biochar traces are flushed out, reverse
osmosis (RO) water will be used to this purpose, due to the significant amount of water that is needed. In spite of being not as pure as DI water, RO water is pure enough not to cause any perturbation when detecting chemicals at low concentration levels. Thus, as it can be observed in figure 3.7, for each reactor, the anode input will be directly connected to the RO water tap and reactors will be left running until visually clean water is observed getting out of the cathode outputs. Then, we have clean reactors prepared to get up and running.

Each reactor will be fed with 7-litre solution batches, which will be prepared in advance, at least 5 hours before its use, so that they have enough time to sit and release all the dissolved oxygen introduced during the stirring of the different compounds. All new substrate batches, prepared according to the formulae and processes described in section 3.3, will be pumped and injected to the anode chamber at a flow rate of 12ml/min., which will give us a substrate supply during 9 hours, 43 minutes and 20 seconds. However, after 9 and a half hours of operation, the "anode pump" (pumping water to the anode) will be stopped in order to make sure that no oxygen is injected to the anode, which would happen in case substrate finished, while keeping the anode chamber filled with substrate to feed the bacteria and allow the proton (and electron, if any) exchange, in the case of the MFC reactor. Although no more substrate enters the reactor, this keeps treating the water by recirculating it through the cathode chamber over and over again. Thus, per each batch water passes through the anode only once but it is recirculated to the cathode continuously, as described in section 3.5, at a flow rate of 300 ml/min.

While this acclimation operation phase is run, per each reactor and each batch, one sample will be taken right before a new batch is used (from the non treated acetate) and more samples will be collected every 24 hours from the treated and continuously recirculating water. The samples will be daily analyzed and their pH, chemical oxygen demand (COD) and occasionally also concentration of phosphate, ammonia and nitrate will be tested. Thus, each substrate solution batch will be treated in each reactor, most of the time then only recirculated, until COD reduction observed is around 90%. In this way, batch after batch the closed MFC-working-like reactor is expected to move forward to its acclimation and stabilization by forming and growing a favourable biofilm into
the anode chamber capable of transferring electrons to the biochar particles and end up working and performing not only as a filter, but also as an MFC. On the other hand, it is trivial that the open circuit reactor will not suffer an evolution in this direction and so, it will be only expected to work as a filter.

Once enough samples are collected and analyzed, and the closed circuit reactor shows a significant and stable cell voltage (if it is the case), it means that it is already acclimated and working as an MFC, so it is time to move to next research experiment, described in next section 4.2. In figures 4.1 and 4.2 images from the experiment are shown.

Figure 4.1: Parallel reactors just before starting the first experiment. MFC’s external circuit still missing.
4.2 Second experiment: MFC reactor energy generating potential

In this second experiment, only the already acclimated MFC reactor will be examined and its maximum performance regarding energy generation will be sought.

In the previous experiment described in section 4.1, due to the limited amount of substrate used (only 7 litre per batch) during each batch operation, the anode chamber is most of the time filled with the last substrate portion injected, which is held while the continuously treated water keeps recirculating through the cathode chamber. The substrate (carbon and energy source) held in anode chamber can feed the bacteria, grow biofilm and be oxidized releasing electrons, but as a consequence it loses its chemical quality composition and resources over time. Then, it is accepted that anode held substrate is not favourable when considering energy generation performance of the MFC reactor. Moreover, it is also not known for how long the substrate is able to "serve" the
microorganism community in the anode, until the extinction of its compounds. Summarising, in the previous experiment the time in which both anode and cathode work optimally together (anode injection pump and recirculation pump working together) is relatively little and so, it is considered that this form of operation is not ideal for letting the reactor achieve its maximum performance regarding energy generation.

For this reason, in this second experiment the MFC reactor will be also fed with acetate, but a slight rearrangement of the operation mode will be necessary to facilitate an expected maximum performance, as described below.

The MFC reactor will be fed this time with 19-litre substrate solution batches that will be also prepared in advance, as described in sections 3.3 and 4.1 to release possible dissolved oxygen content. Unlike the previous experiment, in this case a continuous feeding supply to the reactor will be desired so that the reactor works full time in adequate conditions to achieve its best performance.

The acetate, will be pumped through the anode at the same flow rate of 12ml/min, which will give us 26 hours, 23 minutes and 20 seconds of substrate supply. However, in practice each batch will be switched every 24 hours for a new one in order to make sure that no oxygen is injected to the anode and to establish a more comfortable daily working routine. The switch of substrate buckets will be operated by hand and as quick as possible. On the other hand, the recirculation bucket won’t be changed in order to keep the cathode chamber wet right from the beginning. However, every now and then an eye will be kept on it to avoid that the accumulated treating water pours out, and consequently the bucket will be emptied a bit every time this is about to happen.

All cell voltage data will be recorded by means of the data logger described in section 3.4, which is programmed to collect data every 6 minutes. Cell voltage data, as well as anode and cathode half-cell potentials will be controlled and measured over time with a multimeter. These half-cell potential checks will also be useful to make sure that both anode and cathode chambers work in the desired conditions, which respectively means negative (anaerobic conditions) and positive (presence of dissolved oxygen) half-cell potentials. Furthermore, the difference between these two half-cell potentials should coincide with or be significantly close to the full cell voltage obtained.
Once after several batches are run and the reactor shows a stable behaviour regarding voltage (at 100 Ohm acclimation-phase resistor), it is time to develop its polarization curve, test which consists of checking reactor’s stabilized energy generation performance, specifically voltage, at different resistor values. This test and its results will be further explained in more detail in next section 5.2.

Since the main goal of this second experiment is only to achieve and figure out the reactor’s maximum electrical performance, no samples will be taken along to record its behaviour in regard to water treatment. In fact, collecting samples would not make much sense because, as explained, the treating water contained into the recirculation bucket will be formed of mixtures of the different batches previously run through the system. Figure 4.3 shows the layout of this second experiment.

4.3 Third experiment: MFC Reactor behaviour when using industrial wastewater as substrate

In this third experiment, the objective will be to test the reactor under possible real conditions and observe how it behaves and performs as both an MFC and a wastewater treatment plant, as well as to see if a larger scale-up and its application could be feasible in the future. Thus, for the development of this test a brand new reactor is built and a bunch of fresh biochar is produced, in both cases following the materials, instruments and procedures described through previous sections. Unlike the first and second experiment, respectively described in sections 4.1 and 4.2, industrial wastewater will be used as substrate in this case. This industrial wastewater, collected in 19-litre buckets and directly taken from the aeration tanks of the water treatment plant belonging to and beside Coors brewery industry, located in Golden, CO, will be preserved in the freezer at -20°C until approximately 36 hours before its use, time which is considered enough to be thawed at room temperature and to release all possible dissolved oxygen present in water.

Before anything else, the reactor will be also first run with reverse osmosis water so that it can get rid of the exceeding tiny biochar particles and prepare the device for the experimentation, as it is done with the reactors used in the first experiment, explained in section 4.1.
The reactor will be equipped with an external closed circuit, which will connect anode and cathode chambers through an axial-lead resistor of 500 Ohms, as it can be seen in figure 3.10. Although it is certain that a lower resistor would be more appropriate to facilitate the biofilm growth in the anode and let the reactor reach an earlier stabilization as an MFC, the acclimation phase will be developed with such a resistor, since a lower one is not available.

The batches used to feed reactor will consist of the same 19-litre buckets used to collect and store the brewery industrial wastewater, which will be properly thawed and let sit during 36 hours before being injected, as previously mentioned. The wastewater will be pumped to the anode at a
flow rate of 12ml/min., representing a substrate supply during 26 hours, 23 minutes and 20 seconds. However, like in the first experiment, the "anode pump" will be stopped before the substrate is over in order to avoid the injection of air (oxygen) to the anode and mess up the favourable anaerobic conditions of the chamber. Then, the anode will retain the last injected portion of substrate, keeping the feed of the bacteria and the transfer of electrons, as well as the proton exchange through the PEM. Despite the interruption of the "anode pump", the water will be treated continuously by the recirculation through the cathode chamber thanks to the "recirculation pump" at a flow rate of 300 ml/min.

The acclimation of the reactor will start right from the first batch that is run through the system and samples will be taken along to test its behaviour. Per each batch, a prior sample of the raw wastewater will be taken and then, one sample per day (every 24h) will be collected from the recirculating, non-stop treated water. The samples will be daily analyzed and their pH, chemical oxygen demand (COD) and occasionally, also concentration of phosphate, ammonia and nitrate will be tested.

Thus, each wastewater batch will be treated, most of the time only recirculating, until COD reduction observed is considerable, usually around 90%. As the system works, it will presumably little by little move forward to its acclimation and become an MFC, giving power output thanks to the difference of anode and cathode potentials. At this time, if it happens to occur, the reactor energy generating performance will be tested by means of a polarization curve. Due to a lack of a straight and easy wastewater availability, it will have to be sparingly used and thus, it becomes impossible to develop this energy test with continuous wastewater flow through the reactor. However, it will be done collecting data right from the beginning of each batch and until 24 hours after the substrate depletion, so that values are as representative as possible. Voltage values will be manually controlled and also registered at the voltage logger, at the same time batches are run and samples for wastewater treatment are taken. This method may not give us accurate information, probably underrated, but an idea of its behaviour can be extracted. In figure 4.4 the arrangement for this last experiment is presented.
Figure 4.4: Third experiment layout: Orange bucket containing the raw wastewater, as substrate, and white bucket containing the recirculating and continuously treated water.
Chapter 5

Analysis of results and discussion

In this chapter, the results obtained in each experiment will be presented and analyzed. Thus, an effort will be made to try to understand and explain the behaviour of the reactor in each case.

5.1 First experiment results: Comparison between an MFC-working-like and an open-circuit reactor

Both reactors are built with exactly the same materials and procedures, and electrodes (biochar particles) come from the same production batch and are of the same quantity in respective anode and cathode chambers. At this point, reactors should be expected to show the same or close results. However, one reactor is open circuit and the other is operated under closed circuit conditions, through a resistor of 100 Ohm. Thus, the open circuit reactor is expected to work only as a filter but on the other hand, the closed circuit reactor can also be expected to turn into an MFC. How can this fact affect the reactor wastewater treatment behavior compared to the one from the same device working under open circuit?

A selection of all data collected and tested throughout the experiment has been carried out in order to clarify information and get a representative source of evaluation, from which conclusions can be extracted. This selection consists of data from the beginning, in between and the end of the experiment, in particular from the first, the fourth and the sixth batch used, as explained in section 4.1. Data are shown in tables 5.1, 5.2 and 5.3. Furthermore, the whole extent of data collected during the experiment can be found in section Appendix A, attached at the end of this document.
### Table 5.1: Respective data for each reactor during first batch (Concentrations in mg/l).

#### Closed circuit reactor: first batch; Approx. 0 mV

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>Day</th>
<th>Time</th>
<th>pH</th>
<th>COD</th>
<th>COD Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>eBAC 1.0</td>
<td>feb-21</td>
<td>17:45</td>
<td>6,9</td>
<td>1027</td>
<td>0%</td>
</tr>
<tr>
<td>eBAC 1.1</td>
<td>feb-22</td>
<td>17:45</td>
<td>7</td>
<td>658</td>
<td>36%</td>
</tr>
<tr>
<td>eBAC 1.2</td>
<td>feb-23</td>
<td>17:45</td>
<td>7,39</td>
<td>460</td>
<td>55%</td>
</tr>
<tr>
<td>eBAC 1.3</td>
<td>feb-24</td>
<td>17:45</td>
<td>7,6</td>
<td>298</td>
<td>71%</td>
</tr>
<tr>
<td>eBAC 1.4</td>
<td>feb-25</td>
<td>12</td>
<td>7,8</td>
<td>93</td>
<td>91%</td>
</tr>
</tbody>
</table>

#### Open circuit reactor: first batch

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>Day</th>
<th>Time</th>
<th>pH</th>
<th>COD</th>
<th>COD Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>eBAC C 1.0</td>
<td>feb-21</td>
<td>17:45</td>
<td>6,9</td>
<td>1027</td>
<td>0%</td>
</tr>
<tr>
<td>eBAC C 1.1</td>
<td>feb-22</td>
<td>17:45</td>
<td>7,16</td>
<td>600</td>
<td>42%</td>
</tr>
<tr>
<td>eBAC C 1.2</td>
<td>feb-23</td>
<td>17:45</td>
<td>7,38</td>
<td>468</td>
<td>54%</td>
</tr>
<tr>
<td>eBAC C 1.3</td>
<td>feb-24</td>
<td>17:45</td>
<td>7,61</td>
<td>200</td>
<td>81%</td>
</tr>
<tr>
<td>eBAC C 1.4</td>
<td>feb-25</td>
<td>12</td>
<td>7,76</td>
<td>102</td>
<td>90%</td>
</tr>
</tbody>
</table>

### Table 5.2: Respective data for each reactor during forth batch (Concentrations in mg/l).

#### Closed circuit reactor: forth batch; Approx. 400 mV

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>Day</th>
<th>Time</th>
<th>pH</th>
<th>mV</th>
<th>COD</th>
<th>Phosphate</th>
<th>Ammonia</th>
<th>Nitrate</th>
<th>COD Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>eBAC 4.0</td>
<td>mar-04</td>
<td>14:05</td>
<td>7,09</td>
<td>-5,2</td>
<td>1177</td>
<td>147</td>
<td>Abs. &gt; 3,5</td>
<td>0,466</td>
<td>0%</td>
</tr>
<tr>
<td>eBAC 4.1</td>
<td>mar-05</td>
<td>13:45</td>
<td>7,58</td>
<td>-33,6</td>
<td>336</td>
<td>181</td>
<td>53,4</td>
<td>0,254</td>
<td>71%</td>
</tr>
<tr>
<td>eBAC 4.2</td>
<td>mar-06</td>
<td>14:00</td>
<td>7,84</td>
<td>-49</td>
<td>247</td>
<td>170</td>
<td>45,5</td>
<td>-</td>
<td>79%</td>
</tr>
<tr>
<td>eBAC 4.3</td>
<td>mar-07</td>
<td>10:15</td>
<td>7,94</td>
<td>-51,7</td>
<td>124</td>
<td>147</td>
<td>47</td>
<td>0,656</td>
<td>89%</td>
</tr>
</tbody>
</table>

#### Open circuit reactor: forth batch

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>Day</th>
<th>Time</th>
<th>pH</th>
<th>mV</th>
<th>COD</th>
<th>Phosphate</th>
<th>Ammonia</th>
<th>Nitrate</th>
<th>COD Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>eBAC C 4.0</td>
<td>mar-04</td>
<td>14:05</td>
<td>7,1</td>
<td>-5,6</td>
<td>1144</td>
<td>151</td>
<td>Abs. &gt; 3,5</td>
<td>0,261</td>
<td>0%</td>
</tr>
<tr>
<td>eBAC C 4.1</td>
<td>mar-05</td>
<td>13:45</td>
<td>7,69</td>
<td>-39,5</td>
<td>290</td>
<td>210</td>
<td>53,9</td>
<td>0,165</td>
<td>75%</td>
</tr>
<tr>
<td>eBAC C 4.2</td>
<td>mar-06</td>
<td>14:00</td>
<td>7,97</td>
<td>-56,6</td>
<td>68</td>
<td>136</td>
<td>57</td>
<td>-</td>
<td>94%</td>
</tr>
<tr>
<td>eBAC C 4.3</td>
<td>mar-07</td>
<td>10:15</td>
<td>8,07</td>
<td>-59,3</td>
<td>77</td>
<td>191</td>
<td>60,5</td>
<td>0,209</td>
<td>93%</td>
</tr>
</tbody>
</table>
5.1.1 COD reduction

At the beginning of the experiment both reactors can be considered to work under same conditions, given that the closed circuit reactor is not yet acclimated. Indeed, they show pretty close behaviors: around 40% COD reduction after 24 hours, 55% after two days and approximately 75% the third day. The slight difference between them can be justified due to variables that are difficult to control such as: natural difference of the reactors, sampling action, inaccuracies when sample testing and lack of precision of software and hardware used, among others.

In a more advanced stage of the experiment, at the forth batch, it can be seen that COD reduction efficiency is considerably improved. The open circuit reactor shows a COD reduction of 75%, 94%, 93% corresponding to the first, second and third consecutive day of treatment. The value of 94% of reduction after 48 hours is probably wrong and corresponds to an inaccuracy when preparing the vials to test COD concentration of the sample, given its magnitude. At the same time, the closed circuit reactor shows the similar respective values of 71%, 79% and 89%. However, at this point the MFC-working-like reactor is in the middle of its acclimation phase and gives a considerable voltage (around 400 mV) and so, as it has been claimed by many papers from the MFC community [77, 15, 43] that MFCs tend to show most of the times a significant improvement
in regard to COD removal compared to an open circuit control reactor, it should show an improved behavior too, even though it does not.

In a final phase, when the closed circuit reactor is practically acclimated and works as an MFC, the COD removal performances of both reactors stay still close to each other reaching an average of 75% removal after only 24 hours and 90% after two days of operation. The closed circuit reactor does not show an improved COD removal efficiency compared to the open circuit one in this case, either, even when fully working as a stable MFC.

The overall deductions that can be extracted are:

1st: The percentage of COD removal/time increases over time in both reactors reaching a 90% removal in 24 hours, after six substrate batches and 19 days of operation. This means that biochar can greatly accept and retain substrate particles without getting easily clogged, although it must be said that total biochar adsorption capacity is not reached during this first experiment. The increase in COD removal efficiency over time can be explained by microbiological activity. This means that the more the biofilm grows into the cambers, the more bacteria there are to “eat” the substrate and thus, the COD (“food”) is over sooner.

2nd: Regardless of if the reactor is operated either under closed or open circuit, the responses of both reactors regarding reduction of chemical oxygen demand are very similar and as commented, no improvement is shown by the closed circuit reactor. This fact can be justified by considering that the biochar filtration and adsorption behavior, together with the microbiological activity, play the main role in regard to COD removal. The fact that the closed circuit reactor works as an MFC and has an additional contribution to COD removal is certain, but in this case it is not significant compared to the other parallel mechanisms.

3rd: Taking into account that the COD standard for the effluent of current wastewater treatment plants is COD<125mg/l, the performance of both reactors can be considered pretty good, given that at the end of the experiment they only needs 2 days or even a bit less to reach such a value. However, it is remarkable that acetate is easier to decompose than real wastewater, which means that the reactor performance may not be as good if it were run with real sewage. In
any case, this is tested in the third experiment.

5.1.2 pH

The pH of the different batches prepared to run the reactor show usual pH values around 7, as expected (acetate solution made with reverse osmosis tap water). However, as water keeps treating, in both cases its pH values rise over time and reach values close to 8, turning then into more basic water. This phenomenon has been also observed in experiments where water is treated with activated carbon, whose properties, as explained in previous background section, are very similar to biochar, and it is said it is due to an interaction between the naturally occurring anions and protons in the water and the carbon surface. This interaction can be described as an ion exchange type of phenomenon, in which the carbon surface sorbs the anions and corresponding hydronium ions from the water [56]. Thus, given the similitude of the activated carbon granules and the biochar particles regarding their characteristics, the same explanation can apply in this case.

5.1.3 Ammonia, nitrate and phosphate

According to the occasional tests done for identification of concentrations evolution of ammonia, nitrate and phosphate, the results obtained are unfortunately not easily readable due to their fluctuations. Apparently, a slight decrease of ammonia concentration is observed in most cases but the tendencies that nitrate and phosphate follow, are pretty unclear. The reduction of ammonia could be explained by the nitrification process due to the aerobic environment reactors have in cathode chambers, as noticed in previous MFC studies [72]. However, according to this theory this ammonia reduction should go by the hand with an increase of nitrate, which is not clearly proved by extracted data. It is certain that more data collected for concentration checks and a longer operation time per batch, would have helped to get more interesting conclusions in this direction.
5.1.4 Color and odor

Color and odor are also important aspects to consider when treating wastewater. In this sense, no tools are available this time to test the reactor performance in these aspects. However, it can be said that an important color reduction is observable, as shown in figure 5.1, thanks to biochar’s physical and chemical adsorption capacity. As neither fresh substrate solution nor water after treatment have a particular smell, no relevant observation is made regarding odor.

![Figure 5.1: (a) Initial substrate solution; (b) Treated water after 90% COD removal.](image)

5.1.5 Acclimation stage

The acclimation of the closed circuit reactor is reached after 7 batches and 20 days of operation, at a resistor of 100 Ohm. The reactor gives a stable voltage of 0.680 Volts.

5.2 Second experiment results: MFC reactor energy generating potential

This experiment aims to put the previous acclimated closed circuit reactor under continuous flow rate conditions, so that its maximum power generating performance can be tested by means of a polarization curve.
5.2.1 First polarization curve

In this first trial to develop the polarization profile, the reactor response will be checked under a wide range of different resistors, open circuit included, which will be switched following a descending order. Half-cell potentials (anode and cathode potentials) should ideally be measured setting the reference electrode as close as possible to the electrode (biochar particles) but given the complex design of the reactor, the anode potential will be checked by introducing the reference electrode into the anode through a small hole done on the plastic mesh on top of the cylinder. The reference electrode will be at the same time surrounded by a pipette so direct contact with the biochar particles is avoided. On the other hand, cathode half-cell potential will be measured directly from the cathode-recirculation bucket. The stabilization time left between resistors will be of 60 minutes. Following this operation schedule, the data obtained is presented in next table 5.4.

<table>
<thead>
<tr>
<th>Resistor (Ω)</th>
<th>Voltage (V)</th>
<th>AN h-c Pot. (V)</th>
<th>CATH h-c Pot. (V)</th>
<th>H-c Pot. Difference (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OC</td>
<td>0.748</td>
<td>-0.432</td>
<td>0.335</td>
<td>0.767</td>
</tr>
<tr>
<td>3000</td>
<td>0.746</td>
<td>-0.434</td>
<td>0.335</td>
<td>0.769</td>
</tr>
<tr>
<td>2000</td>
<td>0.744</td>
<td>-0.439</td>
<td>0.329</td>
<td>0.768</td>
</tr>
<tr>
<td>1000</td>
<td>0.739</td>
<td>-0.444</td>
<td>0.325</td>
<td>0.769</td>
</tr>
<tr>
<td>500</td>
<td>0.731</td>
<td>-0.441</td>
<td>0.320</td>
<td>0.761</td>
</tr>
<tr>
<td>300</td>
<td>0.718</td>
<td>-0.444</td>
<td>0.315</td>
<td>0.759</td>
</tr>
<tr>
<td>100</td>
<td>0.681</td>
<td>-0.445</td>
<td>0.310</td>
<td>0.755</td>
</tr>
<tr>
<td>10</td>
<td>0.442</td>
<td>-0.445</td>
<td>0.300</td>
<td>0.745</td>
</tr>
</tbody>
</table>

Table 5.4: Reactor’s polarization curve using acetate solution, first attempt.

Before analyzing data, the first issue to take into account is that the half-cell potentials measured cannot be considered accurate due to the alternative measuring methods used. However, they should be approximate and good enough to have a reference of the reactor status. Thus, the half-cell potentials’ difference should theoretically equal the voltage of the whole system but in this case, if approximate values are proven, they will be considered valid.

The open circuit cell voltage is observed to be around 750 mV, which is a reasonable range for an air-cathode MFC system [65]. It can be also seen that both anode and cathode half-cell potentials
don’t show representative variations and they stay curiously around the same values, though. This observation claims that they have not been measured properly and indeed, they happen to correspond to open circuit values due to a misunderstanding when connecting the multimeter wires with the MFC external circuit. Another prove of it is that the potentials’ difference also stays the same and its values differ from the whole-cell voltage shown, specially at low resistors values.

Moreover, the voltage given by the reactor at low resistors is suspicious because it is significantly too high compared to usual values obtained in other MFC studies. The reason found to explain this magnitude is that not enough time is left before switching resistor, which means that the reactor needs more time for stabilization at each resistor.

After learning from all mistakes committed during this first attempt to extract the polarization curve, it is time to start over and repeat the test to obtain proper results.

5.2.2 Second polarization curve

In this second attempt the system will be also fed continuously with acetate solution and half-cell potentials will be correctly measured at closed circuit conditions. Time between switching resistors will be determined by the time it takes the reactor to stabilize at each resistor. Stabilization is understood as the status at which the device does not show variations of voltage any longer and so, this latter is stable. This time, more resistors will be chosen to conform the curve. The results obtained are shown in table 5.5.

Taking a look at the full-cell voltage column, all values can be considered reasonable and it is proven that the high voltage values obtained at low resistors in the previous attempt were wrong. The cathode half-cell potential data do not show any anomaly, either, and are within a common range for MFC cathode performance, even maybe a bit improvable.

However, the half-cell potential difference is far away from the actual voltage shown by the cell, which again complicates the validity of this second attempt. Thus, in this case the problem comes from the anode half-cell potentials measured, which according to the book ”Microbial Fuel Cells” by Bruce E. Logan [44], has a theoretical minimum voltage around -0,500 Volts. Unfortu-
nately, most of the anode potentials measured are out of this possible range and consequently must be discarded as valid data.

The method used to measure the anode potential is then considered not appropriate for its purpose. This is why it is necessary to carry out another attempt to draw the polarization curve, measuring the anode potential right from the substrate bucket injected to the anode, as done with the cathode potential, methodology which is not accurate, as explained, but should give more actual and representative results.

### 5.2.3 Third polarization curve

In the third and last attempt to extract a valid curve, the voltage will be checked through fewer but still enough resistors in order to complete the test as soon as possible and be able to obtain representative data, due to the fact that an important performance weakening has been already observed after the second attempt, compared to the previous one. With the same purpose of saving time, checked resistors will follow an ascending order given the fact that the device is already stabilized at 10 Ohm at the end of the second attempt and thus, the study can be immediately started. Time between resistors will also depend on reactor’s stabilization period needed and anode and cathode half-cell potentials will be measured by immersing the reference electrode right into
the respective substrate and recirculation buckets. The results are shown in next table 5.6.

<table>
<thead>
<tr>
<th>Resistor (Ω)</th>
<th>Voltage (V)</th>
<th>AN h·c Pot. (V)</th>
<th>CATH h·c Pot. (V)</th>
<th>H·c Pot. Difference (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OC</td>
<td>0.380</td>
<td>-0.200</td>
<td>0.170</td>
<td>0.370</td>
</tr>
<tr>
<td>50000</td>
<td>0.378</td>
<td>-0.205</td>
<td>0.170</td>
<td>0.375</td>
</tr>
<tr>
<td>5000</td>
<td>0.407</td>
<td>-0.200</td>
<td>0.210</td>
<td>0.410</td>
</tr>
<tr>
<td>1000</td>
<td>0.405</td>
<td>-0.200</td>
<td>0.200</td>
<td>0.400</td>
</tr>
<tr>
<td>500</td>
<td>0.391</td>
<td>-0.190</td>
<td>0.200</td>
<td>0.390</td>
</tr>
<tr>
<td>100</td>
<td>0.380</td>
<td>-0.180</td>
<td>0.200</td>
<td>0.380</td>
</tr>
<tr>
<td>50</td>
<td>0.361</td>
<td>-0.170</td>
<td>0.190</td>
<td>0.360</td>
</tr>
<tr>
<td>30</td>
<td>0.337</td>
<td>-0.165</td>
<td>0.170</td>
<td>0.335</td>
</tr>
<tr>
<td>10</td>
<td>0.278</td>
<td>-0.160</td>
<td>0.120</td>
<td>0.280</td>
</tr>
</tbody>
</table>

Table 5.6: Reactor’s polarization curve using acetate solution, third attempt.

After a first look over the half-cell potentials, no strange values are fortunately observed this time and they fall within theoretically possible ranges. Moreover, the half-potentials’ difference is generally pretty close to the voltage showed by the full reactor, which is expected and makes sense. In this direction, it can be said that all data has been properly collected during the test, always considering that the difference between voltage and half-potential values is due to the inaccuracy of the measuring method practised.

However, it can be noticed that the cell voltage has diminished significantly comparing to the values obtained during the first and the second attempt. Thus, this is an usual behaviour of microbial fuel cells and is due to the wearing out of the electrodes, biochar particles in this case, and a possible clogging of the PEM by biofilm formation on its surface.

In fact, this third test has been carried out during an advanced phase of reactor’s performance extinction. As the test is developed, from low to high resistors, it can be observed that the voltage jumps become closer and closer until voltage values at highest resistor and open circuit are lower than the one at 5000 Ohm. The maximum voltage obtained out of an MFC is always at open circuit conditions, which is equivalent to infinite resistance for the electrons. For this reason, the drop of the voltage during the last stage of the test can be explained by this unavoidable wearing out of the reactor.
In this sense, taking a look at the cathode half-cell potentials, it can be seen that the potential grows as the resistor value increases, but at the last part of the test (at 50000 Ohm and open circuit conditions) it sinks and consequently causes a relevant effect to the voltage drop. An important contribution to the cathode wearing out could be explained by the formation of bacteria colonies observed spreading over the stainless steel external mesh (figure 5.2), which clogs the mesh holes, complicates the oxygenation of the water through the cathode and consequently, reduces the electron acceptors present in the chamber and less electrons (less voltage) pass through the external circuit.

![Figure 5.2: Bacteria colonies on cathode external mesh.](image)

The polarization curve is used to characterize current as a function of voltage \(I=V/R\) but doesn’t give clear information about the power output of the reactor. That is why, in order to know its power output a power density curve is developed from the polarization curve. As power is \(P=V\cdot I\) and \(I=V/R\), then \(P=V^2/R\) and this value is normalized together with the current \(I\) so as to obtain respectively the reactor’s power density and current density. The normalization is done by volume, dividing such values by the anode chamber wet (liquid) volume, 695.7 \(cm^3\) (Anode
chamber volume=975.5 cm$^2$; Anode biochar volume= 280 cm$^2$). The resulting graphics are shown in figure 5.3.

![Figure 5.3: Voltage and power density as functions of current density of the reactor using acetate solution.](image)

The voltage obtained with this reactor, specially during the first 2 attempts, is comparable with others obtained from in-series-connected stack MFC systems (using graphite granules electrode and hexacyanoferrate cathode) [50], upflow MFC systems (using reticulated vitreous carbon as electrode) [79] or a single-chamber air-cathode MFC (using carbon cloth as electrode and a mixture of Pt/C catalysts) [63]. However, the configuration of this reactor is very simple and its price is greatly cheaper compared to others that use delicate and expensive electrodes or need not-sustainable catalysts to reach such voltage values.

On the other hand, the power output is usually normalized by surface area of the anode, but this data is not available for the biochar particles used in this study and so, the comparison of this power density curve with other studies is not always direct. However, the shapes of power density curves are comparable and it is remarkable that in most MFC studies the power density curves show a power crest between the lowest and the highest current density values, caused by cathode overpotentials, which means that cathode cannot keep up with the amount of electrons coming from the anode. This phenomenon usually occurs at lower resistances and ends up decreasing the power density, as it can be seen in studies like [79] or [27]. However, this crest curve is not observable in
this case and the reason can be attributed to very little cathode overpotential, which means that the cathode has a good performance in regard to electrons acceptance, even at low resistances.

5.3 Third experiment results: MFC Reactor behaviour when using wastewater as substrate

As explained in section 4, a brand new reactor is built for this last experiment, which consists of testing the reactor performance under real conditions using wastewater from the brewing industry as substrate, source of carbon and energy.

In the first part of the experiment, during the acclimation of the system, its behaviour will be examined in regard to wastewater treatment and its possible performance changes over the acclimation phase. As proceeded for the second experiment analysis of wastewater treatment results, a selection of all data collected and tested along the experiment is done, so as to present representative and clear results and perform a proper evaluation. Thus, data from the first, third and fifth wastewater batch are presented in respective tables 5.7, 5.8 and 5.9. Moreover, all data collected during the experiment can be found attached to this document as Appendix A.

In the second part of the experiment, once the reactor is fully acclimated, its energy generating performance will be tested and a polarization curve study will be developed.

<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C1.0</td>
<td>abr-20</td>
<td>17:00</td>
<td>7.35</td>
<td>-17.5</td>
<td>1210</td>
<td>17,6</td>
<td>1,56</td>
<td>31,7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C1.1</td>
<td>abr-21</td>
<td>17:00</td>
<td>7.74</td>
<td>-39.5</td>
<td>575</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>52%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C1.2</td>
<td>abr-22</td>
<td>17:00</td>
<td>8.67</td>
<td>-93</td>
<td>175</td>
<td>14,8</td>
<td>1,67</td>
<td>19,2</td>
<td>86%</td>
<td>16%</td>
<td>-8%</td>
<td>39%</td>
</tr>
</tbody>
</table>

Table 5.7: Wastewater treatment data along the the first batch (Concentrations in mg/l).

5.3.1 COD reduction

At the beginning of the experiment, at the first contact of sewage with biochar, it seems that the device needs an "adaptation time" and the COD reduction is slower than when biochar particles are completely soaked. However, it still performs a removal of around 50% after 24 hours
Table 5.8: Wastewater treatment data along the third batch (Concentrations in mg/l).

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>C3.0</td>
<td>abr-27</td>
<td>14:00</td>
<td>7.6</td>
<td>-31.4</td>
<td>1250</td>
<td>14.9</td>
<td>1.44</td>
<td>52.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C3.1</td>
<td>abr-28</td>
<td>14:30</td>
<td>8.13</td>
<td>-61</td>
<td>413</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>67%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C3.2</td>
<td>abr-29</td>
<td>14:00</td>
<td>8.24</td>
<td>-68.5</td>
<td>171</td>
<td>6.47</td>
<td>2.95</td>
<td>2.33</td>
<td>86%</td>
<td>57%</td>
<td>-105%</td>
<td>96%</td>
</tr>
<tr>
<td>C3.3</td>
<td>abr-30</td>
<td>14:30</td>
<td>8.44</td>
<td>-79.8</td>
<td>114</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>91%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C3.4</td>
<td>may-01</td>
<td>14:00</td>
<td>8.8</td>
<td>-102.1</td>
<td>42</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>97%</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C3.5</td>
<td>may-02</td>
<td>14:00</td>
<td>8.6</td>
<td>-92.2</td>
<td>14</td>
<td>1.94</td>
<td>13</td>
<td>0.233</td>
<td>99%</td>
<td>87%</td>
<td>-803%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 5.9: Wastewater treatment data along the fifth batch (Concentrations in mg/l).

As of the second batch, the COD removal efficiency after 24 hours increases and reaches a mean of 70%, which stays over the rest of batches. The COD removal after 48 hours of treatment is around 90%, as it is seen in all cases. Using wastewater instead of acetate shows that, although the reactor is surprisingly acclimated much faster, no improvements of the treatment due to the presence of an MFC system are observed, either.

The main extracted conclusions are:

1st: The percentage of COD removal/time increases much faster over time compared to the acetate-run reactor. In fact, after only one batch, the COD reduction in 24 hours is already of 90% and such a performance is repeated through the other batches, without dropping. This can be explained because real wastewater contains a lot more live microorganisms that facilitate and boost the acclimation phase, so the biofilms are rapidly formed and grown into the chambers. Thus, the microbiological activity is high right from the beginning and there are plenty of bacteria available to consume the substrate and consequently reduce the chemical oxygen demand. Again,
biochar particles show a proper behaviour treating real wastewater and accept and retain substrate particles without getting easily clogged. Biochar’s total adsorption capacity is not reached during this experiment, either.

2nd: No improvement regarding COD removal is observed either along the acclimation of the reactor or once fully working as a microbial fuel cell. The explanation for this is the same found for the acetate-run system, so the microbiological activity, biochar’s filtration and adsorption play the main role here, making any other additional contribution to COD removal little significant.

3rd: Considering that the COD standard for the effluent of current wastewater treatment plants is COD<125mg/l and that current wastewater treatment plants are characterized by a hydraulic retention time (HRT) between 8 and 24 hours, depending on their size, the treatment performance of this simple reactor using real wastewater is impressive, given that such a value is reached after only 2 days of treatment, or a little bit more in some cases. It is still far from being comparable to current HRTs but the reactor potential is admirable and cannot be denied in any case.

5.3.2 pH

The pH of the raw wastewater tends to be a little more basic compared to the acetate solution used for the first experiment. Its values are usually closer to pH=8 due to its industrial origin, but the range still stays between 7 and 8. However, as the wastewater is treated the pH values increase and it becomes significantly basic, reaching values over pH=8 after 24 hours and a pH of approximately 8.6 after 4-5 days of treatment in all batches.

This pH evolution has been also previously shown when using acetate in the first experiment and as explained, it is a phenomenon that is commonly seen in experiments where water is treated with activated carbon, material which is very similar to biochar. The reason is that there exists an ion exchange interaction between the biochar and the water, in which the carbon surface sorbs the anions and corresponding hydronium ions from the water [56].

The higher pH range reached using wastewater also results in some not desired consequences,
such as corrosion, which is easily perceptible on the external cathode wall at the end of the experiment, formed of assumed stainless steel mesh (which in practise has turned out to be not as good), as it can be observed in figure 5.4. According to Baylis curve (figure 5.4), the pH values obtained from samples fall into the corrosive area if considering a normal alkalinity, fact that can explain the oxidation of the mesh.

Figure 5.4: (a) Baylis curve; (b) Corrosion observed over cathode outer wall at the end of the experiment.

5.3.3 Ammonia, nitrate and phosphate

Unlike the ambiguous data, regarding minerals, extracted from the first experiment carried out using acetate, this time the reactor behaviour is consistent and surprising.

Data shows a clear nitrification process, usual in cathode chambers of aerobic environment reactors [72], where an ammonia reduction (up to 100% in 4-5 days) is accompanied by a nitrate increase (up to a mean of 550% in 4-5 days), component which remains in water after treatment.
at a mean concentration of 10 mg/l, value that is not negligible and has to be taken into account. Chemical and physical adsorption performed by biochar particles contribute to a faster ammonia drop but a slower ammonia increase.

On the other hand, biochar particles adsorb over 50% of phosphate traces after a couple of days of operation and reach 90% reduction over 4 or 5 days of treatment.

The reactor apparently seems to need a running-in stage during the first batch and as of the second batch, the system performs normally, as it can be observed in tables 5.7, 5.8 and 5.9.

5.3.4 Color and odor

As it has been already proved through the results of the first experiment, reactor potential of eliminating color and odor from wastewater is relevant. In spite of not having the right instruments to test this components along the experiment, a significant color reduction is observable at first sight, as shown in figure 5.5, thanks to biochar’s physical and chemical adsorption capacity. Furthermore, an important reduction of the bad smell of the sewage is remarkable.

Figure 5.5: Right: Raw wastewater; Left: treated wastewater after 4 days of operation.
5.3.5 **Acclimation stage**

The acclimation of the reactor turns out to be much faster using industrial wastewater than acetate solution. At a resistor of 500 Ohm, the system stabilizes at a voltage of 0.650 volts after one batch and 5 days of operation.

5.3.6 **Energy generating performance test**

The electrical potential of the reactor is measured by developing a polarization curve. However, as the system cannot be provided with continuous flow, the data obtained will be considered approximate and guiding, which will allow us to get an idea of the reactor behaviour. Thus, the electrical test will be performed at the same time the samples are taken. Given the fact that the substrate is all already gone by the time the device is stabilized after starting a new batch, it happens to be impossible to collect anode half-cell potentials. Consequently, only the whole cell voltage and the cathode half-cell potential will be measured, this latter with the reference electrode and from the recirculation bucket, as proceeded in the previous experiment.

**Polarization curve**

Resistors will be checked in descending order to conform the polarization curve and time between switches will be determined by the time needed for reactor’s stabilization at each resistor value. The results are shown in next table 5.10.

Voltage and cathode half-cell potential values obtained fall within theoretically possible ranges and they are similar to those obtained during the second experiment using acetate. Thus, at first sight data can be considered reasonable. As anode half-cell potentials are not available, the coincidence of the half-cell potentials’ difference with the voltage cannot be proved. However, assuming that the measured half-cell potentials are correct, anode potentials should be equal or close to each difference between the voltage and the cathode potential (AN. h-c Pot. = CATH. h-c Pot. - Voltage). It is also observed that as the resistor increases, the voltage rises together with the cathode potential.
Table 5.10: Reactor’s polarization curve using industrial wastewater.

<table>
<thead>
<tr>
<th>Resistor (Ω)</th>
<th>Voltage (V)</th>
<th>CATH h-c Pot. (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OC</td>
<td>0.671</td>
<td>0.310</td>
</tr>
<tr>
<td>50000</td>
<td>0.670</td>
<td>0.310</td>
</tr>
<tr>
<td>5000</td>
<td>0.668</td>
<td>0.300</td>
</tr>
<tr>
<td>1000</td>
<td>0.665</td>
<td>0.295</td>
</tr>
<tr>
<td>500</td>
<td>0.627</td>
<td>0.221</td>
</tr>
<tr>
<td>100</td>
<td>0.550</td>
<td>0.237</td>
</tr>
<tr>
<td>50</td>
<td>0.387</td>
<td>0.230</td>
</tr>
<tr>
<td>30</td>
<td>0.228</td>
<td>0.250</td>
</tr>
<tr>
<td>10</td>
<td>0.115</td>
<td>0.235</td>
</tr>
</tbody>
</table>

Power density curve

In order to analyze the power output extracted from the wastewater-run reactor, a power density curve is developed from the polarization curve by applying the formula $P = \frac{V^2}{R}$ and normalizing the result, and also the respective current ($I = \frac{V}{R}$), by dividing such values by the anode chamber wet (liquid) volume, 695.7 cm$^3$. The resulting graphics are shown in figure 5.6.

Figure 5.6: Voltage and power density as functions of current density of reactor using wastewater.

The voltage obtained with this industrial-brewery-wastewater-run reactor, is comparable to the values obtained in other studies also using wastewater as substrate but utilising much more complex and expensive systems, such as an upflow microbial fuel cell with an interior cathode (using
activated carbon granules as electrodes and platinum-coated carbon paper) [80], a single chamber membrane-free microbial fuel cell (using carbon cloth anode electrode and air cathode containing Pt catalyst) [74] or a normal two-chambered MFC (using electrodes made of carbon paper and continuous aeration and a Pt catalyst at the cathode) [8]. The simplicity and economy of this reactor make it a truly competitive alternative.

Furthermore, the shape of the power density curve is this time similar and comparable to most obtained in other studies, showing a power crest at a middle resistor, 100 Ohm in this case, which determines the optimum resistance for the system regarding energy production. The power density drop at low resistors is caused by a cathode overpotential in which cathode cannot keep up with the electrons coming from the anode chamber and thus, less power is produced.
Chapter 6

Conclusions

The results extracted from the experiments carried out to test the performance of this novel reactor, have been surprising and not disappointing at all. Regarding wastewater treatment its potential is outstanding in spite of being a bit slower than the aeration method of current sewage treatment plants, reaching 90% COD reduction in only 48 hours of operation and an excellent mineral removal (>90%) of ammonia and phosphate after 4-5 days of recirculation. However, a nitrification process has also been observed when treating real wastewater and consequently a considerable increase of nitrate traces is produced, issue that should be considered in a hypothetical future reactor design improvement study. In regard to electricity generation, reactor’s behaviour is acceptable but still far from being representative, with a maximum power density of 4.5 W/m$^3$.

The materials used to build the system are all accessible, economic and easy to assemble. No expensive electrode or catalyst (like graphite foams, reticulated vitreous carbon electrodes or Pt catalyst) is used, which makes it a lot competitive compared to other MFC designs. The proton exchange membrane is the main element that sets its cost, due to its 20 US$ foot$^{-2}$ market price.

Biochar granules tested as MFC electrode material have shown good behaviour allowing a satisfactory maximum power density (4.5 W/m$^3$) comparable to other experiments’ values obtained with graphite granules (6.5 W/m$^3$ [70]) or granular activated carbon electrodes(7.32 W/m$^3$ [70]). The cost of biochar is significantly less than the two above mentioned materials due to its cheap and accessible raw material and its quick and easy manufacturing process. Moreover, biochar has environmental benefits such as biowaste feedstock, energy positive manufacturing, carbon seques-
tration capacity or land application as fertilizer, but more research has to be still developed to improve the electrode production manufacturing method, as well as its performance.

The success of specific MFC applications in wastewater treatment depends on the concentration and biodegradability of the organic matter in the influent, the wastewater temperature, and the absence of toxic chemicals. Waste-driven applications require mainly significant removal of the waste substrate, objective that is clearly achieved with this novel MFC reactor design. However, an equilibrium of the processes the carbon source is removed from water should be found, because all organic and inorganic matter adsorbed and retained by biochar particles are not converted to electrons and so, power. As seen through the experiments’ results, biochar adsorption plays a huge role in regard to COD reduction, fact that on one hand is very positive for water treatment but, on the other hand, diminishes the carbon supply for the MFC system, destined to produce energy.

Currently, when applying conventional aerobic treatment, ~1 kWh of energy is needed for oxidation per kilogram of carbohydrate present. For example, treatment of domestic wastewater represents an aeration energy cost of ~0.5 kWh per m$^3$, amounting to an energy use of the order of 30 kWh per capita per year (about 4 US$ energy cost per capita per year) [38]. For practical implementation high voltages and currents are required but neither the ones showed by the tested reactor nor any others from alternative MFC studies are of this order of magnitude, yet.

Thus the reactor design tested in this research is characterized more by its simplicity, its economy and the fact of merging two research fields, such as microbial fuel cells and wastewater treatment, rather than its electrical and treatment performance, which can be overall considered still good taking into account its easy configuration. Although the reactor design is still far from any feasible real-scale application, there would exist enough reasons to develop further research on the design aspects and the materials used for its configuration, so that performance improvements could be achieved in the future.

In the long run, microbial fuel cells have potential to evolve to convert wastewater treatment from an energy consumer to a net energy producer, although much research and many technological improvements are needed to enhance the biological understanding, improve the electrochemical
technology, decrease the overall electrode prices, build viable scale-ups and finally increase the power output towards a stable 1kW per $m^3$ of reactor, which is thought the MFC feasibility threshold.

Over the next upcoming years, it may be that the electricity produced by MFCs will not be a cost effective source of energy in its own right but reactors may be used to contribute to reduce the costs of energy used in wastewater treatment, specially obviating the need to aerate the sludge produced as a by-product. Taking into account that wastewater treatment represents 3-5\% of the total energy consumption of a developed country (It is estimated that about 45 US$ billion is needed in the U.S. for wastewater infrastructure improvements in addition to current annual costs of 25 US$ billion. [26]) , any contribution done by MFC systems could be seriously relevant.
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