Integrated Microbial Electrolysis Cell (MEC) with an anaerobic Membrane Bioreactor (MBR) for low strength wastewater treatment, energy harvesting and water reclamation

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ABSTRACT

Integrated Microbial Electrolysis Cell (MEC) with an anaerobic Membrane Bioreactor (MBR) for low strength wastewater treatment, energy harvesting and water reclamation

Rodrigo José Jiménez Sandoval

Shortage of potable water is a problem that affects many nations in the world and it will aggravate in a near future if pertinent actions are not carried out. Decrease in consumption, improvements in water distribution systems to avoid losses and more efficient water treatment processes are some actions that can be implemented to attack this problem. Membrane technology and biological processes are used in wastewater treatment to achieve high water quality standards. Some other technologies, besides water treatment, attempt to obtain energy from organic wastes present in water.

In this study, a proof-of-concept was accomplished demonstrating that a Microbial Electrolysis Cell can be fully integrated with a Membrane Bioreactor to achieve wastewater treatment and harvest energy. Conductive hollow fiber membranes made of nickel functioned as both filter material for treated water reclamation and as a cathode to catalyze hydrogen production reaction. The produced hydrogen was subsequently converted into methane by hydrogenotrophic methanogens. Organic removal was 98.9% irrespective of operation mode. Maximum volumetric hydrogen production rate was 0.2 m$^3$/m$^3$d, while maximum current density achieved was 6.1 A/m$^2$ (based on cathode surface area). Biofouling, an unavoidable phenomenon in traditional MBRs, can be minimized in this system through self-cleaning approach of hybrid
membranes by hydrogen production. The increased rate of hydrogen evolution at high applied voltage (0.9 V) reduces the membrane fouling. Improvements can be done in the system to make it as a promising net energy positive technology for the low strength wastewater treatment.
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1.0. INTRODUCTION

Nowadays, the world is facing a number of problems related to the quantity and quality of water that is available for human consumption. Drinking and agricultural water that comes from fresh water represents only 2.5% of the total water available on the planet. Throughout the world, water is being consumed in large amounts at such a fast rate that it cannot be replenished by precipitation, therefore, the scarcity of freshwater is increasing. The United Nations has estimated that two-thirds of the Earth’s population will be living in water-stressed conditions by the year 2025. Hence, the reclamation, reuse and recycling of water remain important processes that can help avoid or diminish water scarcity problems. The reuse and recycling of wastewater can result in a source of water that in many cases is lost when it returns to the natural environment. Wastewater should not be considered as waste because from it, water, nutrients, energy and even bioplastics can be recovered (Pechan et al, 2013). Therefore, wastewater must be reclassified as a renewable resource instead of as simple waste (Abu Madi et al, 2009).

Saudi Arabia has not enough freshwater (from rivers or lakes) to sustain its population of 25.7 million. In 2010, Saudi Arabia consumed approximately ten times more water than its renewable water resources (20 billion m$^3$ consumed compared to 2.4 billion m$^3$ renewable water resources), making it the third largest country with exceeded water usage per capita. Most of the water supply in Saudi Arabia comes from non-renewable groundwater aquifers. Due to the rapid population growth and the expansion of cities, water demands have increased in recent years and are expected to double over the next 20 years, with municipal and industrial sectors increasingly reliant on water desalination. Considering the above, the government of Saudi Arabia should encourage the reuse and recycling of wastewater in industries through economic incentives and better cleaning standards. (Kajenthira et al, 2011).

1.1. Wastewater treatment generalities

Adequate treatment of wastewater is required for proper reuse and recycling. Currently, a variety of methods have been developed and new methods are being developed for water treatment. Water is usually treated both for public supplies and also before it goes back to the environment. Conventionally, water treatment is a sequence of processes that help to remove pollutants in water (Figure 1). The methods used depend on the cleaning objectives and the type (quality) of wastewater needed. In general, the methods used to remove constituents in water and wastewater can be classified as physical, chemical and biological (Crittenden et al, 2005).
In a domestic wastewater treatment plant, the wastewater needs to pass through different stages: pretreatment or preliminary treatment, primary treatment, secondary treatment and advanced or tertiary treatment (EPA, 2004).

During the preliminary treatment a screen is used to remove big coarse solids like cans, bottles or sticks. When the screening is completed, the water flows into a chamber where the sand, grit and other small stones settle down. At the end of this stage, the water still contains dissolved organic and inorganic matter and suspended solids (EPA, 2004).

The next stage is the primary treatment. Water flows into a primary sedimentation tank where the flow slows down to allow the sedimentation of suspended solids to the bottom, to form primary sludge. Instead of use gravity force to remove suspended solids, the chemical processes of coagulation and flocculation can be applied (EPA, 2004).

After the primary treatment is completed, the following stage (the secondary treatment) is based on biological processes that can remove up to 90% of the organic matter in wastewater. There are two main methods that are used to achieve organic matter removal: attached growth processes and suspended growth processes. In the attached growth process, bacteria form a
biofilm in a plastic surface or stones. Wastewater passes over the microorganisms along with air, needed by them to metabolize the organic matter that is found in the wastewater. In the suspended growth process, microorganisms grow in suspension in a chamber where air is vigorously mixed with wastewater: the aeration tank (EPA, 2004). All the microorganisms (bacteria, protozoa and other microbes) are collectively referred as activated sludge. The growth of the microorganisms in the aeration tank is a consequence of the uptake of small organic carbon molecules by the bacteria. The final result is clean water (Davies, 2005).

After the mixed liquor, i.e. microbial mass together with wastewater, is retained for a determined time in the aeration tank, it flows into a settling tank or secondary clarifier, in which the activated sludge settles down. This process produces an effluent that can be treated further depending on the desired water quality (Sustarsic, 2009).

To kill pathogens that are present in wastewater, a disinfection process is used. Chlorine, ozone and ultraviolet radiation are some techniques that are used to achieve disinfection (EPA, 2004).

Following these processes, wastewater occasionally needs to be further treated in what is called tertiary or advanced treatment. Tertiary treatment is used to remove the constituents of wastewater that could not be significantly removed during secondary treatment. These include residual suspended solids or biological nutrients (nitrogen, phosphorous) treated with chemical methods and biological processes respectively (EPA, 2004).

Several new technologies have been developed to improve the wastewater treatment process, specifically referring to biological processes. One of the new technologies that is still developing for wastewater treatment is the membrane bioreactor (MBR). This technology combines a physical method, that is the membrane separation process, and a biological method, using activated sludge to degrade organic compounds. Before discussing this technology further, it is necessary to elucidate what membrane process entails.

1.2. Membranes

Generally, membranes are structures permeable for at least one component and impermeable for others, and they are mostly used to filtrate water. The driving force to transport water across the membrane can be gradients of concentrations or applied pressure. Pressure-driven membrane processes are used in wastewater treatment (Wiesmann et al, 2006). Membranes can be
classified as following, according to their separation ranges (Radenovic et al, 2008):

1) Microfiltration membranes: 100-1000nm
2) Ultrafiltration membranes: 5-100nm
3) Nanofiltration membranes: 1-5nm
4) Reverse osmosis membranes: 0.1-1nm

The wastewater feed can be divided into the filtrate or permeate (the cleaned water) and the concentrate or retentate (the rejected water) (Wiesmann et al, 2006).

Selection of membrane materials is a very important decision because this factor influences the efficiency of the filtration process. The kinds of materials that can be used to construct a membrane are organic or inorganic solids. The most important type of materials used are ceramics, refined steel and glass due to its resistance to high temperatures and chemical stress, all of them are inorganic solids (Wiesmann et al, 2006).

According to the arrangement of the membrane modules, the membranes can be categorized in five principle configurations (Wiesmann et al, 2006):

1. Plate and frame modules: used frequently for micro- and ultrafiltration
2. Spiral wound modules: standard configuration for nanofiltration and reverse osmosis membranes.
4. Hollow fibre and capillary modules: these are self-supporting membranes. The hollow fibre membranes have an out-to-in flow configuration, and the capillary module an in-to-out flow configuration.
5. Tubular modules: they are used because of their good cleaning characteristics and resistance to high turbulence.

Pressure-driven membranes operate on one of two modes: dead-end or cross-flow. In dead-end mode any retentate stream is formed (Jude et al, 2011) due to the orthogonal feeding of the wastewater to the membrane (Wiesmann et al, 2006). In cross-flow mode the retentate is continuously flowing from the membrane outlet. This implies that not all wastewater is being converted into permeate product (Judd et al, 2011).

Contaminants in wastewater that are rejected by the membrane tend to accumulate on its surface. This leads to various phenomena that can reduce the water flow through the membrane (i.e. the flux) at a given transmembrane
pressure (TMP) or an increase of the TMP at a given flux. These phenomena are known as membrane fouling, and it's one of the main limitations to membrane operation (Judd et al, 2011).

Meng et al, 2009 classified membrane fouling in three categories according to the effectiveness of controlling the fouling: removable fouling, irremovable fouling and irreversible fouling. Removable fouling can be easily removed by physical methods like backwashing. Irremovable fouling can be solved by chemical cleaning. Irreversible fouling cannot be eliminated by any approach (Figure 2).

Fouling can also be classified according to the composition of the foulants or substances that cause fouling:

1. Inorganic fouling: or mineral scaling is the precipitation of inorganic salts on the membrane surface due to an excess of their concentration that causes precipitation (Shirazi et al, 2010).
2. Organic fouling: is the deposition of biological polymers such as proteins or some carbohydrates on the membrane surface (Meng et al, 2009).
3. Microbial fouling or biofouling: the biofouling occurs when microorganisms (mainly bacteria) grow in the surface of the membrane forming a biofilm that causes clogs in the pores of the membrane (Meng et al, 2009).
1.3. Membrane Bioreactor

To produce reusable water, pollutants in wastewater can be removed effectively by membrane filtration coupled with a biological treatment to degrade organic pollutants (Parameshwaran et al, 1998), this technology is called membrane bioreactor (MBR).

MBR technology is able to treat many types of wastewater and for that reason it has become more popular in recent years. In the other hand, conventional activated sludge has a more restricted way of action and it cannot deal with different types of wastewater or flow rates in wastewater (Radjenovic et al, 2008).

According to the membrane location, the MBR can be divided in two categories (Marrot et al, 2004):

a) Side-stream or external configuration: in which a pressure-driven force is needed to carry out the separation process. This configuration works under the cross flow mode. Permeate flux is between 50 and 120 Lh\(^{-1}\)m\(^{-2}\) and the TMP is in the range of 100-400 KPa (Figure 3A).

b) Immersed or submerged configuration: a vacuum-driven force is applied to separate the solid-liquid phases. This configuration operates in a dead-end mode. Permeate flux is from 15-50 Lh\(^{-1}\)m\(^{-2}\) and the TMP is about 50 KPa (Figure 3B).

![Figure 3. MBR configurations a) Side stream configuration, and b) submerged configuration. Source: Radjenovic et al, 2008.](image)

The submerged MBR configuration consumes less energy for filtration than the side-stream configuration making it more popular for wastewater treatment (Marrot et al, 2004; Radjenovic et al, 2008).
The most common types of membrane used by MBRs are hollow fibre membranes, plate-and-frame/flat sheet membranes and tubular membranes in the range of micro- and ultrafiltration (Judd et al, 2011).

In both MBR configurations, a shear over the membrane is needed to scour the membrane to prevent fouling. To provide the necessary shear to avoid membrane fouling, external MBRs use the pumping force, while the immersed configurations employ the aeration in the tank (Radjenovic et al, 2008). In an anaerobic configuration the aeration in the bioreactor cannot be used as a shear, instead, a liquid pumping or the generated biogas is used (Judd, 2011).

In the previous lines, the general physical processes that happen in an MBR have been presented, but as it name says, the MBRs need biological processes to accomplish their water cleaning goals.

A biotreatment process consists of the removal of suspended organic chemicals in wastewater through biodegradation and physical separation or uptake by microorganisms. To carry out a biological process in a bioreactor, this must provide appropriate conditions to keep the microorganism alive and active to achieve the removal of organic compounds. Organic compounds are usually measured as biochemical oxygen demand (BOD) or chemical oxygen demand (COD). Those are measurements of the amount of oxygen that is needed to oxidize an organic compound. Biotreatment processes can be classified according to their process configuration, feeding regime and oxidation state (Judd et al, 2011).

A process configuration refers to the type of growth of the microorganisms in wastewater: a biofilm formation in a supporting material, in suspension in the reactor, or in some cases a combination of both (Judd et al, 2011).

Feeding regime refers to the way in which feeding solution is introduced to the system. It can be continuous or batch-wise. With the later one, the same vessel can be used for biodegradation and membrane separation, like the sequence batch reactor (Judd et al, 2011).

The reduction-oxidation (redox) conditions of the medium influence the microbial communities growing in the system, thus affecting the treatment process. The redox conditions are determined by the presence of oxygen (aerobic conditions), the presence of a molecule that can provide oxygen to the microorganisms (anoxic conditions) and the complete absence of oxygen (anaerobic conditions) (Judd et al, 2011).
When an aerobic treatment is applied, the organic compounds (BOD or COD) are removed and the ammonia present in wastewater is converted into nitrate (nitrification). The aerobic treatment can be combined with anaerobic process to produce biological nutrient removal (BNR) (Judd et al, 2011), which is the removal form wastewater of nitrogen and phosphorous (microorganism´s nutrients). Nowadays this is an important part of conventional wastewater treatment because the presence of nitrogen and phosphorous can lead to the bloom of algae and other microorganism, sometimes is performed as an advanced treatment. Also, BNR has a smaller footprint comparing to chemical treatments because it produces less waste solids and consumes less energy (Metcalf and Eddy, 2003).

1.3.1. Anaerobic Membrane Bioreactors

There are some MBR configurations that only work in anaerobic conditions, these are called anaerobic membrane bioreactors AnMBRs (Figure 4). When compared with the aerobic process, the anaerobic treatment has some advantages: less production of sludge, operation at lower quantities of nutrients and biogas (mainly CH\textsubscript{4}) production. Nevertheless, the investment costs are high and the operation of an anaerobic process is complex, therefore, the anaerobic treatment is not implemented very often. Municipal and industrial wastewater treatment with different contaminant loads (i.e. low-, medium, and high-strength wastewaters) has been tested using AnMBRs (Skouteris et al, 2012).

![AnMBR configurations](image)

**Figure 4.** AnMBR configurations a) Side stream configuration, and b) submerged configuration. Source: Skouteris et al, 2012.

One parameter to consider when operating an AnMBR is the biomass retention in the reactor. Anaerobic microorganisms used to perform the biological reactions have a low growth rate. Hence, the retention of the biomass is an important parameter to maintain high biomass concentrations in the reactor to be
able to remove high organic loads. Granule and biofilm-based configurations are used to achieve good biomass retention.

Despite the small sludge production, AnMBRs can recover energy from wastewater converting up to 98% of the organic content in the water into biogas. This biogas is of excellent fuel quality (80-90% CH₄ composition) and it can be used to produce electricity. In few cases, the biogas production is so high that the energy can be used for water filtration and the wastewater treatment plant is still energy positive and this energy can be used in other processes, e.g. pumping, filtration among other processes (Skouteris et al, 2012).

To obtain these large quantities of CH₄, the AnMBR must operate at a long solid retention time (SRT) because a short SRT decreases the magnitude of the reaction. Most of AnMBRs operate at a mesophilic range of temperatures, nevertheless at high temperatures, e.g. 50° C, high removal rates of COD are kept (Skouteris et al, 2012).

Despite the benefits of using MBRs (with either aerobic or anaerobic processes) there are still some drawbacks. Membrane fouling (Fangang et al, 2009), high price of membranes (Radjenovic et al, 2008), and its apparently big footprint on the environment due to its energy consumption for operation (Wenzel et al, 2008) are some of the disadvantages found to this technologies. More studies have to be done in order to improve these and other aspects.

Due to the necessary improvement of the present technologies that are used to treat water, different new water technologies are being studied. These new tools represent potential approaches to effectively treat water by removing organic compounds but also because of their capacity to produce energy. One of these technologies are the Microbial Electrochemical Systems (MXCs), that use microbial reduction-oxidation (redox) reactions to carry out water treatment (Pinto et al, 2011). A review on this technology will be presented in the next section.

1.4. Microbial Electrochemical Systems

The energy coming from electrochemical technologies is a promising renewable/alternative energy source because this is generated from waste streams and it can reduce pollution and costs during wastewater treatment. The utilization of bacteria capable of using solid electrodes as an electron acceptor, allows the removal of organic pollutants from wastewater and at the same time the production of bioenergy (Pant et al, 2011).
Microbial electrochemical systems (MXCs) are not only used to treat wastewater and produce energy, they can also be used to desalinate water (Cao et al., 2009), and to biosynthesized chemical products (Rabaey et al., 2010).

The most common MXCs include Microbial Fuel Cells (MFCs), Microbial Electrolysis Cells (MECs), and others that will not be included in this work, e.g. Microbial Desalination Cells (MDCs) or Microbial Electrosynthesis Cells (MESCs) (Pant et al., 2011; Pinto et al., 2011).

1.4.1. Microbial Fuel Cells

A microbial fuel cell (MFC) is a device that converts organic compounds into electricity (Rabaey et al., 2005). The general configuration of a two chambered MFC is presented in Figure 5. It consists of an anode, a cathode, and a proton exchange membrane (PEM) that sometimes acts more generally as a cation exchange membrane (CEM) (Kim et al., 2008). In some configurations the membrane is removed, referred as single chambered MFC.

![Figure 5. Microbial fuel cell in a double chamber configuration and working principles. Source: Rabaey et al., 2005.](image)

Generation of electrical energy is carried out when some microorganisms (electrochemically active bacteria or exoelectricigens) oxidize an organic substrate and transfer the electrons generated during the metabolism into an inert insoluble surface, i.e. the MFC anode, instead of using oxygen or nitrate
Electrons can be transferred to the anode by different mechanism:

1. Direct transfer: this process occurs when the outer bacteria membranes enter into direct contact with the electrode surface. Some electron transport proteins like type c cytochromes are the ones that make possible the electron transfer (Schröder, 2007).

2. Conductive nanowires produced by some bacteria: these structures, called pili, are produced by the bacteria to allow them reach the electron acceptors, i.e. the electrode surface, at long distances (Schröder, 2007).

3. Soluble mediators (exogenous or bacterial-origin): the exogenous mediators are redox mediators that facilitate the electron transfer from the bacteria to the electrode. They have to be added to the media culture, some examples are quinones, phenazines or phenothiazines. The bacteria-origin soluble redox mediators are primary or secondary metabolites used as electron shuttling compounds (Schröder, 2007).

After the electrons are transferred to the electrode, they flow through an external circuit with a resistor towards the cathode where the final electron acceptor is reduced (usually oxygen) (Rabaey et al, 2005).

Oxidation of substrates also generates protons that diffuse through the electrolyte in the anode (anolyte) and the CEM to the cathode chamber where they combine with oxygen and electrons to produce water (Kim et al, 2008).

An electrolyte is a conductive solution in which the electrodes are immersed. Generally, for the anode it is used a solution with organic compounds that can be oxidized by bacteria (e.g. acetate or wastewater). For the cathode solutions that can be reduced like ferrycianide (Li et al, 2010) or potassium permanganate (Jadhav et al, 2008) are used catholyte. In some configurations the cathode is directly exposed to the air (Kim et al, 2008), termed as air-cathode MFCs.

As mentioned in the previous paragraph, wastewater and acetate can be used as substrate or feed solution in an MFC. Besides these substrates, there are several other solutions that are being used. Substrates used in MFC ranged from pure compounds to very complex solutions. Some pure compounds used are: acetate, glucose, sucrose, lactate, propionate, etc. Some complex solutions are: landfill leachate, farm manure, corn stover biomass, etc (Pant et al, 2010).

Selection of the substrate for an MFC is very important because it influences the bacteria community composition, and the electrical performance of the MFC, i.e. power density (PD) and coulombic efficiency (CE).
Generation of electricity in an MFC depends on many factors and can be described and analysed by different techniques. Electricity in an MFC is only generated if the reaction is thermodynamically favourable. The overall reactions in an MFC can be evaluated as an Electromotive force ($E_{emf}$) that is the potential difference (V) that exists between the anode and the cathode and doesn’t take into account the resistance of the system (Logan et al, 2006). The electrons in the circuit flow from the electrode of less redox potential (the anode) to the electrode of more redox potential (the cathode).

Reactions that occur in an MFC are known as half-cell reactions because the total reaction is spatially separated: half of the reaction occurs in the anode (oxidation reaction) and the other half in the cathode (reduction reaction) (Logan et al, 2006).

As mentioned previously, electrons flow from a place with less electrical potential to a place with more electrical potential. To determine the electrical potential, or more accurate the standard electrode potential, it is necessary to compare the desired half reaction potential with a reference electrode. One example of a reference electrode is the standard hydrogen electrode (SHE) or normal hydrogen electrode (NHE) that has a potential of 0V at standard conditions (Logan et al, 2006).

There are some phenomena that decrease the performance of an MFC:

1. **Ohmic losses**: this concept refers to the resistance of the electrons to flow through the electrodes and the rest of the circuit, and the resistance of the ions to flow through the CEM. When the space between both electrodes is reduced the ohmic losses are reduced as well (Logan et al, 2006).

2. **Activation losses**: to carry on a redox reaction in an MFC is necessary a source of energy that is called activation energy. The use of this energy represents a potential loss of energy in the system. The addition of a catalyst, increase the temperature of the system or the increase of the surface area of the electrodes (Lens et al, 2005; Logan et al, 2006).

3. **Concentration losses**: this kind of loss occurs when the mass transport limits the current production, more specifically, when the compounds are being oxidized faster than they can be transported to the electrode surface. When poor mixture systems or thick non-conductive biofilms exist this kind of losses appear (Lens et al, 2005; Logan et al, 2006).

Some modifications have been done to the traditional MFC configuration in order to achieve different goals beyond the electricity production. One of these important modifications is the one that allows hydrogen production. This modified
MFC is known as microbial electrolysis cell (MEC). The hydrogen produced in an MEC can be used as a fuel (biofuel). Hydrogen is a zero-emissions fuel, therefore, it’s environmental friendly, and it’s a renewable resource (Johnston et al, 2005).

1.4.2. Microbial Electrolysis Cells

A microbial electrolysis cell (MEC) is a device that contains electrochemically active bacteria that grow in the form of a biofilm in the surface of the anode where they covert organic matter into electrons, protons, and carbon dioxide. As in the MFC technology, the electrons travel through the external circuit to the cathode and the protons through the solution to the cathode. The difference is that the electrons and protons combine to generate hydrogen (Liu et al, 2010), the protons act as the electron acceptor (Figure 6). This system is completely anaerobic to avoid oxygen reduction.

![Microbial electrolysis cell in a single chamber configuration and working principles. Source: Liu et al, 2010.](image)

In an MFC, the redox reaction is spontaneous because the redox potential of the oxygen is higher than the redox potential of the organic substrates used in the anode chamber. Nevertheless, in an MEC the occurring redox reaction is thermodynamically unfavourable because the hydrogen reduction has a lower potential than the potential in the anode chamber. Therefore, the reaction is not spontaneous and external energy is necessary to carry out it. For that reason, an external voltage is applied to overcome this thermodynamic barrier (Kim et al, 2008; Liu et al, 2010). In practice, the applied voltage must be more than 0.2V (Logan et al, 2008).
Another important modification of an MEC, comparing it to an MFC, is that a compartment is needed for the collection of the generated hydrogen (Logan et al, 2008). In a laboratory scale MEC, a sealed bag can be used to collect the hydrogen.

The same anode and cathode materials of an MFC can be used in an MEC. For example for anode is common to use carbon-based materials such as carbon cloth, carbon paper, graphite felt, graphite granules, and graphite brushes. For cathode materials, is common to use platinum, nevertheless, this material is expensive and its extraction is not environmental friendly (Logan et al, 2008). For that reason some investigators have used a biofilm in the cathode surface to act as a biocathode, the bacteria in the biofilm will catalyse the hydrogen reduction reaction (Jeremiasse et al, 2010).

Different configurations are also found in MECs. A double chamber configuration is usually constructed with a CEM separating both anode and cathode cambers. The use of a membrane helps to keep separated the hydrogen that is being produced and thus, maintaining the purity of the biogas. Other type of membranes can be used to increase the performance of the MEC. Nevertheless, a single chamber configuration is also used (Liu et al, 2010).

When a single chamber configuration MEC is constructed, the potential losses caused by the membrane are avoided and the current density increases. Membrane drawbacks such as fouling or high costs are also eliminated in a single camber MEC. However, the produced hydrogen can be consumed by other microorganisms localised in the reactor vessel, and be converted into methane. This biochemical activity is performed by groups of microorganisms called hydrogenotrophic methanogens or by electromethanogenic bacteria (Liu et al, 2010).

Several solutions can be applied to overcome the previous situation. Addition of 2-bromoethanesulfonate (BES) to the culture has demonstrated that inhibits the methanogenesis. When 1µmol of BES is added, acetoclastic methanogenesis is inhibited, but when 50 µmol of BES is added hydrogenotrophic methanogenesis is also inhibited (Zinder et al, 1984). Periodic aeration of the anode culture can be used to inhibit methanogenic bacteria, but when the anode biofilm is very thick, this method is not useful (Zhuang et al, 2012). Other authors have proposed to manipulate bacterial mixed cultures (Liu et al, 2010) to reduce hydrogen consumption. Nevertheless, a deep understanding of the microbial ecology is necessary to carry on this approach successfully.
1.4.3. Microbial ecology of electrochemically active bacteria

Electrochemically active bacteria (EAB), also known as exoelectrogens, electricigens or anode-respiring bacteria (ARB) (Liu et al, 2010), can transfer electrons produced during oxidation of an organic substrate to an MEC/MFC anode directly or indirectly (See MFC section) (Goswami et al, 2009). In nature, these types of bacteria reduce metals (final electron acceptor) to produce energy for anaerobic growth (Biffinger et al, 2008).

In an anaerobic system (for example an MEC or an AnMBR) the production of methane (methanogenesis) is carried out and at the same time other byproducts are generated. This process is only possible when different types of microorganisms interact with each other. Fermentative bacteria, acetogenic bacteria, acetate oxidizing bacteria, hydrogenothrophic methanogens, and acetoclastic methanogens are the main groups of microorganisms that participate in the whole anaerobic digestion process (Demirel et al, 2008).

Molecules like glucose, proteins or lipids are hydrolysed by fermentative bacteria and converted into monomers that can be taken up by other fermentative microorganisms or by anaerobic oxidizers. Products of these reactions are acetate and hydrogen, but also intermediate products like butyrate, and propionate that can also be converted in acetate and hydrogen. After this, methane can be generated from acetate or hydrogen along with carbon dioxide. Methanogens can compete with acetate oxidizing bacteria to uptake acetate. Acetate oxidizing bacteria convert acetate into hydrogen and carbon dioxide, while acetoclastic methanogens transform acetate into methane. In the other hand, hydrogenothrophic methanogens use hydrogen as electron donor to reduce carbon dioxide to produce methane (Figure 7) (Demirel et al, 2008).

Different types of EAB are used in MECs and MFCs. The decision about which type of bacteria will be inoculated depends on the kind of feeding solution that is going to be used. The use of pure cultures and mixed cultures is commonly found. One of the bacteria that is most used is *Geobacter sulfurreducens*. This species is capable of oxidizing acetate completely into carbon dioxide with more than 90% of electrons recovered to produce electricity. *G. sulfurreducens* can transfer electrons with a direct contact of its membrane to the anode surface. It has been noticed that species inside the *Geobacter* genus are the first colonizers of an anode biofilm when aquatic sediments and swine waste is used as feeding solution. *G. sulfurreducens* is also important because it produces the highest current densities comparing it to other pure or mixed cultures when using graphite as anode (Richter et al, 2008).
G. sulfurreducens can oxidizes efficiently acetate, but also formate, lactate and hydrogen can act as electron donors. However acetate showed to be an effective electron donor for high rate of current generation and yielding maximum current (Speers et al, 2012). When the feeding solution is other than specified substrates, this species needs syntrophic interactions with other bacteria to carry out its anode-respiration. Fermentative bacteria can convert complex organic substrates like glucose into smaller compounds that G. sulfurreducens can metabolize. This interaction helps to convert effectively the organic substrate into electricity (Kim et al, 2008).

If the final objective is hydrogen production, interactions between EAB and methanogenic bacteria can result detrimental for biogas generation. When methanogenic bacteria are present, the electrons from electrical current can be diverted into hydrogen but also into methane. Hydrogen generated from fermentation can be used by hydrogenotrophic methanogens as a substrate to produce methane, instead of being used by EAB to produce electrical current. This causes a divergence in electrons, which ends in a decrease in coulombic efficiency (Parameswaran et al, 2009).

As noted before, there are many advantages but also drawbacks when biological systems are used to produce energy or treat water. Optimization of each technology depends on the use of new materials, the better understanding of the biological processes, and the sustainability of the system among others. However, there are several examples in which the hybridization of two
technologies results in the improvement of the former configurations. In the next section some of these examples will be presented.

1.5. Hybrid technologies for energy production and wastewater treatment

Microbial fuel cells are energy recovery systems using wastewater as their source of energy. Nevertheless, the water treatment in these systems is not very efficient. Wang et al, 2011, proposed a hybrid system between an MFC and an MBR to improve COD and ammonium removal efficiency and to generate energy to partially sustain the energy consumption of the system. They called this system Bioelectrochemical membrane reactor (BEMR), using a stainless steel mesh as a membrane and a biocathode. The highest power density obtained was only 4.35 W/m$^3$ and a current of 18.32 A/m$^3$. The great achievement after using this system was the high-quality effluent obtained. The COD and ammonium removal were 92.4% and 95.6% respectively, and permeate turbidity was less than 1NTU. However system required energy extensive aeration step for achieving the high quality permeate.

Wang et al, 2012 developed another integrated system between an MFC and an MBR. This system was constructed with low-cost materials and a nylon mesh was used as a membrane. When comparing it with the previous example, they made improvements in power generation (6.0 W/m$^3$), however, COD removal and turbidity remained similar.

Recently, Malaeb et al, 2013 proposed what they called a truly integrated MFC-MBR. In this integrated system a conductive ultrafiltration membrane was used as an air-biocathode, avoiding the need of aeration. The maximum power density reached in this system was 6.8 W/m$^3$. The soluble chemical oxygen demand (SCOD) removal was 97.3% and the ammonium removal was 97.3% as well.

Many solutions have been proposed to improve the wastewater treatment process. The hybridization of MFC and MBR is an important achievement to increase the quality of the permeate water. Nevertheless, the energy recovery from wastewater in these types of systems is still low, thus, an opportunity is presented to further modify these systems to increase the energy production.

In this work, an anaerobic electrochemical membrane bioreactor (MEC-MBR) system is proposed, as a proof-of-concept to recover water, energy (as methane and hydrogen) and simultaneous removal of chemical oxygen demand (COD) from low strength synthetic medium. This hybrid system is a truly integrated and effective single-chambered configuration that avoided the need for
wastewater aeration or separate membrane modules. The system consisted of an electrically conductive nickel made membrane that functioned as both a cathode for hydrogen generation, and as a membrane for wastewater filtration.
2.0. OBJECTIVES

1. To integrate a microbial electrolysis cell (MEC) with a membrane bioreactor (MBR), using a conductive membrane that acts as cathode and as the filter material at the same time for artificial wastewater treatment and energy harvesting.

2. To evaluate the robustness and integrity of the hybrid unit in terms of renewable energy generation (biogas and electricity) and effluent water quality.

3. To evaluate the performance of the system under various operational parameters and its influence on (i) organics removal, (ii) biogas yield, (iii) biofouling mitigation and (iii) TMP changes.
3.0. MATERIALS AND METHODS

3.1. MEC-MBR configuration

The photograph of the single chambered reactor setup is presented in Figure 8. The MEC-MBR consisted of a vertical cylinder made of plexi glass with 0.24m length and 0.046m diameter. The total volume of the reactor was 400ml, but the liquid working volume was 350ml. For the anode, a brush made of carbon fibers (PANEX33 160K, ZOLTEK) was used (0.15 m length and 0.06 m diameter). The distance between the cathode and the anode was 0.005 m. The cathode was nickel made hollow fibers (Ni-HFM), that acted as both cathode and filter material. The cathode/membrane and the anode were placed in the middle of the cylinder, with the anode at the bottom. Control reactor (as conventional anaerobic MBR) was similarly operated (i.e. configuration and anode/cathode similar to MEC-MBR), but as open circuit mode.

3.1.1. Cathode assembly

The cathode/membrane consisted of 5 Ni-HFM placed vertically on top of the anode. Each HFM was 0.1m length, with a total surface area of 0.00141 m$^2$. The average pore size in the Ni-HFM was \( \mu \text{m} \), so it can be considered a microfiltration membrane.

To assemble the fibers, they were clustered and tightened at one end with a titanium wire to keep them together. After that, silver epoxy glue was added to the same end in which the wire was tied. Then, this part of the fiber bundle was

![Figure 8. MEC-MBR setup. 1) Peristaltic pump. 2) Gas collector bag. 3) Permeate tube. 4) Ni-HFM/cathode. 5) Carbon brush anode. 6) Power supplier. 7) Feeding (recirculating) tubes.](image)
inserted into a flexible tube and sealed with conventional epoxy glue. This tube was connected to the pump used to draw the treated effluents through the HFM.

3.2. Ni-HFM preparation

The Ni-HFMs are comprised of almost pure nickel (99.9%) with a small percentage of unknown content (0.1%). Ni-HFMs were fabricated using a combined phase-inversion/sintering method. Nickel powders, 1-methyl-2-pyrrolidinone (NMP, HPLC grade, 99.5%, Alfa Aesar), Polyether Sulfone (PES, Ultrason® E6020P, BASF) and Polyvinylpyrrolidone (PVP, Alfa Aesar) were mixed and well dispersed by ball milling for 18 h, followed by degassing under vacuum for 24 h. After that, the suspension was extruded through a spinneret using water as the inner and outer coagulant. The black body of the hollow fiber was dried at room temperature and then sintered at 560 °C for 6 h to remove organic compounds in air flow of 500 ml/min. After cooling to room temperature, the fiber was reduced from the metal oxide state to the metal state at 810 °C for 6 h in pure hydrogen of 500 ml min⁻¹. The fabrication process is not a patented process and a description of the process has been reported in the literature (Meng et al., 2009).

3.3. MEC-MBR operation

The MEC-MBR was inoculated with a 10% of mixed anaerobic sludge, which was collected from the effluent of MECs that were previously inoculated with an anaerobic digester effluent. The reactor was operated with a low strength synthetic medium (artificial wastewater) at 30 °C. At the end of each batch, i.e. when the voltage decreased to <1mV, the synthetic medium was pulled through out of the membrane using a pump (Masterflex L/S, Cole-Parmer, Vernon Hills, IL.). After the end of each batch, transmembrane pressure (TMP) was measured using a pressure transducer from Cole Parmer Instruments Inc. The flux to perform the TMP analysis was fixed at 70 Lmh with a flow of 0.16 ml/min. The LJStream UD software was used to record the data coming from the transducer.

3.4. Anaerobic artificial wastewater preparation

The composition of the artificial wastewater was 1.5 g/L NH₄Cl, 0.6 g/L Na₂HPO₄, 0.1 g/L KCl, 0.41 g/L Na-acetate, 10 ml trace elements, 10 ml vitamin solution, 2.5 g/L NaHCO₃ (Katuri et al, 2010). This solution was heated to the boiling point to remove the dissolved oxygen in water. Then it was cooled down at room temperature by sparging with a gas mix (80% N₂ and 20% CO₂). Prior to use, it was sterilized using an autoclave.
3.5. SEM imaging

To characterize the virgin and biofouled HFM’s, a small sample of the membrane was removed from the reactor for subsequent scanning electron microscopy (SEM). Prior to SEM imaging, fixation was undertaken by placing the sample in the following solutions: (a) 1% glutaraldehyde, 2% paraformaldehyde, 0.2% picric acid, 10 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES, pH 7.4) for 1 h, (b) 50 mM NaN3 for 1 h, (c) 2% tannic acid for 1 h, (d) 1% osmium tetroxide for 2 h, (e) 1% thiocarbohydrazide for 30 min, and (f) 1% osmium tetroxide overnight, with washing using 10 mM HEPES buffer (pH 7.4) between steps (all Sigma-Aldrich). The samples were then dehydrated in a graded series of aqueous ethanol solutions (10−100%) and oven-dried for 2 h at 40 °C to remove residual moisture. The dried samples were mounted over SEM stubs with double-sided conductivity tape and a thin layer of gold metal applied using an automated sputter coater (Emitech, K550) for 1 min and imaged using a model 4700 SEM instrument (Hitachi, Japan).

3.6. Electrochemical measurements and analyses

Through all the operation of the MBR-MEC, the voltage was measured and recorded every 20 min using a PicoLog high resolution data logger. To provide the voltage required to carry out the hydrogen reduction reaction an external DC Power Supplier model 3646A from Circuit Specialists Inc. was used.

The current was calculated from the measured voltage with ohm’s law (I=V/R) at a constant resistance of 10Ω. The power was calculated multiplying the current by the voltage (P=IV). Current and power densities were normalized to the cathode surface (0.00141m²) and to the total working volume (350ml).

To calculate the cathode coulombic efficiency (CE), the moles of hydrogen recovered based on the generated current (nCE) was divided by the theoretical moles produced based on substrate removal (nTH). Taken from Call et al, 2008.

\[ n_{TH} = \frac{b_{H_2/S} V_L \Delta S}{M_s} \]

Where \( b_{H_2/S} \) is 4 mol/mol (4 moles of hydrogen produced by 1 mole of acetate), \( V_L \) is the working volume of the reactor, \( \Delta S \) is the change in substrate concentration, and \( M_s \) is the molecular weight of acetate.

\[ n_{CE} = \frac{\int_{t=0}^{t} I dt}{2F} \]
Where I is the current integrated over the time (t), 2 represents the number of moles of electrons to produce one mole of hydrogen and F is the Faraday’s constant (96485 C/mol e⁻).

\[ CE = \frac{nCE}{nTH} \]

To calculate volumetric hydrogen production rate (Q), cathodic hydrogen recovery (rCAT) was calculate first:

\[ rCAT = \frac{nH_2}{nCE} \]

Where \( nH_2 \) is the number of moles of hydrogen recovered during a batch cycle. Then Q was calculated:

\[ Q = \frac{43.2I_vrCAT}{FC_g(T)} \]

Where \( I_v \) is the current density normalized to the working volume, \( C_g \) is the concentration of gas at a temperature \( T \) using ideal gas law, 43.2 is for unit conversion.

3.7. Biogas measurement

The biogas produced in both reactors was collected in a gas bag from Calibrated Instruments Inc. The gas bag was changed when it was full or at the end of a batch. A 200µl sample was taken from the bag and it was injected into an SRI 310C Gas Chromatographer (GC). The gas analysis was performed using a 6’MS column with argon as a carrier to detect hydrogen, oxygen, nitrogen and methane with an oven column temperature of 100 °C; and a 3’ silica gel column with helium as a carrier to detect carbon dioxide with an oven column temperature of 80 °C.

3.8. Acetate measurement

Acetate concentration from the artificial wastewater was measured using high performance liquid chromatography (HPLC, Thermo Scientific). Organic acids analysis column Aminex® HPX-87H Ion exclusion column was used for measurements using a 5mM sulphuric acid mobile phase.

3.9. Suspended solids

Suspended solids content in samples was determined by this equation:
\[
SS = \frac{W_2 - W_1}{V}
\]

Where \(W_1\) is the weight of the filter paper (Glass microfiber filters, 0.2 µm, GE healthcare life sciences) and \(W_2\) is the final weight of the filter after drying it at 100°C for 1h followed by filtration of known volume of sample. \(V\) is the volume of the sample taken for filtration.

### 3.10. Nickel determination

For Ni detection in the samples, an Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) analysis was done. Relevant protocols were obtained from the environmental protection agency (EPA, 1994).

### 3.11. DNA extraction and analysis

Biofilm samples from the cathode (i.e. HFMs) were taken at different time points of the reactor operation for quantitative PCR (qPCR) analyses. To extract the DNA from the samples, a Power Soil™ DNA isolation kit from MO BIO Laboratories Inc. was used. After the DNA extraction, a DNA quality analysis was performed with a NanoDrop 2000 spectrophotometer from Thermo Scientific. The target organisms for qPCR were:

- **Methanosarcinaceae** (primers: MST 702 F and MST 863 R, probe: MST 753).
- **Methanomicrobiales** (primers: MMB 282 F and MMB 832 R, probe: MMB 749).

qPCR was performed using a total volume of 25µl containing: 12.5µl of iQ Supermix from Bio-Rad laboratories, 0.25µl of each primer (forward an reverse), 0.25µl of probe, 1µl of DNA sample and 10.9µl of RNase free sterile water. For total archaea, *methanosarcinales*, *methanobacteriales*, and *methanomicrobiales*, the qPCR procedure was followed as per Yu et al, 2005. For total bacteria analysis Ritalahti et al, 2006 procedure was followed. Amplifications were created using a CFX96 real-time PCR detection system (Bio-Rad Laboratories, Hercules, CA).
4.0. RESULTS

4.1. Membrane characterization

Platinum and hydrogenase enzymes are excellent cathode catalysts for the production of hydrogen ($H_2$). Platinum, however, is a precious metal with high cost and low abundance on earth. More abundant metals, such as nickel, cobalt, molybdenum and iron have shown to be promising electrocatalyst for $H_2$ production. In this study, nickel (Ni) based hollow-fiber membranes (Ni-HFMs) were evaluated as cathode materials for $H_2$ production. Details pertaining to Ni-HFM structure are shown in Figure 9. The catalytic activity of nickel has been well explored (Hambourger et al, 2009; Le Goff et al, 2009) as a potential replacement for costly platinum catalysts for fuel cell applications.

In order to determine the Ni-HFM pore size and pore distribution, a scanning electron microscope (SEM) was used. To visualize the Ni-HFM structure in detail, a scanning electron microscope (SEM) was used to obtain images coming from fresh membranes. Figures 9a and 9b show a cross section

![Figure 9](image_url)

**Figure 9.** Micrographs at different magnifications and different sides of a fresh Ni-HFM sample. A) Cross section of the membrane. B) Amplification of one side of the cross section. C) Frontal view of the membrane surface. D) Close up to the membrane surface, pores can be appreciated.
of the virgin membrane, and Figure 9c shows the surface of the membrane. Based on SEM imaging, the membrane dimensions were found to be: wall thickness 0.01 cm, internal diameter of hollow fiber 0.09 cm and outer diameter was 0.1 cm. The surface of the membrane was covered with unevenly shaped pores (Figure 9d), and the average of the pore size measured was 1 µm. SEM images revealed that the pore density was low, a factor that usually affects water flux and transmembrane pressure (TMP).

4.2. MEC-MBR performance

The performance of the novel hybrid system was assessed in terms of current generation, coulombic efficiency, biogas (CH4 and H2) production, substrate removal and permeate quality.

4.2.1 Electrical parameters and biogas analysis

A profile of current generation and relevant biogas formation during the batch mode operation of the MEC-MBR is presented in Figure 10. The reactor was acclimatised using graphene coated Ni-HFMs as cathode/membrane under 0.7 V applied voltage with 5 mM acetate as substrate (electron donor). During this phase, a variable current and biogas production was observed between the batch tests. Perhaps, this unstable reactor performance was due to the biofilm attachment/detachment mechanism and biofilm/planktonic microbial communities adaptation to the reactor’s condition with time. After 52 days of operation, the reactor showed a stable performance in terms of current and biogas generation, meaning that the reactor was acclimatised. At this point, graphene coated Ni-HFMs were replaced with virgin uncoated Ni-HFMs as cathode/membrane for experimental test. To investigate the efficacy of the hybrid MEC-MBR configuration with catalytic Ni-HFMs, a variety of operational conditions were examined in series over the test period. The variations in the reactor performance observed in terms of current, H2, CH4 generation and TMP with respect to changes in the operational conditions are shown in Figures 10 and 11.

After 67 days of reactor operation, the recovered biogas was comprised predominantly of CH4 gas (points 1, 3 and 4 of Figure 11). Availability of H2 production (from cathode) and CO2 (from bacterial respiration and bicarbonate from the reactor medium), enabled hydrogenotrophic methanogens to enrich over the period of reactor operation and reduced CO2 to CH4 using H2 as the electron donor. Methane generation is a common problem when mixed culture inoculums are used as a source of bacteria for the anodic biofilm development. It is possible to enrich slow growing hydrogenotrophic methanogens in these systems (predominantly in single chambered cells) which convert energy rich and
environmentally friendly H₂ gas to the lower energy containing greenhouse gas CH₄. Thus, variations in H₂ production over time and under different operational conditions were closely related to CH₄ production: when low levels of hydrogen were detected higher levels of methane were detected and vice versa (point 7, 8 and 9 of Figure 11).

At open circuit voltage (OCV) growth mode (point 2 Figure 11), H₂ cannot be produced at the hybrid cathode/membrane due to the lack of electrons necessary to drive the catalytic reaction and, consistent to this, H₂ was not observed in the system. Since the electrodes were disconnected from the external circuit, electricigens were unable to use the anode as an electron acceptor. Moreover, trace amounts of CH₄ were observed during the first batch of this phase but not detected in the successive 2nd and 3rd batches. These observations indicate that CH₄ production was carried out by hydrogenotrophic methanogens and not via acetoclastic methanogenesis, which is usually the dominant route in traditional anaerobic digestion processes. qPCR analyses presented below confirmed the presence of hydrogenotrophic methanogens.

Figure 10. Current generation in the MEC-MBR with time under 10Ω external resistance (red line) and biogas production (black dots) at different operational conditions. 1) Start-up phase with graphene coated Ni-HFM at 0.7V. 2) Reproducible performance with Graphene coated Ni-HFM at 0.7V. 3) Graphene coated Ni-HFMs replaced with Ni-HFM and applied voltage of 0.7V. 4) Under OCV mode of operation (i.e. electrodes disconnected from the external circuit). 5) At 0.7V. 6) At 0.7V with 1mM of BES. 7) At 0.7V with 50mM of BES. 8) At 0.7V. 9) At 0.5V. 10) At 0.9V. 11) At 0.9V with 50mM of BES.
Methanogenesis inhibitor 2-bromoethane sulfonate (BES) was added to the feed solution to test if hydrogen and current production increase by reducing the substrate electron flow to methanogenesis. It is well known that electrons diverted from substrate into methane production decreases system coulombic efficiency (CE) (Darus, 2011). When reactor medium was supplemented with 1 mM of BES (day 42), no noticeable increase in the H₂ yield was observed due to its low concentration. H₂ detection was the same as observed in the previous batches operated without BES, suggesting that hydrogenotrophic methanogenesis was not inhibited. The addition of 50 mM of BES increased H₂ production, current and power densities at both 0.7V (day 45) and 0.9V (day 70) by suppressing methanogens activity (see Table 1). The maximum hydrogen production rate achieved in this system (0.2 m³/m³·day at 0.9 V) is comparable with some systems using Pt as the catalyst material (Liu et al, 2010). It is likely that the lack of a proton exchange membrane between the electrodes in the reactor resulted in methane production by hydrogenotrophic methanogens. However it has been suggested that methane production cannot be avoided even with the use of a membrane separation in the system (Call et al, 2008).
As expected, higher applied voltages resulted in increased current generation. A maximum current density of 8.7 mA (equivalent to 5 A/m²) at 0.9 V was achieved (Figure 10, 11 and Table 1). Concurrently, H₂ production rate also increased with increased applied voltage (from 0.007 m³/m³ day at 0.7 V to 0.08 m³/m³ day at 0.9 V, Table 1) due to higher efficiencies at the anode and cathode catalysts. The total biogas production (H₂ + CH₄ + CO₂) increased linearly with increases in applied voltage (Figure 12). Similar positive relationships between applied voltage and total biogas recovery has been observed in the literature.

### Table 1. Coulombic efficiency, current and power densities, volumetric hydrogen and methane production rates in different experimental conditions of the MEC-MBR.

<table>
<thead>
<tr>
<th>Experimental Conditions</th>
<th>CE (%)</th>
<th>I (A/m²)</th>
<th>I (A/m³)</th>
<th>P (W/m²)</th>
<th>P (W/m³)</th>
<th>H₂ m³/m³ day</th>
<th>CH₄ m³/m³ day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coated Ni-HFM 0.7V</td>
<td>68.1</td>
<td>2.7</td>
<td>10.9</td>
<td>0.1</td>
<td>0.4</td>
<td>0.002</td>
<td>0.06</td>
</tr>
<tr>
<td>Uncoated Ni-HFM 0.7V</td>
<td>115.3</td>
<td>3.0</td>
<td>12.0</td>
<td>0.1</td>
<td>0.5</td>
<td>0.007</td>
<td>0.1</td>
</tr>
<tr>
<td>Uncoated Ni-HFM 0.7V 1 mM BES</td>
<td>19.2</td>
<td>3.0</td>
<td>12.3</td>
<td>0.1</td>
<td>0.5</td>
<td>0.000005</td>
<td>0.08</td>
</tr>
<tr>
<td>Uncoated Ni-HFM 0.7V 50 mM BES</td>
<td>80.6</td>
<td>3.9</td>
<td>15.6</td>
<td>0.2</td>
<td>0.8</td>
<td>0.08</td>
<td>0.02</td>
</tr>
<tr>
<td>Uncoated Ni-HFM 0.5V</td>
<td>35.1</td>
<td>1.6</td>
<td>6.6</td>
<td>0.03</td>
<td>0.2</td>
<td>0.0007</td>
<td>0.04</td>
</tr>
<tr>
<td>Uncoated Ni-HFM 0.9V</td>
<td>60.7</td>
<td>5.1</td>
<td>20.4</td>
<td>0.4</td>
<td>1.4</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Uncoated Ni-HFM 0.9V 50 mM BES</td>
<td>95.1</td>
<td>6.1</td>
<td>24.6</td>
<td>0.5</td>
<td>2.1</td>
<td>0.2</td>
<td>0.03</td>
</tr>
</tbody>
</table>

As expected, higher applied voltages resulted in increased current generation. A maximum current density of 8.7 mA (equivalent to 5 A/m²) at 0.9 V was achieved (Figure 10, 11 and Table 1). Concurrently, H₂ production rate also increased with increased applied voltage (from 0.007 m³/m³ day at 0.7 V to 0.08 m³/m³ day at 0.9 V, Table 1) due to higher efficiencies at the anode and cathode catalysts. The total biogas production (H₂ + CH₄ + CO₂) increased linearly with increases in applied voltage (Figure 12). Similar positive relationships between applied voltage and total biogas recovery has been observed in the literature.

**Figure 12.** Linear relationship between the applied voltage (V) and the biogas production (ml). The data used to perform the relationship come from biogas measurements at 0V (OCV), 0.5V, 0.7V, and 0.9V with uncoated Ni.HFM.
however, they used expensive platinum as cathode catalyst for hydrogen evolution reaction (Call et al, 2008).

In addition to biogas yield, the system performance in terms of current and power densities improved when increasing the applied voltage. When BES was added to the reactor medium (respective time points presented in Figure 11), the recovery of substrate electrons to current, power and total biogas was higher at 0.7 V and 0.9 V, than the batch cycles operated without BES. In general, when the medium was supplemented with BES, methanogenesis was inhibited moderately by arresting the methane-mono-oxygenase enzyme activity, which is responsible for producing methane by methanogens, leading to the production of green biogas rich with H₂ content. Although methane was not detected in the following operation at OCV, acetoclastic methanogenesis cannot be discounted, because CH₄ can be soluble in liquid (as much as 16 mg/L) and would not be detectable by gas chromatography.

4.2.2. Comparison of MEC-MBR with conventional anaerobic MBR

A control reactor was constructed and operated in the same conditions as the MEC-MBR reactor, but in open circuit mode (without electrical connection). Ni-HFM were used in both cases as membranes, but they acted only as filter material in the control reactor. The control reactor was configured to resemble a conventional anaerobic MBR and in this configuration methanogenesis is the only way to recover energy from wastewaters.

![Fig. 13](image-url) Biogas production in MEC-MBR (purple squares) and in AnMBR (green squares). TMP data from MEC-MBR (dotted line) and AnMBR (black line).
Figure 13 compares the control anaerobic MBR and MEC-MBR reactors in terms of substrate removal, biogas recovery and TMP. The results clearly demonstrate that the start-up of the control reactor was longer than the tested MEC-MBR configuration and biogas recovery was almost negligible in the control reactor over the test period of 53 days. It is well known that the growth rates of methanogens and kinetics of anaerobic metabolism are slow, which usually increase the start-up time of anaerobic digestion processes and biogas recovery. In MEC-MBR, the recovery of substrate chemical energy into biogas was more rapid from the beginning of the reactor start-up than in the anaerobic MBR, and stable performance was achieved after 30 days of reactor operation. The predominant electricigen in BES systems, *Geobacter sulfurreducens*, can outcompete acetoclastic methanogens for fermentative intermediates of the anaerobic digestion process, such as acetate. Also, *G sulfurreducens* utilizes trace amounts of acetate more effectively due to higher substrate affinity (low Ks value) and higher growth yield than methanogens (Jung et al, 2011). Thus the overall start-up time for BES is shorter.

Figure 13 shows the relationship between TMP and biogas production between both reactors. As expected, TMP increased gradually with the time of reactor operation due to biofouling of membrane (Chen et al, 2013). The fast increment of TMP in the control reactor indicates a higher rate of fouling on the membrane compared to the MEC-MBR, in which TMP increment was slower. The TMP increased to 50 KPa over the period of 60 days of operation, and it was reduced suddenly to 33 KPa when the applied voltage increased to 0.9 V. Increased rate of hydrogen production under 0.9 V applied voltage (Figure 14) probably created a sheer force which removed part of the biofilm from the

![Figure 14](image_url)

*Figure 14.* SEM micrographs at different magnifications (A and B) of the Ni-HFMs collected at the end of the operation of the reactor, i.e. day 125.
membrane surface (Figure 14). Overall, this observation confirmed, to some extent, the importance of hydrogen evolution rate on mitigation of the biofouling, however, additional studies are needed to confirm this observation.

Biofouling is an unavoidable phenomenon in traditional membrane bioreactor technology due to bacterial adhesions, which leads to reduction in membrane permeate fluxes and, therefore, increases costs and prevents MBRs from faster commercialization. However, biofouling could potentially be minimized through this proposed new hybrid membrane bioreactor configuration:

(i) H$_2$ generation from the membrane surface creates the natural scouring mechanism, which in turn reduces the accumulation of biomass in HFMs. However, this phenomenon depends strongly on the rate of hydrogen evolution which is directly related to the anode reaction and applied voltage.

(ii) The imposed low electrode potential (< -0.7 V vs NHE) and localized high pH (> 9) on the cathode HFMs due to the cathodic hydrogen evolution reaction disfavors bacterial adhesion and growth. Thus, integrating conductive membranes with MEC process provides an additional form to mitigate biofouling.

The rate of substrate removal (mM/day) was higher in MEC-MBR irrespective of conditions (except OCV growth mode) tested [i.e. 0.5 V (1.8 mM/day), 0.7 V (1.5 mM/day), and 0.9 V (3.5 mM/day)] than control (Figure 15). Though the control reactor removed 99.6% substrate, the rate of removal was considered to

![Figure 15](image_url). Comparison of the substrate removal between the MEC-MBR (open circles) and the MBR (black squares). The red box indicates the OCV phase of the MEC-MBR. Both reactors fed with 5 mM...
be slow (0.8 mM/day) and no biogas was recovered from the substrate removed.

During OCV phase of the MEC-MBR (red box in Figure 15), the substrate removal decreased due to the lack of electrons necessary to drive the electron transfer between bacteria and electrode, therefore, the metabolism of electricgens was supressed. Anode potential must be higher than substrate potential in order to allow the electron transfer to the anode (Torres et al, 2007).

4.3. Suspended solids and nickel in permeate

Traditional MBR processes use suspended solids (SS) and turbidity as standard measurements for testing permeate quality. In this study, the quality of MEC-MBR permeate was assessed using the SS measurement. In average, the permeate contained 18 mg/L SS, close to the observed values (< 10 mg/L) in the literature where they used microfiltration/ultrafiltration membranes in traditional MBRs (Poyatos et al, 2007; Falsanisi et al, 2007). Since the pore size of conductive Ni-HFMs was relatively large (1µm), bacteria were able to pass through the pores. Permeate quality can be improved further by reducing the pore size of the membranes.

Nickel (Ni$^{2+}$) was analysed in permeate samples (using ICP-MS) filtered through both graphene coated and uncoated HFMs to test the degradability of the membrane. It was found that nickel concentration is very low in permeates, 0.1 µg/ml to 0.2 µg/ml.

4.4. Preliminary microbial community insights on membrane fouling in MEC-MBR

Membrane fouling in MBR is an unavoidable phenomenon. However, in this configuration, the hydrogen availability on membranes accommodates the hydrogenotrophic heterotrophs, which may play a dominant role in biofouling.

In order to study the microbial composition of the biofouling layer on the membrane surface, biomass was collected at different time intervals. Samples were analysed for total bacteria, archaea and possible hydrogenotrophic methanogens such as *methanobacteriales*, *methanomicrobiales* and *methanosarcinales* using DNA based quantitative PCR.

The qPCR results of the biofilm in graphene coated Ni-HFMs confirmed that the relative abundance of archaea was 74% of the biofouled biomass of which 85% belongs to the hydrogenotrophic methanogen group *methanobacteriales* (Figures 16 and 17).
Similarly, the qPCR analysis of Ni-HFMs (MEC-MBR had been in operation for nearly two months with graphene coated Ni-HFMs prior to inserting the fresh Ni-HFMs) revealed that:

(i) The ratio of archaea/bacteria increased significantly from 0.06 to 0.45 from day 24 to day 69 from the time point when Ni-HFMs were immersed in the reactor (Figure 16), demonstrating that the composition of the biofouled-microorganisms shifted from a bacteria dominated community structure to a one dominated by archaea.

(ii) Archaea play a strategic role in fouling the membranes by outcompeting the bacteria for space over the time period, and the archaea community was dominated by the hydrogenotrophic methanogens *methanobacteriales* (Figure 17). This suggests that localised H\(_2\) availability on the cathode surface favoured the growth of *methanobacteriales*. Some species of *methanobacteriales* are known to be alkaliphilic, thriving at pH values between 8.1 and 9.1 (Dworkin et al, 2006), which is the range of pH found in the medium consistently.

![Figure 16](image1.png)  ![Figure 17](image2.png)

**Figure 16.** Archaea/bacteria ratio of the biofilm sample coming from graphene Ni-HFMs and Ni-HFMs at different days of the reactors operation.

**Figure 17.** Methanobacteriales/archaea ratio of the biofilm sample coming from graphene Ni-HFMs and Ni-HFMs at different days of the reactors operation.
5.0. CONCLUSION

The microbial electrolysis cell coupled with a membrane bioreactor represents a fully integrated system that is capable of organic removal from wastewater and biofuel production at the same time. A nickel-made hollow fiber membrane was used as filter material and cathode due to the catalytic properties of nickel, making the integration of the two systems possible. One of the major challenges in the MEC technology is the high cost of catalyst materials such as Pt. The use of Ni instead of Pt has a great advantage in terms of avoiding high capital costs. Removal of organics (98.9% of substrate removal) using this hybrid system is comparable to fully developed technologies. Biogas generated in this system rich with CH$_4$ content, is an added advantage compared to the traditional anaerobic digestion. qPCR results show that archaea community is likely to play a substantial role in the biofouling phenomenon. Archaea, predominantly the hydrogenotrophic methanogens *Methanobacteriales*, were able to outcompete bacteria for space on the membrane surface over the course of the experiment. Conductive membranes offer additional advantages like biofouling mitigation in addition to energy recovery from low strength organic artificial wastewaters. Observations from this study suggest that biofouling could be mitigated by increasing rates of biogas production, which can be controlled by regulating the applied voltage. More studies are needed in order to optimize this system performance and project this hybrid technology as an energy positive technology.
6.0. BIBLIOGRAPHY


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