MICROBIAL CATALYSIS OF METHANE FROM CARBON DIOXIDE

The Future of Renewable Energy is Inside You

A THESIS

Presented in Partial Fulfillment of the Requirements for Graduating with the Degree of Bachelors of Science with Distinction from the Program in Chemical and Biomolecular Engineering from The Ohio State University

By

Julia Ruth Mueller

****

The Ohio State University

2012

Examination Committee:

Dr. Jeffrey J. Chalmers

Dr. James F. Rathman

ABSTRACT

The world has energy problems, including insufficient sustainable sources, and problems associated with the waste from energy use, including emission of greenhouse gases. The ideal solution is a sustainable energy source with no subsequent waste. As it happens, Nature has offered a means to our ideal end: microbes that can turn waste into energy. However, Nature's solution was not custom made for the scale of humans' problems; we cannot apply microbe-mediated waste to energy on a large enough scale to eliminate our leftovers. Thus we must apply our scientific skill to elucidate the means with which to direct microbes to create resources in tandem with our waste.

The goal of this work was the production of reduced hydrocarbon fuels, specifically methane, from carbon dioxide (CO_2) in a microbial fuel cell using the microbes naturally present in wastewater and to evaluate system parameters for continuous flow operation. The reaction of interest was the reduction of CO_2 to CH_4, performed by microbial catalysts on the cathode. The hypothesis was that if one provides electrons and CO_2 then one could control methane production by promoting growth of the microbes. The approach was to build an MFC to investigate what happens with adjustments to the inputs, e.g. amount of electrons, carbon source, amount of carbon or frequency of carbon addition.

The microbial catalysis of methane production would be most efficient with microbes capable of extracellular electron transfer. The specific factor to examine then was the use of hydrogen as an electron shuttle by (1) examining the relationship between methane production and the availability of electrons as hydrogen, (2) the consumption of CO_2 below the hydrogen evolution point, (3) the behaviour of the microbes to lower the hydrogen evolution point and continue to preferentially produce methane or other fuels leading to methane.

It was found that the microbial community naturally present in the inoculum wastewater was capable of autotrophic methanogenesis in the presence of hydrogen, homoacetogenesis in concert with the production of other VFAs and methanogenesis in response to the sufficient presence of VFAs. The change in rate of methanogenesis before and after hydrogen evolution suggests the biofilm was successfully absorbing all hydrogen shuttles or directly transferring electrons. In support of the data, the hydrogen overpotential was significantly reduced from the un-colonized overpotential but still remained above the theoretical hydrogen evolution level.

The conclusion, barring further microbe analysis, was that methanogens were not forming a direct biofilm, but instead remained biofilm-associated inside the reactor volume and outside the reactor as a planktonic community in the sampling bottle. Coulombic efficiencies for mixed VFA products by autotrophic processes (including homoacetogenesis) ranged from 2% to 90% given the operating conditions. Coulombic efficiencies for methanogenesis ranged from 0% to 60% during production. Using a sequencing batch reactor method, 98% of the mixed VFA products could be converted to

methane. Of particular interest was the result that the rate of methanogenesis could also be minimized using this system in a continuous flow mode by controlling the flux.

ACKNOWLEDGMENTS

I am very much indebted to my advisors Prof. Jurg Keller of The University of Queensland and Prof. Jeffrey Chalmers of The Ohio State University. With their help, I have gained invaluable experience designing research and communicating my findings.

I would also like to extend my gratefulness to Prof. James Rathman and Brian Endres of The Ohio State University. I would have drowned in drudgery if not for their help, advice and guidance.

I wish to thank Dr. Yang Mu and many other researchers within The Advanced Water Management Center at The University of Queensland. My growth as a scientist and the strength of my research was aided by the many conversations I have had. Dr. Damien Batstone deserves special mention for his help and guidance in thinking about microbes from a different perspective.

I would like to thank The Advanced Water Management Center, the Lettinga Foundation and Project Neptune for funding my research.

Finally, I would like to thank my husband for his encouragement, support and patience while I delved into my research. Words cannot satisfy my gratitude.

VITA

March 2012…….....…….B.S. Chemical Engineering

March 2012……....Minor, Environmental Engineering

Field of Study

Major Field: Chemical Engineering

Minor Field: Environmental Engineering

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

1.1. A Concise History of Wastewater Treatment

Modern wastewater treatment began in the 1800s when British statisticians determined the cause of cholera outbreaks to be the result of contact with contaminated water: the anti-cholera acts of parliament led to the building of pump stations (Cambridge Museum of Technology, 2012). Pump stations used the city's solid waste to produce steam, which was then used to pump raw sewage waste to a nearby sewage farm where it could be spread over the land and thereby treated. This was the first wastewater treatment plant. Since then, sewage treatment and sewers have proven to be the greatest invention of modern science. Removing the organic material and its associated nutrient content from wastewater is the primary means to human health protection and environmental safety.

Wastewater is 99.9% water and 0.1% organics, salts and nutrients, for example nitrogen and phosphorous (Tchobanoglous et al. 2003). The organic content comprises bacteria and degraded organic mass from human faeces. Despite the small fraction of organics in wastewater, humans so densely inhabit the planet that the organic matter and nutrients must be reduced to much lower levels.

Wastewater treatment has advanced since the days of spraying on farmland (thought we still do that too). The two forms of secondary treatment used to remove organics are aerobic technology and anaerobic technology. Aerobic treatment is the conventional practice and uses oxygen to promote bacteria growth. The bacteria process the waste and produce CO_2. The inputs required are oxygen (air) and electricity to pump the air into the aerobic tanks. The outputs are clean wastewater (needing to be sterilized still) and sludge. Sludge is the bacteria left over from processing and is not sterile. It is expensive to dispose of and is usually sent to a landfill. Landfilling sludge is a large part of the solid waste that goes into landfills and a significant cost to operating utilities (Tchobanoglous et al. 2003).

Anaerobic treatment is the method for current best practice. Anaerobic treatment limits the oxygen available. The bacteria process waste, in the absence of oxygen, and produce methane, CH_4,, and carbon dioxide, CO_2. Electricity is required to maintain the flow and recirculation of bacteria and to operate the equipment used to remove the hydrogen sulphide gas, H_2S, present in biogas. The outputs are clean wastewater, a small amount of sludge and biogas. Biogas is approximately 60% CH_4 and 40% CO_2. It can be burned to produce heat. The CO_2 content, however, reduces the efficiency and thereby makes it unsuitable to sell or use for much more than running the wastewater treatment facility. Anaerobic digestion requires at least three unit operations and professionally trained staff. Further, the small amount of sludge produced still requires disposal.

Neither aerobic nor anaerobic treatment has the ability to lower the concentration of organics in their effluent without increasing the retention time of the wastewater

significantly. There is then a practical limit to the lower concentration levels these technologies are able to achieve.

To compare aerobic to anaerobic treatment we use the metric of Chemical Oxygen Demand, COD, which is a measure of the degradable material in the wastewater. Figure 1 gives a visual representation of the effectiveness of each treatment at converting the COD to a useful product.

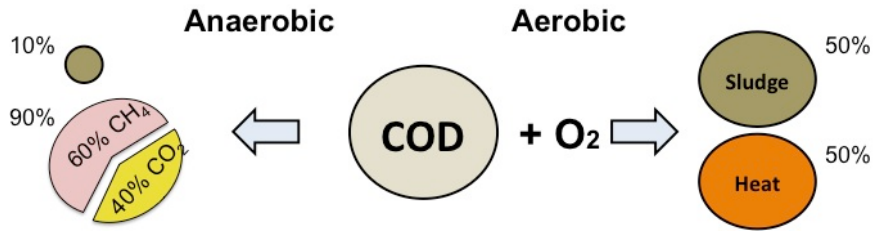


Figure 1: Comparing outputs from aerobic and anaerobic technologies based on Chemical Oxygen Demand, COD

Aerobic and anaerobic processes have strengths and weaknesses as effective wastewater treatment technologies. Financially, their weaknesses outweigh their strengths. The sludge produced and the CO_2 content released, in each process, are not preferred products and so we may conclude that a better method of wastewater treatment is warranted. MFCs are that better method.

Microbial Fuel Cells, MFCs, are a specific form of a Bio-Electrochemical System, BES, which has been proven to treat the organics and nutrients in wastewater using an electroactive biofilm (Habermann and Pommer, 1991). The major drawback to aerobic and anaerobic treatments is the use of free form (i.e. free floating) bacteria, which must

be periodically removed to maintain a constant mass balance in the system. These systems also require the intensive use of pumps to recycle large quantities of water to maintain the culture. The bacteria in these treatment systems are not present in high concentration when the wastewater arrives at the treatment plant; they are intentionally cultivated to feed on the organic concentration of the wastewater. The aerobic and anaerobic wastewater treatment processes rely on the fact that these (and all) bacteria follow Monod kinetics. The growth phase, which implies a great amount of reproduction, is cultivated by processing at the plant. This growth phase is good for cleaning the wastewater quickly, but bad because it then requires the removal of the resulting mass of bacteria (microbes).

The advantage of the MFC is that it retains the bacteria on a solid, stationary surface. Biofilm growth is limited to the available surface area, so they colonize the surface and then remain in the stasis phase of growth. The loss of cellular biomass is only from dead cells at the end of their life cycle and not the result of any sudden population growth followed by subsequent death, requiring removal.

Biofilms are diffusion limited, so the bulk phase concentration of substrate and the flow rate (and corresponding boundary layer thickness) determine the rate of mass transfer through the biofilm. The biofilms in MFCs are able to feed on lower organic concentrations than traditional wastewater treatment techniques and thereby reduce the effluent waste concentration further. The microbes develop niche abilities and produce continuously in either an oxidative or reductive capacity.

1.2. Current Best Practice and Biogas Upgrading: The Lettinga Project

The current best practice for wastewater treatment uses fermentation and Anaerobic Digestion, AD, combined with the CAMBI thermal hydrolysis process, which reduces the biosolids fraction created by fermentation and anaerobic digestion. Wastewater fermentation refers to the conversion of organic substrates into alcohols, organic acids and biosolids. The ratios and types of volatile fatty acids (alcohols and organic acids) produced in fermentation can be predicted based on the composition of the substrate, regardless of operating changes (IWA, 2002). Anaerobic digestion, AD, delineates the wastewater treatment process to convert the carbon in wastewater, assumptive of the volatile fatty acids produced in fermentation, into biogas and biosolids. Biogas is a mixture of methane, carbon dioxide and trace hydrogen sulfide. The fermentation process can be performed along with AD in one unit as a sequencing batch reactor process, SBR. Alternatively, fermentation and the completion of AD can be performed in separate units placed in series. The transfer of material between the two units in series must then be timed accurately, in order to maintain their distinct process separation. Anaerobic digestion following fermentation produces methane from volatile fatty acids to the limit of available compounds present in the wastewater feed.

Biosolids must be disposed of in sanitary landfills, as is standard federal practice worldwide, and are an expensive burden on municipal wastewater treatment operations. Biosolids can alternatively be treated as a high volume, renewable carbon and energy source. A major problem with using biosolids as a substrate for resource production is that 55% to 80% is non-degradable. The CAMBI thermal hydrolysis process reduces the

fraction of non-degradable material to 25% to 40%, where the now degradable fraction can be further fermented and anaerobically digested. The CAMBI process is being applied worldwide.

Biogas is not of high enough quality to sell and distribute as natural gas. Pipeline quality for natural gas is 99.999% methane. Biogas additionally has trace levels of hydrogen sulfide that must be removed before burning, as it is a controlled pollutant and corrosive. Increasing the methane content of biogas is called biogas upgrading. Currently, upgrading can be achieved by removing the CO_2, usually with pressure swing adsorption. This process involves compressing gases and is energy intensive. Using pressure swing adsorption for upgrading is only economically feasible for very large wastewater treatment plants in areas where natural gas is in demand. Removing the hydrogen sulfide is already a part of biogas treatment and must be done before distribution or burning on site. Methane is also a controlled pollutant, so flaring is the minimum required practice. More often the biogas is burned on site to use for heating and operating the wastewater treatment plant. Instead of removing the CO_2 to upgrade the biogas, if all the CO_2 in the biogas were reduced to methane, then pure methane gas would result.

The amount of hydrogen and number of electrons naturally present in wastewater limits the fermentation and anaerobic digestion of the available carbon into methane, instead resulting in carbon dioxide. A microbial fuel cell, integrated into the wastewater treatment process, could supply the previously limited electrons and hydrogen to a biofilm of wastewater treatment microbes, allowing them to continue to upgrade the biogas without creating biosolids.

This project was funded for the expressed purpose of integrating wastewater treatment units based on the concept of a Microbial Fuel Cell (Figure 2) to (1) remove the residual organic fraction in the digestate, and (2) convert the biogas CO_2 fraction to CH_4. The work to remove the residual organic fraction in the digestate (1) was completed prior to this work. This work focused on the conversion of the biogas CO_2 fraction to CH_4 (2). The terms used in the diagram are detailed in Appendix 1.

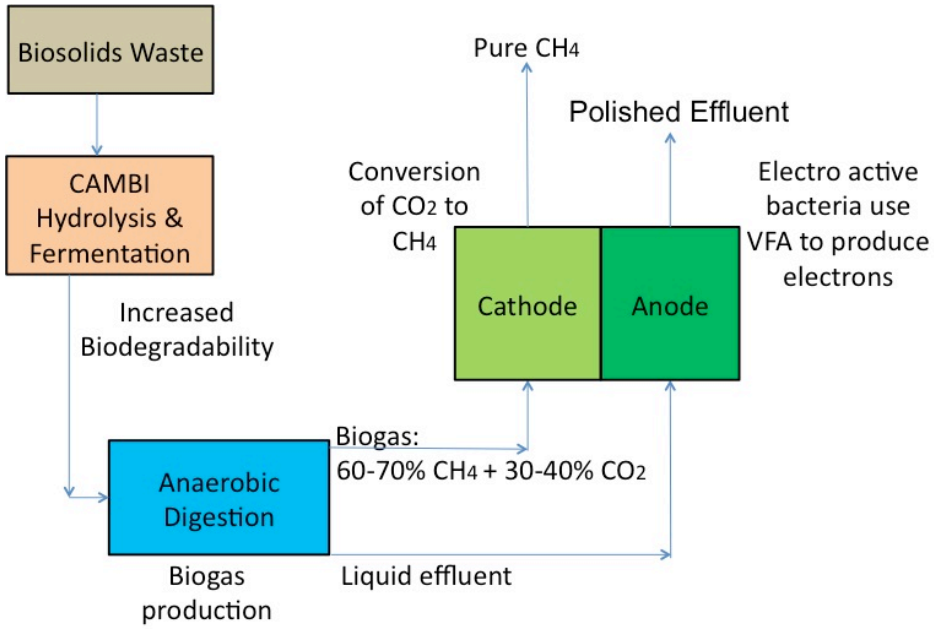


Figure 2: Integrated concept for the treatment of waste sludge with biogas upgrading using an MFC

Using an MFC to clean wastewater is not the end of the quest for economic wastewater treatment with no further environmental impact or unusable wastes (biosolids and CO_2). The power of an MFC lies in the ability to create resources by completely using wastes. To create resources we turn to the cathode side of the MFC, were investigation is still new and the creation of reduced products is possible. Using the

(unknowably) large flora of bacteria we have readily available everywhere on earth, human flora, we know we can create methane and other fuels on the MFC cathode. The nutrients remaining in the wastewater could support continued microbial production of methane from CO_2 (in the cathode), beyond the consumption of the carbon in the wastewater (performed in the anode). The MFC becomes a fuel factory, run on energy from wastewater and CO_2, producing until it completely exhausts the nutrient content of the wastewater. Investigation into the controls for optimization, scale up, and reliability are the focus of this work.

The following chapters complete the review of research work done as proposed here. Chapter 2 expands the basic knowledge required for experimentation, detailing the electrochemistry of a fuel cell, the equations to measure efficiency, the extension of microbes into fuel cells to create microbial fuel cells, the biochemical processes performed by microbes in normal wastewater treatment (fermentation and AD), and an overview of methane producing microbes, methanogens. In Chapter 3 the experimental methods will be explained with special focus on the operating parameters of interest for this work. In Chapter 4 the results will be presented followed by a discussion. Chapter 5 will offer conclusions and directions for future work. Finally, various Appendices are included to explain terms, provide equations relevant to the wastewater used for an MFC but not included in this research, and finally list protocols for MFC specific activities.

Bibliography

Cambridge Museum of Technology, http://www.museumoftechnology.com/, accessed Feb. 24, 2012.

Habermann, W. and Pommer, E.-H. (1991) Biological fuel cells with sulphide storage capacity. Appl. Microbiol. Biotechnol. 35, 128-133.

IWA Task Group for Mathematical Modelling of Anaerobic Digestion Processes (2002) Anaerobic Digestion Model No. 1 (ADM1). IWA Publishing.

Tchobanoglous, G., Burton, F., Stensel, H. (2003) Wastewater Engineering: treatment and reuse. Metcalf & Eddy. McGraw-Hill.

2. MICROBIAL FUEL CELLS

2.1. Introduction

The third law of thermodynamics is the mathematical expression of the phenomenon that the universe abhors a gradient and wherever gradients exist the universe will induce a flow (of the appropriate material or energy form) to reduce the gradient. The third law is applied to conditions of difference in pressure (a force, specifically of fluids), differences in thermal energy (heat and by extension light), differences in mass concentration (more specifically activity) and differences in charge (coulombs), also known as electrical potential difference (volts). Chemical engineering studies the first three categories of differences. Electrical engineering and electrochemistry study the fourth category primarily. *See definitions in Appendix I for further explanation of electrical engineering and electrochemistry terms.*

Third law measurements use Gibbs free energy, where a negative change in Gibbs free energy implies a spontaneous process and the overall energy state within the system is minimized (e.g. the gradient is reduced between chemical species or energy concentrations). The final energy state being less than the initial energy state means a release of energy has happened. In between the final state and the initial state, the energy path of the reaction has to first overcome activation energy. When there is no activation

energy preventing a reaction this is called a spontaneous reaction, because it happens spontaneously or automatically.

All of life takes advantage of the third law of thermodynamics. All life performs reduction and oxidation reactions, called metabolism, to use the flow of energy for continued existence and reproduction. The third law independently dictates the release of energy by the movement of electrons (energy) and/or mass. Life positions itself between the initial and final state to allow the flow of electrons to pass through it and activate the reactions to continue its life. Life attempts to use spontaneous redox reactions or to lower the activation energy of these redox reactions using various means including catalytic enzymes, communal interactions, and for people the additional use of fire and tools. Microbes preforming redox reactions transfer electrons from an electron donor, at lower potential, to an electron acceptor, at higher potential. The resulting flow of charge is used for regeneration and reproduction.

The electrochemical behaviour of microbes can be applied to an electrochemical cell, the basic electrochemical system, to create a Bio-Electrochemical System (BES). The purpose of an electrochemical cell is to either drive a chemical reaction by supplying energy (electrolytic cell) or to gain electricity from a chemical reaction (galvanic cell, e.g. battery).

An electrochemical cell separates the reduction reaction from the oxidation reaction in a redox reaction. The reduction and oxidation reactions occur separately on the surface of two electrodes. These electrodes are separated either in two distinct compartments or by space in two distinct zones within the same compartment. The

electrons passed to complete the reduction and oxidation reactions are carried between the two electrodes, external to compartments or zones, using a conducting material, usually wire. Oxidation reactions occur on an anode electrode in an anode compartment (or zone). Reduction reactions occur on a cathode electrode in a cathode compartment (or zone). A membrane that allows ions (of some type) to pass between compartments must separate the compartments, if the cell has physically separated compartments.

Just as Lewis acids and bases describe the transfer of electrons in traditional redox reactions, so too can Lewis acids and bases generally describe the transfer of electrons in an electrochemical cell, specifically in a closed electrochemical cell system. The oxidation reaction on the anode (electrode) creates a net loss of electrons and therefore a net positive charge. This makes the anode compartment solution (or solution in the anode zone) become a Lewis acid. The reduction reaction on the cathode (electrode) creates a net gain of electrons and therefore a net negative charge. This makes the cathode compartment solution (or solution in the cathode zone) become a Lewis base.

An electrochemical cell must have a completed circuit to operate, meaning to maintain the flow of electrons the charges cannot accumulate indefinitely and must be balanced. This happens naturally (according to the third law of thermodynamics) by the migration of charged ions towards the two electrodes. This migration of ions occurs to try to balance (neutralize) the charge at either electrode. Either a negatively charged ion (anion) will migrate toward the anode, which has a net positive charge, or a positively charged ion (cation) will migrate toward the cathode, which has a net negative charge.

In an open electrochemical cell the continuous flow of new substrate, to be oxidized or reduced, past an electrode will eliminate most of the accumulation of charge around the electrode. Some minimal, localized charge accumulation will continue to occur close to the electrode surface based on the fluid dynamics and electrochemistry.

A fuel cell denotes an electrochemical cell that provides a useful fuel as the final product. Fuel cells are a convenient way to extract electricity from a naturally spontaneous reaction (galvanic cell). However, any form of useful fuel can be the final product of a fuel cell, so long as the electrochemical reactions in the cell are overall spontaneous. When a chemical reaction is spontaneous this means there is no activation energy to stop it from occurring. Supplying a net amount of energy (work) to drive a chemical reaction means the reaction has an activation energy and is not spontaneous. Gaining energy (work) from a chemical reaction means the reaction has no activation energy to overcome and is spontaneous. Production of chemical fuels, or the energetically neutral production of useful chemicals, in a fuel cell requires the free flow of electrons. This necessitates two spontaneous reactions: the spontaneous oxidation reaction at a higher potential supplies electrons to the spontaneous reduction reaction at a lower potential. The fuel is presumably the reduced species, but either chemical product could be useful (and hopefully both). Catalysts are used to make desirable non-spontaneous reactions occur spontaneously by overcoming the activation energy and nearly spontaneously by lowering the activation energy. Catalysts can be organic or inorganic. Catalysts can be used to drive chemical reactions using less energy in electrolytic cells. Catalysts can be used to gain more energy from the chemical reactions in galvanic cells.

Spontaneous reactions provide a spontaneous exchange of electrons between the oxidized and reduced substrates. In electrochemical cells, this means spontaneous reactions causing a flow of electrons when there is a completed circuit between the anode and the cathode (with negligible resistance). Conversely, when potential is freely available (but not forced) in a completed circuit a spontaneous reaction will proceed at its thermodynamically determined potential.

The biological drive to live and reproduce is a universal law. Live cells, capable of accessing electrons and/or potential, will react spontaneously to access available electrons and/or appropriate potential, or they will use a biocatalyst to overcome the activation energy needed to react. Freshly dead cells and tissues, which possess all that same biochemical apparatus, will react biochemically only upon the external application of current or potential and are eventually denatured instead of revived. The drive to live and reproduce means microbes can act as biocatalysts for otherwise non-spontaneous reactions. More importantly, these microbe biocatalysts and the catalytic enzymes cells use are continuously regenerated.

A Bio-Electrochemical System (BES) is any form of the electrochemical cell that uses microbes interfaced with the electrode to perform electrochemical reactions, including extracting electricity and producing chemicals. Microbes perform redox reactions on one or both of the electrodes. Supplying current to drive the microbial production of specific chemicals is an example of a microbial electrolysis cell (Logan et al. 2008). Bioelectrodes can be an effective replacement for some chemical electrodes because the microbes act as catalysts to lower the activation energy of reaction (an

overpotential) and are less expensive. Chemical catalysts such as platinum can be prohibitively expensive and limit the commercial potential of even high efficiency electrochemical systems. Electrochemically active bacteria and microbes, capable of useful or exotic reactions, create a better alternative. The physical separation of the cathode and anode by an ion exchange membrane allows for the separation of the associated environments and, in a BES, of the species present. This means that a mixed culture, appropriate to treat wastewater, can be used on the anode, while a different culture can exist on the cathode, appropriate to catalyse another feed stream. Figure 3 illustrates the idea of a BES with an ion exchange membrane, where both the anode and cathode perform microbial electrosynthesis.

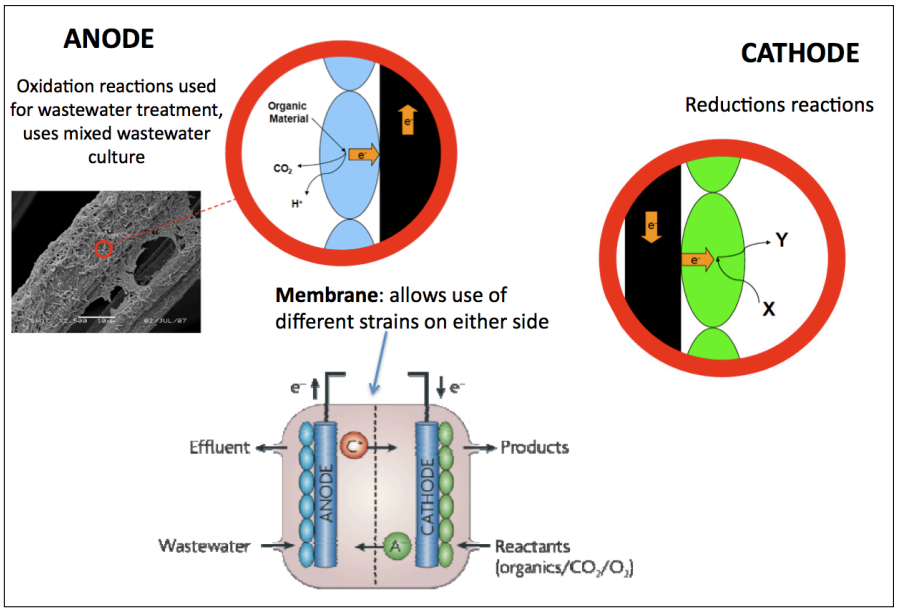


Figure 3: Concept of Microbial Electrosynthesis. Adapted from Rabaey & Rozendal 2010

A Microbial Fuel Cell, MFC, is a classic fuel cell that uses microbes as catalysts, to either extract electricity and/or produce chemical fuels. A MFC is specifically the fuel

cell version of a BES. Microbes catalyse redox reactions on one or both of the electrodes. The production of (reduced) chemical fuels in a microbial fuel cell similarly requires the spontaneous flow of electrons and necessitates both electrodes have spontaneous reactions, where the potential of the reaction on the anode is higher (overall) than the potential of the reaction on the cathode.

The number of research papers in the last decade on BES systems is expansive. The majority of the research has been on the anode processes. The research has led, thus far, to a couple of successful industrial scale-up projects using bioelectrochemical anodes and electrochemical cathodes, such as Bilexys, in Australia, where caustic soda and hydrogen peroxide are produced on the cathode.

The research concerning the cathode processes is relatively new and growing. The production of methane specifically has been primarily in the attempt to minimize it, in favour of higher value products, produced on both the anode and the cathode. Methane producing bacteria are hardy and common, making them a problem bacterium in reactor conditions that would facilitate their growth (Logan et al. 2008). The syntrophy between electroactive bacteria, fermenters and methanogens has been shown on the anode (Freguia et al. 2008). Initial studies have recently shown that methane can result from biocatalysis in anaerobic treatment wastewaters (Clauwaert et al. 2008).

While methane may be a lower value product than other potential chemicals or fuels, biogas is a wasted renewable fuel whose purity and value could benefit from treatment using a MFC.

2.2. Microbes in Methanogenesis

2.2.1. Fermentation, Respiration, and Extracellular Electron Transfer

Microbes transfer electrons from an electron donor at lower potential, comparatively, to an electron acceptor at higher potential, comparatively. Respiration and fermentation are the two modes of cellular transport used by microbes in redox reactions to transport the combined substrate/electron. Respiration denotes external redox reactions. Fermentation denotes internal redox reactions. If the electron acceptor/donor is external, this metabolism is called respiration. If the electron acceptor/donor is internal, and excreted later after use, this metabolism is called fermentation.

Thermodynamics dictates that some electron donors must be used externally, respired, and while other electron donors are used internally, fermented (Heijnen, 1999). Traditional cellular respiration works by creating an electrochemical gradient across the cell membrane, using protons, and results in a hydrogen potential or pH difference across the membrane. Membrane bound proteins with sequentially increasing reduction potentials oxidize a reduced species. In aerobic respiration the terminal electron acceptor is oxygen. In anaerobic respiration the terminal electron acceptor is usually another microbe. Fermentation is an entirely internal process where the electron acceptor, NAD+, and the electron donor, NADH, are continuous regeneration during the production of ATP using the glycolysis pathway.

Most microbes are capable of both modes of combined substrate/electron transport and use each mode for different reactions based on the greatest ΔG value they

can achieve in their environment. Biochemical enzymatic redox reactions depend on the oxidation-reduction potential (ORP) state of the environment, or the environments reduction potential (E_h). The redox state of the environment affects the solubility of nutrients, particularly metal ions that can act as trace nutrients or trace poisons. Strictly aerobic microorganisms are generally active at positive E_h values, whereas strict anaerobes are generally active at negative E_h values.

Fermentation and respiration are cellular transport terms distinct from whether or not the system is aerobic or anaerobic. In aerobic conditions microbes are able to use O_2 as their electron acceptor, in anaerobic conditions they cannot. Commonly an anaerobic system promotes the growth of microbes that use fermentation, however this is only a common occurrence and anaerobic respiration is possible for some microbes. Many microbes will use respiration when soluble electron acceptors (i.e. O_2) are present in the environment and switch to fermentation when their preferred soluble electron acceptor is depleted. Anaerobic respiration simply implies there are soluble electron acceptors available for respiration other than oxygen, such as sulphate (SO_4^{2-}), nitrate (NO_3^-), or sulfur (S). These electron acceptors have smaller reduction potentials than O_2, so less energy is released per molecule oxidized. Anaerobic respiration is generally less energetically efficient than aerobic respiration. The energy output per mole of fermented material is far less than the energy output from the complete respirative oxidation of the same substrate, however the rate of ATP production in fermentation can be up to a 100 times faster than for certain forms of anaerobic respiration (*Voet et al.,* 2002). Therefore, organisms that require fast consumption of ATP use fermentation over anaerobic

respiration. Facultative anaerobic organisms are an example of microbes capable of aerobic respiration and anaerobic fermentation.

It is important to note that wastewater engineering defines anaerobic conditions as those where no oxygen, Fe (III), nitrate, or sulphate (which have oxygen in the molecule) are present and anoxic denotes the absence of only oxygen.

The microbes of interest here include several that perform anaerobic respiration. Methanogenesis by anaerobic respiration for the reduction of carbonate (CO_2) to methane (CH_4) can be performed by Methanothrix thermophile (now, *Methanosaeta*) at a reduction potential ($E^{0'}$) of -0.25 V. Acetogenesis, specifically homoacetogenesis, for the reduction of carbonate (CO_2) to acetate can be performed by respiration by Acetobacterium woodii at a reduction potential of -0.30 V. Sulphate respiration for the reduction of sulfate (SO_4^{2-}) to sulfide (HS^-) can be performed by Desulfobacter latus and Desulfovibrio, which are obligate anaerobes, at the reduction potential of -0.22V. Sulphur respiration for the reduction of sulfur (S^0) to sulfide (HS^-) can be performed by Desulfuromonadales, among other facultative aerobes and obligate anaerobes, at the reduction potential of -0.27 V. These potentials are for reactions where hydrogen gas is present. The theoretical potential for hydrogen gas production by electrolysis is -0.41 V. Therefore the availability of hydrogen gas, or electrons in aqueous solution, limits methane production when abundant carbon is available.

Microbes can transfer electrons separately to the chemical substrates used in their redox reactions. When redox by respiration is no longer possible, microbes can either ferment or use a non-soluble electron acceptor/donor. Using a non-soluble electron

acceptor/donor means transporting electrons to the outside of the cell. This is called extracellular electron transfer, EET. An example of EET in nature is Shewanella, a marine bacterium capable of the respiration of metals and EET. By saturating the metal with electrons, the metal expands and softens, which allows the Shewanella to process it and release an electrical charge. Geobacter are one genus of bacteria capable of anaerobic respiration and EET. Many microbes are capable of some form of EET (Aelterman et al. 2008).

The modes of extracellular electron transfer are direct electron transfer and indirect electron transfer (Rabaey et al. 2007). Direct electron transfer uses membrane bound (or membrane associated) enzymes to attach the microbe to the surface, pili, and pili-like structures (nanowires). Indirect electron transfer uses shuttles, either organic or inorganic, to transfer oxidized and reduced compounds back and forth to the cell surface. The shuttle may be regenerative or not. Indirect electron transfer is diffusion limited. Direct extracellular electron transfer is the most efficient mode of electron transfer. Figure 4 shows examples of the three modes of electron transport as they would apply to the reduction of CO_2 into CH_4.

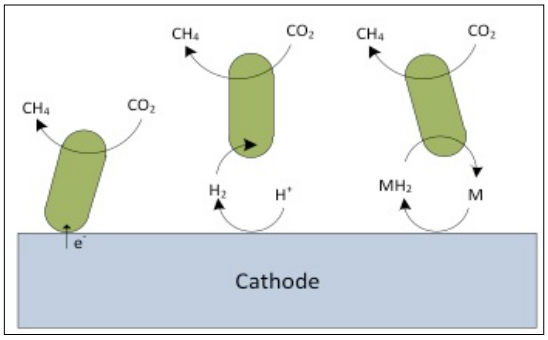


Figure 4: Conception of three possible extracelluar electron transport mechanisms as applied to the bioelectrochemical reduction of CO_2 to CH_4

In anodes, the syntrophy between mixed culture microbes is significant (Pham et al. 2008). Not all microbes are capable of producing pili or nanowires for direct EET however. More are capable of having membrane bound enzymes that can attach them to the surface and/or excreting enzymes to create conductive glue for themselves. Many are capable of using shuttles, indirect transfer, with the surface enzymes they normally use to perform respiration. Finding microbes capable of direct extracellular electron transfer is an essential step to creating highly efficient microbial fuel cells.

When microbes and engineers separate electron transfer from substrate transfer (essential carbon, nutrients and trace minerals), then the optimization of efficiency with which cells produce can be simplified and focused to (1) increase the rate of electron transfer between the cell and the electrode and (2) increase the availability of chemical substrates.

2.2.2. Methane Fermentation (AD) Process

In wastewater engineering, fermentation refers to the fermentation process used to produce methane, collectively called AD: a multi-stage bioprocess capable of the consecutive biochemical breakdown of almost all types of polymeric materials to methane and carbon dioxide (biogas) under anaerobic conditions. Methane fermentation (AD) is achieved in an environment in which a variety of microorganisms which include fermentative microbes (acidogens); hydrogen-producing, acetate-forming microbes (acetogens); and methane-producing microbes (methanogens) grow in symbiosis to produce reduced end-products (*FAO, 1997*). While methane fermentation (AD) implies the degradation of organic solids using internal electron/substrate transfer, only the first step and most important step in methane fermentation is a guaranteed fermentation redox reaction. The microbes present in the different stages of the methane fermentation process may or may not be strictly fermentative in their redox reactions. Cells use fermentation and/or respiration depending on the species and their environment, and in accordance to the greatest thermodynamic gain they can achieve. The use of fermentation or respiration loosely follows the original purpose of the microbe in its larger community, or the stage the microbe functions within. In the degradation of solid organics, those microbes at the beginning of the degradation chain are most likely to perform fermentation. Those microbes at the end of the degradation chain (methanogens) are more likely to perform respiration and/or fermentation.

The stages of methane fermentation are shown below in Figure 5, the percentages are general reference.

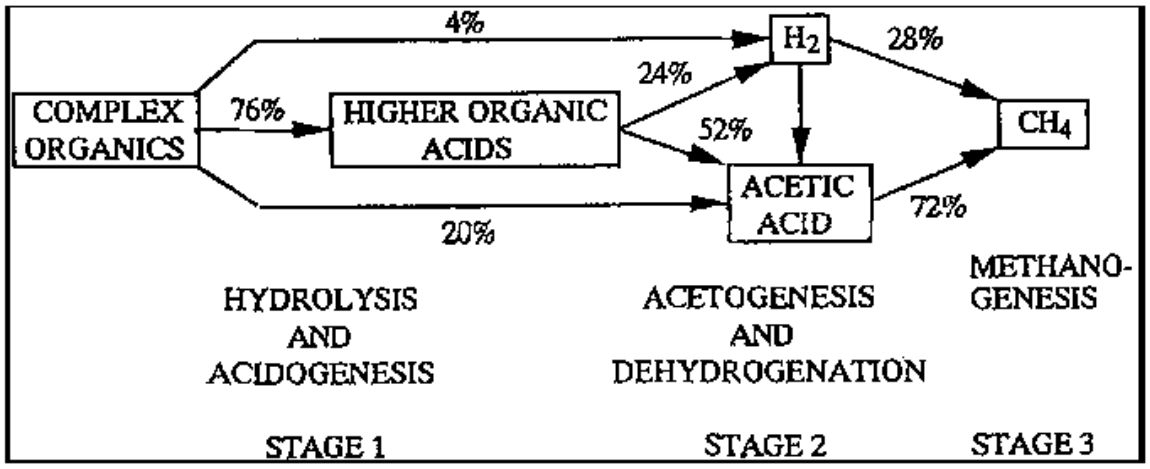


Figure 5: Anaerobic Digestion (AD) stages. *Figure referenced from McCarty, 1981*

Hydrolysis and acidogenesis (Stage 1): Microbes excrete extracellular hydrolytic enzymes to hydrolyse the polymeric material, usually lipids, proteins and carbohydrates, into monomeric material, sugars, amino acids, and fatty acids. These monomers are metabolised by the bacteria and are primarily fermented to acetate, propionate, butyrate, lactate, ethanol, carbon dioxide, and hydrogen (FAO, 1997). Acetate, propionate, butyrate, lactate, and ethanol are collectively referred to as (types of) volatile fatty acids. Different polymeric materials require different hydrolytic enzymes to degrade them, which are usually excreted by different bacteria, all of which are present in the consortium.

Acetogenesis and dehydrogenation (Stage 2): Microbes convert the propionate, butyrate, lactate, ethanol, carbon dioxide, and hydrogen to acetate (and water). The majority of the acetate and hydrogen are produced at this stage. Hydrogen production by acetogens is energetically unfavourable ($\Delta G>0$). Obligate hydrogen producing acetogenic

bacteria can produce both H_2 and acetate. In the presence of hydrogen consuming microbes, methanogens and sulphate-reducing bacteria, the co-culture can reduce the fatty acids to acetate, CH_4 and H_2S, with H_2 as an intermediate. Acetate and H_2 accumulation inhibit acetogens, thus acetate and hydrogen consumption by the methanogens (stage 3) is important to maintain consortium health.

Methanogenesis (Stage 3): Microbes convert acetate, carbon dioxide and hydrogen into methane and carbon dioxide. Methanogens can also convert formate, methanol, methylamines and carbon monoxide into methane. Formate is a food source of note for studying methanogens. Some of the hydrogenotrophs are capable of converting formate. Acetate consumers are incapable of using formate. The final product of methane fermentation is biogas, a combination of methane and carbon dioxide at about a 60:40 ratio. Increasing this ratio in favour of methane is only limited by the availability of electrons (and the associate H^+ freely available in water).

2.2.3. Methanogenesis

Methanogens are classified in the domain of archaea. Archaea were previously classified as a prokaryote along with the bacteria, named archaebacteria, but have since been found to be distinct. Methanogens are obligate anaerobes and require a redox potential of less than ~-250 mV for growth. Methanogens can be divided into two groups: Hydrogenotrophs (H_2/CO_2-consumers) and Acetotrophs (acetate-consumers). The two types of methanogens produce two sets of products. The acetotrophic methanogens ferment acetate to produce methane and carbon dioxide. They are classified as chemotrophs. Acetate, produced in stage two of methane fermentation, is consumed by a

limited number of strains, such as *Methanosarcina* spp. and *Methanosaeta* spp. (formerly *Methanothrix).* The hydrogenotrophic methanogens respire carbon dioxide and hydrogen to produce methane and water. They are classified as autotrophs. Most species of methanogens are hydrogenotrophic autotrophs, meaning they can only grow on CO_2 and H_2. Acetotrophic methanogens are more abundant in human flora and wastes, however both are present. Examples of strict hydrogenotrophs include Methanococcus and Methanopyrus. Methanogens that convert methylated compounds such as methylamines, methanol with H_2, and methanethiol are called Methylotrophic archaea.

The continued reduction of CO_2 to CH_4 requires more H_2, which inhibits acetogens at high partial pressures. In regular wastewater fermentation (AD) using increased hydrogen partial pressures would result in un-degraded (wasted) fermentation material. A separate aqueous system with a primary culture of hydrogenotrophic methanogens and abundant H_2, and/or H^+ and electrons, could continuously produce methane from the CO_2 in biogas.

Hydrogenotrophic methanogens were the main microbes of interest for this work. The metabolic pathway for hydrogenotrophic methanogenesis is presented below in Figure 6.

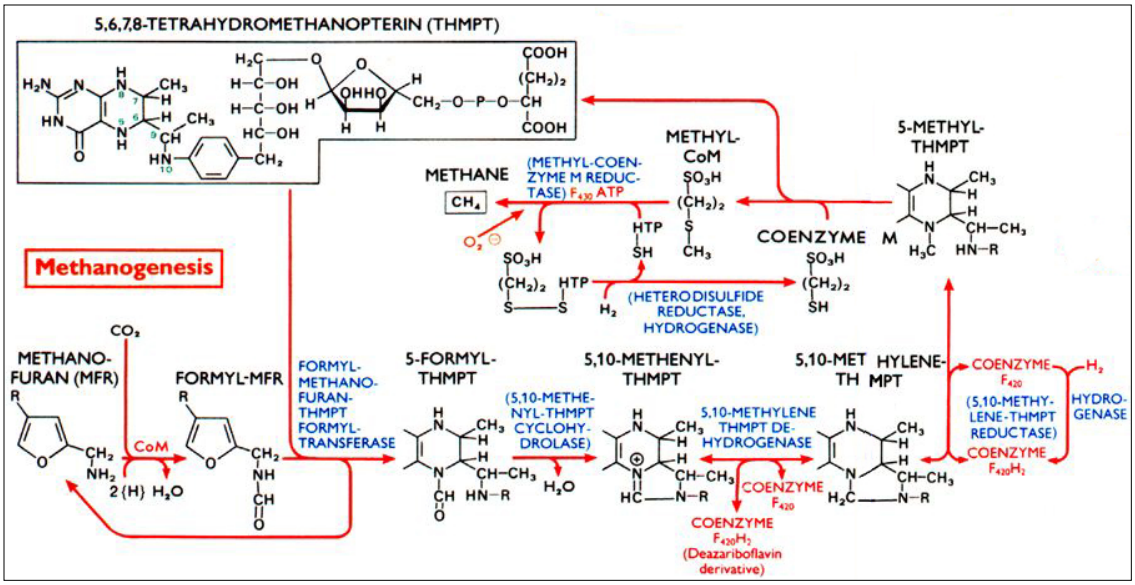


Figure 6: Metabolic pathway of autotrophic methanogenesis. *Referenced from Roche, 2012*

There are three known metabolic pathways for methanogenesis. Most methanogens have one of the three pathways and can use two or three substrates with that pathway. The most common pathway is the hydrogenotrophic (autotrophic) pathway. Being able to switch between substrates, using one pathway, to function as a chemotroph or as an autotroph is a benefit to the methanogens in the methane fermentation process. For example, Methanofollis can use H_2/CO_2, formate, 2-propanol/CO_2, and 2-butanol/CO_2. Methanoculleus can use H_2/CO_2, formate or acetate. Methanobrevibacter smithii is the most common methanogen in the human flora. Methanosarcina, also present in human flora, is a unique primary acetotroph that has all three methanogenesis pathways and the limited ability for cell-cell adhesion.

2.2.4. Energy metabolisms and their incidences

In methane fermentation, the ΔG values gained by microbes relies the combination of VFA production with methanogenesis using the hydrogen (communal cooperation). The communal degradation of VFAs to methane requires low partial pressures of hydrogen. For propionate an extremely low partial pressure (10^{-5} atm) is required. The conversion of CO_2 and H_2 to methane is not as sensitive to the partial pressure of hydrogen. In the MFC, the conversion by microbes depends on the ability of any species of microbe to both perform some version of EET and complete the desired conversion given the potential of the electrode is within the range of the potential the microbe requires to perform the reaction. For an anode, this means the potential is slightly lower than the microbe performs at and can pass an electron off to it. For the cathode, this means the potential is slightly higher than the microbe performs at and can accept an electron from the electrode. The theoretical potential the reaction occurs at is compared to the potential the microbe can perform at and production is evaluated for a given electrode material. Microbes can often adjust the potential losses (overpotential) of the bare electrode by using EET and/or unique enzymes. This is especially probable when using organic electrodes like carbon or another naturally preferred electrode. Below is a diagram the oxidation reactions (anode) and reduction reactions (cathode) that have biologically relevant reaction potentials, Figure 7.

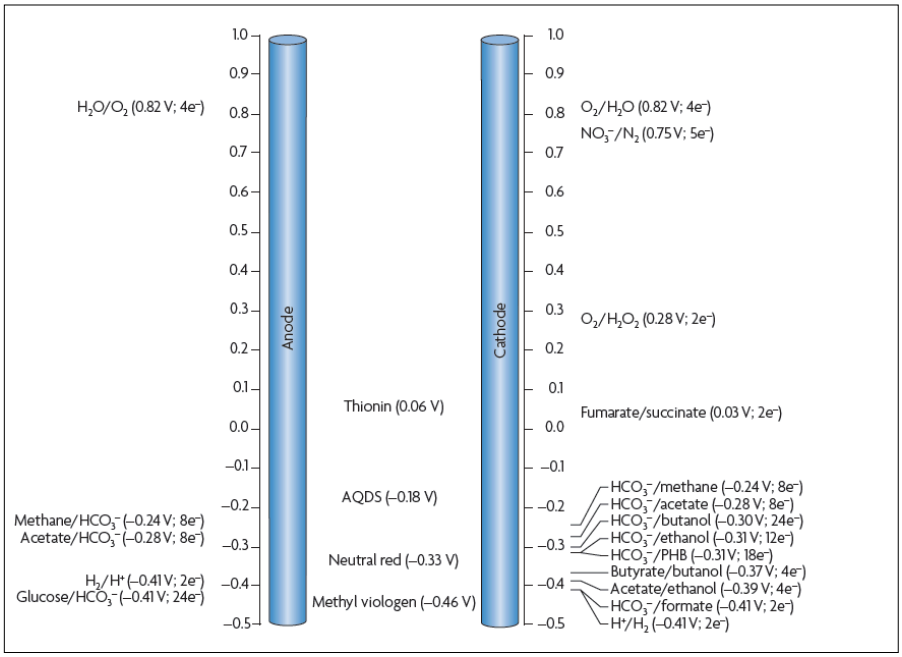


Figure 7: Redox potentials of biological reactions of interest. *Referenced from Rabaey and Rozendal, 2010*

The question is when a mixed culture of microbes (presumably) capable of EET are in an autotrophic environment, will they continue to require low partial pressures of hydrogen, as they did in the fermentation environment? If so, the likelihood is that electrolysis will produce hydrogen and be used by those more tolerant of higher hydrogen partial pressures (methanogens). If not, those capable of EET and autotrophic production will compete with one another and produce any variety of organic products including methane, given sufficient CO_2 is present. Autotrophic methanogens capable of direct EET would give the best methane production efficiency and be slightly more competitive than microbes producing similar organic products, based on the theoretical production potential. Given the range of species of methanogens and their hardiness, it is likely that in such a situation methanogens will persist and turn any products excreted by an

electroactive biofilm into methane. This is further supported by the fact that most microbes are inhibited by their own waste products, so an electroactive biofilm would benefit from a syntrophy with chemotrophic methanogens.

2.3. The MFC performance - Parameters defining the efficiency of the MFC

The theoretical energy gain for microbes is directly related to the potential difference between the electron donor and acceptor. Microorganisms try to maximize their energy gain (or the flow of energy through them) by using the available electron donor that has the lowest potential and the available electron acceptor that has the highest potential, within reason for their metabolism. Reactions go forward if the ΔG available to the microbe is negative. For example, ATP metabolism (by hydrolysis) releases -7.3 kcal/mole. Therefore, a microbe could theoretically use ATP to make any reaction go forward if that reaction's ΔG was less than +7.3 kcal/mole. The equation to measure the theoretical potential difference is:

$$\Delta G = -nFE_{emf} \quad [1]$$

- n is the number of electrons exchanged in the reaction (using a mole balanced equation)
- F is Faraday's constant - the charge of one mole of electrons (96485 C/mol)
- E_{emf} is the potential difference (V) between the electron donor and acceptor

Coulombic efficiency refers to the efficiency of electrosynthesis and is the ratio of current that is converted into products, as a percentage. It is calculated as the total number of electrons used to create product(s), in coulombs, divided by the total current

supplied, in Coulombs, times 100. To find the number of electrons used to create the product we write the redox reaction for the product from the reactant and, in the cathode, use the stoichiometric number of electrons. In the anode we would use the stoichiometric number plus one, since at least one electron charge must be transferred to the anode for an oxidation reaction to happen by extracellular electron transfer, or EET. The coulombic efficiency at the cathode is:

$$\frac{Coulombs\ transfered\ into\ products}{Coulombs\ supplied\ to\ electrode} * 100 \quad [2]$$

In the cathode microbes receive energy, catabolism, by reducing the electron shuttle, hydrogen or a renewable mediator, which is generated or regenerated at the cathode. The microbes metabolize the reduced electron shuttle with their biological pathways. To receive the substrates needed for continued life, i.e. carbon, nitrogen, phosphorous, and other trace nutrients, the atoms must be separately consumed. Substrate consumption still follows the Monod kinetics:

$$R_i = q_{i,\max} C_{bac} \frac{C_i}{C_i + K_i} \quad [3]$$

- $q_{i,\max}$ is the maximum specific uptake for chemical species i
- C_{bac} is the density or concentration of the microbe(s)
- K_i is the half saturation constant

The separation of the cell into chambers, and the increasing concentrations of ions including pH, creates resistance within the solutions, which affects the potential of the substrate(s).

The Nernst-Planck equation is used to calculate the potential of electrolyte based on a standard redox potential at reference conditions, and can include the resistance changes in an iterative model. The Nernst-Planck equation is:

$$\frac{\delta C_i}{\delta t} = \nabla\left(D_i \nabla C_i + z_i u_{m,i} F c_i \nabla V\right) - \boldsymbol{u}\nabla C_{i,F} + R_i \quad [4]$$

For any species i, the change in concentration is equal to the summation of four concurrent mechanisms:

- Diffusion ($\mathrm{D_i}\nabla^2 C_i$),
- Migration in an electric field ($z_i u_{m,i} F C_i \nabla^2 V$), where z_i is the charge; mobility of the species $u_{m,i}$ is calculated using the temperature T and diffusion coefficient D_i, giving $u_{m,i} = \frac{D_i}{RT}$; velocity field $\boldsymbol{u}$ is taken as zero for the domain; and Faraday's constant is denoted F.
- Convection ($-\boldsymbol{u}\nabla C_{i,F}$), and
- Reaction (R_i).

Ohm's law governs voltage across the entire cell:

$$V = I.R_{ext} \quad [5]$$

- I is the total current
- R_{ext} is the external resistance

This paper focuses on the cathode. Therefore, the external resistance will be disregarded for this work.

Maximum electrode voltage, $V^{\max}$, is the difference between EA, the potential at the terminal, and the supplied voltage,

$$\mathrm{V}^{\max} = \mathrm{V}_{\mathrm{supplied}} - \mathrm{E}_{\mathrm{EA}} \quad [6]$$

The real electrode voltage is lower than $V^{\max}$, due to resistances inherent to all the materials used in the electrochemical system. The real electrode voltage is:

$$V = V^{\max} - I.R_{int} - \eta^{act} - \eta^{cp} \quad [7]$$

- η^{act} is the activation overpotential resistance or activation loss
- η^{p} is the polarisation overpotential resistance or polarization loss
- $I.R_{int}$ is the ohmic resistance or ohmic loss(es)

Ohmic resistance includes the resistance created by the transfer of ions in the solution and losses due to concentration gradients. The resistance from the transfer of ions is proportional to the current produced. The general equation for ohmic resistance created by the transfer of ions in the cell is:

$$R_{int} = r_m + r_e.d_{cm} \quad [8]$$

- r_m is the resistance of the membrane

- r_e times d_{cm} is the resistance of the electrolyte using the average distance between the cathode and the membrane that the ions travel, in a packed bed this could be considerable

The cathode potential then is the supplied voltage minus the internal or ohmic resistance:

$$E_{CA} = V_{\text{supplied}} - I.R_{int} \quad [9]$$

Polarisation resistance describes the ability, or lack thereof, of reactants to transfer to the electrode. Polarization is calculated using the Nernst equation in the bulk fluid as:

$$E_M^E = E_M^0 - \frac{RT}{n_{es}F} \ln\left(\frac{C_{MH2}^E}{C_M^E}\left(\frac{C_{Href}^E}{C_H^E}\right)^2\right) \quad [10]$$

$$\eta_M^{cp} = E_M^E - E_{HCO3}^B \quad [11]$$

Activation overpotential refers to the dissipated energy lost by the reactions at the electrode, and is the difference between the electrode potential and the species potential reacting at the electrode:

$$\eta_M^{act} = E_{CA} - E_M^E \quad [12]$$

Current density, i_{CA} as A/m^2, is calculated using the Butler-Volmer equation:

$$i_{CA} = i_{CA}^0\left[\exp\left(\frac{-\beta_{CA}n_{ES}F}{RT}\eta^{act}\right) - \exp\left(\frac{(1-\beta_{CA})n_{ES}F}{RT}\eta^{act}\right)\right] \quad [13]$$

- i_{CA}^{0} is the exchange current density
- η_{ES} is the number of electrons transferred by the shuttle
- β_{CA} is a symmetry factor
- η_{CA}^{act} is the activation overpotential of the cathode.

In a bulk fluid, with perfect mixing and constant volume, the mass balance varies depending upon either batch or continuous flow through the BES. For a batch solution initially saturated with CO_2, the mass transfer at the boundary between the biofilm and the bulk liquid is governed by the differential equation:

$$\frac{dC_i}{dt} = \frac{N'' A_{ca}}{V} \quad [14]$$

- A_{ca} is the area of the cathode biofilm with flux N''

For a continuous solution, assuming constant saturation with CO_2, the concentrations in the bulk liquid is a constant:

$$C_i = S \quad [15]$$

Mass transfer is not as important for understanding the bioelectrochemical system except at large external voltages or prohibitively low concentrations to microbe consumption.

Bibliography

Aeltermann, P., Rabaey, K., De Schamphelaire, L., Clauwaert, P., Boon, N. and Verstraete, W. (2008) Bioenergy. Wall, J.D., Harwood, C.S. and Demain, A.L. (eds).

Clauwaert, P., Toledo, R., Van Der Ha, D., Crab, R., Verstaete, W., Hu, H., Udert, K.M. and Rabaey, K. (2008) Combining biocatalyzed electrolysis with anaerobic digestion. Water Sci. Technol. 57 (4), 575-579.

Food and Agricultural Organization of the United Nations. (1997) Renewable biological systems for alternative sustainable energy production (FAO Agricultural Services Bulletin - 128). Miyamoto, K. (ed.). FAO Corporate Document Repository.

Freguia, S., Rabaey, K., Yaun, Z.G. and Keller, J. (2008) Syntrophic processes drive the conversion of glucose in microbial fuel cell anodes. Environ. Sci. Technol. 42 (21), 7937-7943.

Heijnen, J.J. (1999) Bioprocess Technology: fermentation, biocatalysis and bioseparation. Flickinger, M.C. and Drew, S.W. (eds). John Wiley & Sons, Inc., New York, pp. 267-291.

Logan, B.E., Call, D., Cheng, S., Hamelers, H.V.M., Sleutels, T., Jeremiasse, A.W. and Rozendal, R.A. (2008) Microbial electrolysis cells for high yield hydrogen gas production from organic matter. Environ. Sci. Technol. 42(23), 8630-8640.

McCarty, P.L. (1981) Anaerobic Digestion. Hughes, D.E. et.al. (eds.). p3-22. Elsevier Biomedical Press, Amsterdam, New York, Oxford.

Pham, T.H., Boon, N., Aeltermann, P., Clauwaert, P., De Schamphelaire, L., Vanhaecke, L., De Maeyer, K., Hofte, M., Verstraete, W. and Rabaey, K. (2008) Metabolites

produced by Pseudomonas sp. Enable a Gram-positive bacterium to achieve extracellular electron transfer. Appl. Microbiol. Biotechnol. 77(5), 1119-1129.

Rabaey, K., Rozendal, R. (2010) Microbial electrosynthesis – revisiting the electrical route for microbial production. Nature Reviews Microbiology. 8, 706-716.

Rabaey, K., Rodriguez, J., Blackall, L., Keller, J., Gross, P., Batstone, D., Verstraete, W., Nealson, K. (2007) Microbial ecology meets electrochemistry: electricity-driven and driving communities. ISME J 1(1) 9-18.

Roche Applied Science Biochemical Pathways wall chart. http://web.expasy.org/pathways/, accessed Feb. 24, 2012.

Voet, D., Voet, J., Pratt, C. (2002). *Principles of Biochemistry*. Wiley.

3. EXPERIMENTAL DESIGN

The goal of this work was the production of reduced chemical fuels, specifically methane, in a microbial fuel cell using CO_2 and the bacteria naturally present in wastewater. The objectives were to upgrade the biogas from anaerobic digestion and evaluate system parameters for continuous flow operation. The reaction of interest was the reduction of CO_2 to CH_4, performed by a microbial catalysis on the cathode. The hypothesis was that if one provides electrons and CO_2 then one could control methane production by promoting growth of the bacteria already capable of this reduction. The approach was to build an MFC to investigate what happens with adjustments to the inputs, e.g. amount of electrons, carbon source, and amount of carbon or frequency of carbon addition. The microbial catalysis of methane production would be most efficient with microbes capable of extracellular electron transfer. The specific factor to examine was the use of hydrogen as an electron shuttle by (1) examining the relationship between methane production and the availability of electrons as hydrogen, (2) the consumption of CO_2 below the hydrogen evolution point, (3) the behaviour of the microbes to lower the hydrogen evolution point and continue to preferentially produce methane or other fuels leading to methane. This system used a granular packed bed to increase the surface area available for bioactivity. As such, extracting biofilm samples during testing was not possible.

3.1. Materials

3.1.1. Reactor Design

The production of reduced chemical fuels in the microbial fuel cell requires the spontaneous flow of electrons and necessitated both electrodes have spontaneous reactions, where the potential of the anode reaction was higher than the potential of the cathode reaction. The spontaneous supply of current from the anode was performed initially using the microbially catalysed oxidation of (synthetic) wastewater. In methane fermentation hydrogen is provided to the methanogens by the acetogens when they perform dehydrogenation. In this system hydrogen was provided by the electrolysis of water at the cathode.

The MFC anode and cathode were to be catalysed by separate biofilms, grown on (electrode) beds of packed graphite granules. The cathode biofilm was retained. After a series of experiments the anode biofilm was allowed to die (see results section). Graphite rods served as the conducting electrodes and were inserted into the packed graphite beds, which became electrodes by extension. An anion exchange membrane separated the two compartments. The flow of charge was carried by dissolved CO_2, which is HCO_3^-, through the membrane into the anode. The choice to use dissolved CO_2 or bicarbonate would further decrease the carbon dioxide concentration in the biogas and eliminated the requirement for a separate, possibly economically limited, charge carrier.

The charge flow, to complete the circuit, proceeds as shown below in Figure 8. Note that the conversion reactions at the electrodes result in the gases shown leaving the top of the reactor chambers.

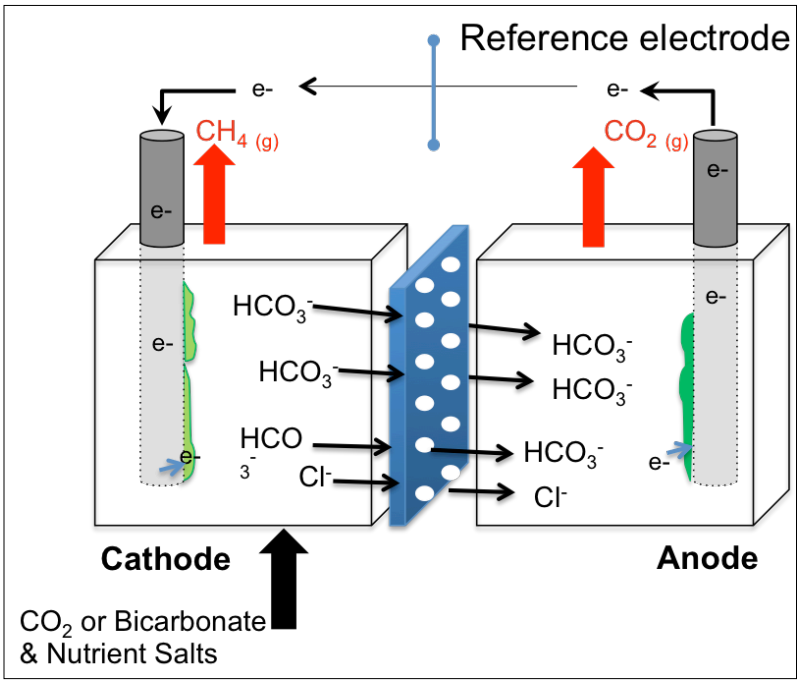


Figure 8: Biogas enrichment BES, detail of charge flow to complete the circuit

This system differs from others used for the conversion of CO_2 to CH_4 in two ways. One, previous systems used cation exchange membranes and did not attempt to use CO_2 as the charge carrier (Mieke et al. 2011; Villano et al. 2010). Two, these previous systems were batch systems and did not investigate the possibility of continuous flow production.

The colonization of the cathode electrode planned for the investigation of two possible autotrophic methane production reactions by the microbes, both of which rely on the microbe's ability to perform a version of EET.

$$CO_2 + 8H^+ + 8e^- \leftrightarrow CH_4 + 2H_2O \qquad E^{0'} = -0.244V\ vs\ SHE \qquad \text{i.}$$

$$8H^+ + 8e^- \leftrightarrow 4H_2 \qquad E^{0'} = -0.414\ vs\ SHE \qquad \text{iia.}$$

$$4H_2 + CO_2 \leftrightarrow CH_4 + 2H_20 \qquad \text{iib.}$$

Methane production using hydrogen gas (ii), produced at the cathode electrochemically, assumes the biofilm uses hydrogen as a shuttle for electrons. The biofilm would grow very, very close to the electrode to reduce the resistance (distance) of mass transfer and be able to absorb the hydrogen gas easily. For this reason granular graphite was chosen for the electrode material. The bioavailable surface area of porous materials is significantly less than the actual surface area measured by chemical testing, such as gas adsorption tests. Aside from the large surface area available in a small volume and the economic price, the porosity of graphite granules is such that microbes can only attach to the top layers of the graphite. The rest of the porosity is small enough only for chemical diffusion, ideally hydrogen gas, captured by the biofilm above. The best efficiency for microbes is still direct electron transfer, but having porous electrodes should help increase efficiency for indirect electron transfer when the shuttle is (non-regenerated) hydrogen gas. This leads to the second possible reaction, the production of methane using direct electron transfer (i). The theoretical potential for the reaction of CO_2 to CH_4 is below the theoretical potential for the electrolysis reaction of H_2. Therefore,

methanogens capable of direct electron transfer would have a greater energy gain than methanogens only able to use a hydrogen shuttle. From an engineering standpoint, the lower the overall potential used to supply the same amount of electrons, the less power is used and the more efficient the reaction. Therefore, for the same reaction, CO_2 to CH_4, using a lower potential is preferable and cultivating direct electron transfer is desired. If the microbes are able to attach directly to the electrode, then when supplied with a current they will control the electrode's potential to their preferred potential, which would be less than the electrochemical hydrogen evolution potential. In that case, hydrogen gas would not be produced at all. The hydrogen ions would come from water, or the waste of other microbes in a mixed culture. Figure 9 illustrates the two expected microbe reactions, with direct EET on the left and indirect EET on the right.

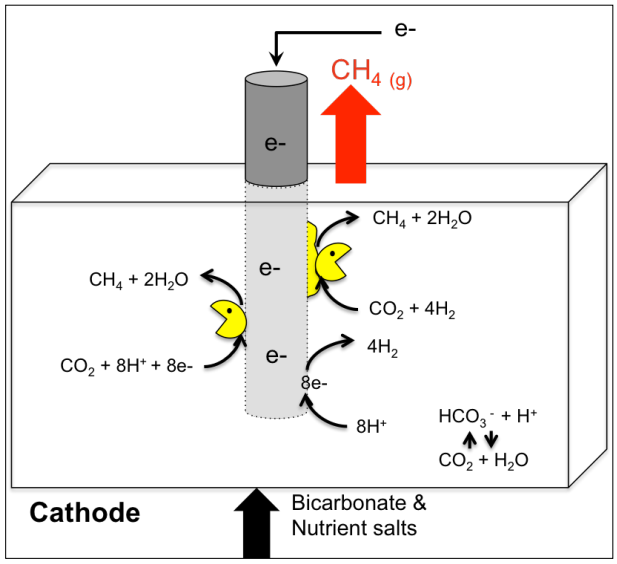


Figure 9: Possible methane production (autotrophic) reactions by EET

A square plate and frame reactor was built of smooth, translucent, hardened acrylic and sealed between the acrylic faces with flat Teflon seals, Figure 10. The interior volume of the each compartment was 288 mL (12cm*12cm*2cm). An anion exchange membrane of 144 cm^2 separated the two compartments. In the anode, liquid flow ran from an inlet at the lower right side and exited at the upper left. The dissolved CO_2 transferred to the anode could only leave with the liquid flow out. In the cathode the liquid flow was set up in the same manner, from the lower right to the upper left, with an additional outlet at the top right for evolved gases. Having parallel inlets lower than the parallel outlets would encourage CO_2 to transfer through the length of the bed while it diffused through the membrane to the outlet in the (upper left) anode compartment. Long threaded rods and wing nuts compressed the plates and frames together. The seals later had tile caulk and BluTak added to the outside of the reactor to seal small gas leaks while the biofilm grew.

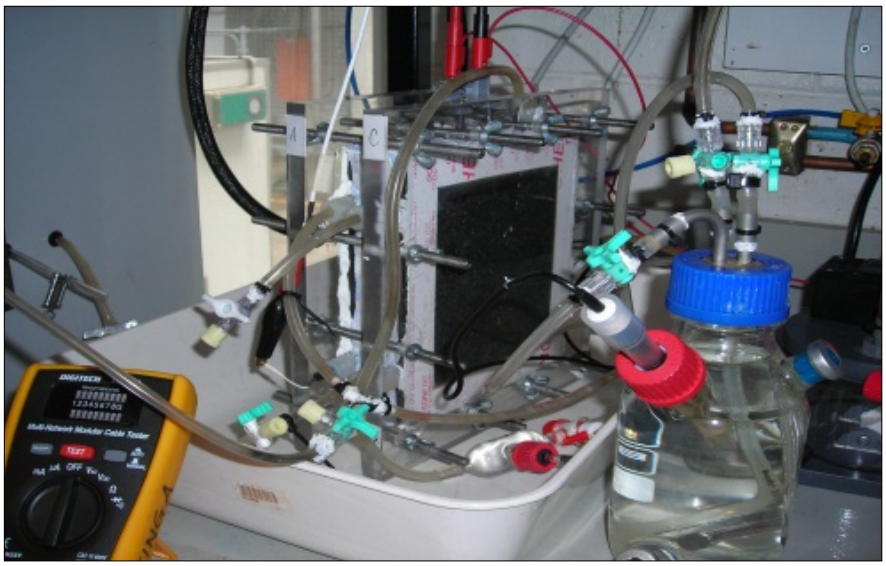


Figure 10: Plate and frame reactor with packed graphite granule bed as electrode, shown facing the cathode

Each electrode was made with 300 dry, bulk millilitres of graphite granules. The granules were soaked in the (sterile) buffer solution that would be used during testing and were then able to be packed into 254 mL of volume. The total volume of free liquid held by the (previously) saturated packed bed was 140 mL. The each extended 12 cm into the tightly packed beds. Dual Ag/AgCl reference electrodes were installed in both the anode and cathode to assist in accurate measurements. A 24 mL headspace above both electrodes was intended to allow gas produced in the cathode to accumulate and leave separately to the liquid flow. After testing began, it was found that the liquid flow consistently flowed out of the gas exit because the liquid accumulation space required for the packed bed, above the bed and below the liquid outlet point, was insufficient and had

a tendency to clog with small granules. As a result, tests were run on the cathode side with the liquid flow adjusted to run in the same point at lower left side and out the top left, through the original gas outlet point. This change in flow pattern effectively halved the bed size subject to continuous liquid flow, however the entire bed was still an active electrode.

The external hydraulic system was designed to eliminate the artificial transfer of liquid or ions through the membrane upon gas and/or liquid sampling. A sampling bottle was used for both the liquid and the gas measurements. The bottle was installed in the liquid recirculation line immediately following the outlet. Samples taken from the liquid, or gas, in the bottle did not affect the flow of liquid and/or ions through the membrane. Gas samples were assumed to be in equilibrium with the liquid in the bottle. The recirculation rate was set (permanently) to minimize the hydraulic retention time, HRT, in the sampling bottle and time sensitive measurements accounted for the bottle HRT. Gas collection and measurement used 50 mL syringe tubes connected to the outlet gas lines and inverted in a beaker of inert liquid (high viscosity silicone oil designed for use in manometers). This static gas system could accurately measure changes to the gas volume and remain at atmospheric pressure in response sampling of liquid and sampling or evolution of gas. This system thereby maintained the partial pressure of dissolved gases upon sampling as well. All gas flows used EZ-Flow gas flow controllers. For higher flow rates a Swagelok adjustable gas valve, flow meter and timer were used.

The cathode was operated as batch liquid using bicarbonate, with the purpose of later switching the developed biofilm to a continuous CO_2 gas flow in batch liquid,

Figure 11. The cathode side had static gas measurement. A jacketed bottle was used for temperature control (34 °C), gas and liquid sampling, dosing, and had an optional CO_2 or N_2 sparge into the bottle (not shown).

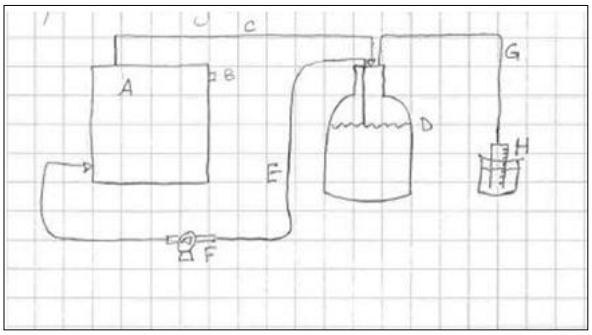


Figure 11: Cathode as batch liquid system with static gas measurement (*not drawn to scale*)

The anode was operated in two fluid flow modes. The first (open) used continuous liquid flow and passive gas flow, meaning the dissolved CO_2 was removed by the flow of liquid out (anolyte effluent) and could be sampled from a small a gas head, Figure 12. This open anode set up had an optional one-way N_2 gas purge for the anode bottle (not shown) to clear the headspace between tests.

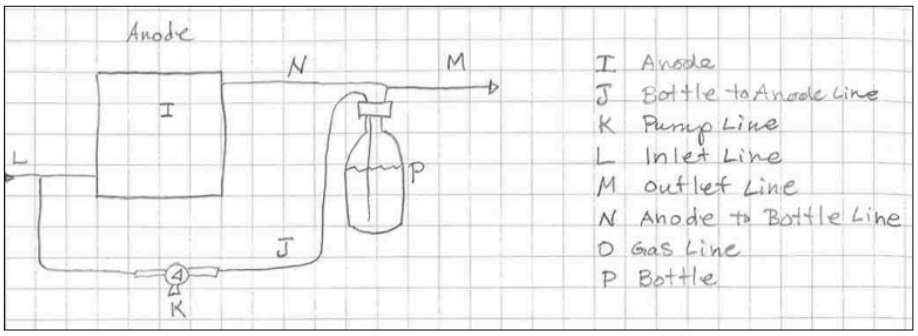


Figure 12: Continuous liquid flow anode with passive gas flow (*not drawn to scale*)

The second (closed) anode flow mode used batch liquid flow and active gas flow (sparging) with inert nitrogen gas, N_2. The controlled flow of N_2 was used to maintain the gradient and flux of dissolved CO_2 through the membrane, and to remove other compounds. For the closed anode gas outlet a 50 mL inverted syringe tube was chosen to permit the switch to static gas measurement as part of a test (Figure 13).

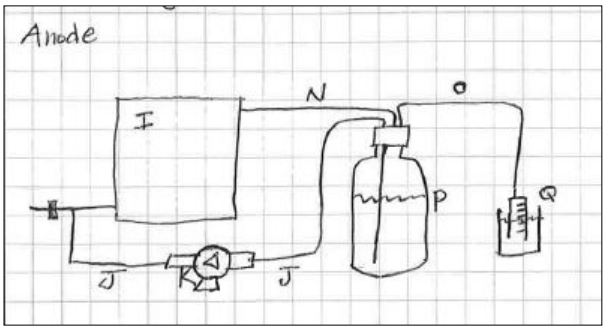


Figure 13: Batch liquid anode with active gas flow, N_2 sparge to bottle not shown (*not drawn to scale*)

3.1.2. Components

The sampling reservoir bottle for the cathode was a 1.2 L glass reactor with a reverse-flow heating jacket. The sampling reservoir bottle for the anode an 1100 mL Schott bottle.

Pumps used included a Watson Marlow peristaltic pump set at 0.43 mL/min for the continuous flow of the anode. A Stauff Telemecanique Altivar peristaltic pump with multiple heads was used for simultaneously recirculating both electrode compartments, set at 90 mL/min. The ProMinent beta/4 fluids control pump was occasionally used for dosing. Tubing used masterflex tygon tubing at $1/8^{th}$ inch diameter for conveying liquids and gas, and ½ inch tubing for the recirculation pump heads. All hose connections were zip-tied and coated in tile caulk and then silicone caulk. This was not the ideal material for gases, however other materials were not available at the time.

A VersaSTAT 3 Ametek potentiostat by Princeton Applied Research was used to control the electrodes. An Agilent data logger recorded the cell potential. The pH probe was a inserted into the sampling bottle liquid.

The pH range of the best electrochemical cell operation was between pH 6.4 and pH 8.0. The pH range for testing was chosen to accommodate the methanogens in the cathode and required shortened tests for batch operation given the electrochemical behaviour of the system. The pH range for mixed culture wastewater bacteria is between pH 5.0 to 8.0. The pH range for methanogens is smaller, 6.8 to 7.5 or less. This narrow pH range supported shorter tests, particularly if they were to be batch tests.

The microbes and the their limited pH range were supported using a buffer and nutrient solution as follows:

Table 1: Nutrient solution chemistry

Nutrient or buffer	Concentration
Na_2HPO_4	6 g/L
KH_2PO_4	3 g/L
NaCl	0.5 g/L
NH_4Cl	0.5 g/L
$MgSO_4$, $7H_2O$	0.465 g/L
Trace nutrients (stock solution)	1 mL/L
$CaCl_2$, $4H_2O$ (stock solution)	1 mL/L
CO_2 (cathode)	20mM as bicarbonate, or saturation by gas

3.2. Experimental Design and Plan

The goal was to test the MFC operation parameters of current, voltage, and retention time, for optimal fuel production efficiency. Before all tests the sampling bottles, MFC liquid, and any feed containers were sparged with N_2 eliminate dissolved O_2. The run time for a test was limited to 3 to 4 days by the combination of the total amount of gas required and the systematic error of the GC.

The anode would run acetate and nutrient salts (synthetic wastewater) and the cathode would run first bicarbonate with nutrient salts, and then CO_2 gas with electrolyte salts and nutrient salts. Then the anode was switched to electrolysis for the remainder of

the experiments. The cathode would be inoculated and run at a constant current for several weeks. The current would be progressively increased. The tests on the cathode were then planned to run at varying potentials and currents. These tests were not randomized. They proceeded in a progressive order.

3.2.1. Microbe production efficiency with potential

The hydrogen evolution potential of the electrode was an important indicator of the electrochemical behaviour (connection) of the microbes with the electrode. The actual electrode potential, meaning overpotential, of the reaction is increased by an electrode's material. Lowering the overpotential directly increases the production efficiency. The electrode potential should lower with colonization; ideally the potential will reach a value close to the theoretical potential for the reaction occurring in the electroactive biofilm. If the electrode's potential were to go below the theoretical hydrogen potential then there is no possibility of hydrogen being made. If the electrode's potential is above the theoretical, -414 mV SHE, then one can only know for sure if hydrogen is being produced by measuring the potential at which hydrogen gas evolves. Since hydrogen is the energy source and CO_2 is the carbon source, normal H_2 evolution should only occur at the end of a (batch) test when the carbon has run out is not available for the microbes to reduce it, either directly or indirectly.

The un-colonized hydrogen overpotential for the graphite granule electrode was determined by setting a progressive series of potentials and measuring for hydrogen gas. The gas headspace was measured using GC. The liquid was measured using evacuated tubes with GC.

Once the biofilm was established, production efficiency was tested at -650 mV, 700 mV, 750 mV, and -800 mV vs. SHE. The gas headspace was measured using GC. The liquid was measured using evacuated tubes with GC. Regular gas measurements during all tests, indicated when the electrode's hydrogen overpotential had changed or was reached at the end of a test. Methane and VFA production was tested before and after the consumption of charge by the biofilm. VFAs were tested using HPLC. For start up, energy was initially supplied as current to provide a negative potential for the reduction reaction while the biofilm grew. This means the growing cathode BES was an electrolytic cell during start up.

3.2.2. Microbe production efficiency with current, change in carbon source for testing

The CO_2 absorption kinetics in the electrochemical system were measured against a current change. We used 0.1mM NaCl solution and varied the current each day: 0, 5, 10, 20, and 30 mA. The gas volume was measure using the inverted 50 mL syringe connected to the system and the gas concentration was measured with GC.

Bicarbonate was used for testing purposes at 20 mM (liquid) CO_2 concentration, which is just below the saturation limit. The liquid was replaced between tests in twice the volume of the system, using recirculation to flush the reactor liquid. The headspace was purged between tests with N_2 gas, before and after the liquid was replaced. After the liquid was replaced, at least 10 minutes was allowed for the gas headspace to equilibrate before testing began.

The current will dictate the rate at which CO_2 will absorb in an electrochemical system. The best rate for CO_2 absorption for the system was determined to be 5mA. The headspace was not doped with CO_2 gas for most of the tests for various reasons, including the fact that the absorption of the gas created a negative pressure. It risked, and occasionally resulted in, air leaks into the gas headspace of the sampling bottle through the gas outlet. *See the results section for more discussion of the choice to use bicarbonate over CO_2 gas for testing.* The syringe used to measure the change in gas volume was a standard 50 mL plastic syringe tube. Adding CO_2 during a test was done selectively to test the biochemical response of the system.

Once microbes are added, the rate of absorption will increase to include the rate of microbe consumption. A series of efficiency tests were done on the developed biofilm at 5 mA, and 10 mA. Methane and VFA production was tested before and after the consumption of charge by the biofilm. VFAs were tested using HPLC.

3.3. Methods of Analysis

The analysis of efficiency requires the concentrations of products produced by the microbes. For sampling, the system volume was recorded, liquid and gas samples were taken and their volumes recorded, and then the final system volume was recorded. Concentrations were measured with GC and HPLC, respectively. The whole liquid or gas volume of the system at the time of sampling was then used to calculate the total amount and percentage volume of product present, using the concentration measurements obtained. For reporting purposes it is often more appropriate to use the per cent volume since the total volume changed significantly with each gas measurement. Reporting the

total volume of material would be inappropriate since it does not indicate whether changes in volume are artificial or system based. Reporting only the volume of material generated is similarly prone to artificial volume changes. Showing these values remains helpful to estimate the relative gas volumes when significant shifts in concentration occur.

3.3.1. Gas Analysis Method

The Shimadzu GC1 gas chromatograph was used to measure gas concentrations. The GC used nitrogen as the carrier gas and was more appropriate for the system since the background gas of the system was nitrogen and a 5 mL gas sample, which was unfortunate because this necessitated a large amount of gas for long tests. The larger the total gas volume at the beginning of the test, the lower the initial methane concentration was, making it subject to systematic error. For unusual measurement results triplicates were taken, otherwise duplicates were taken.

The total gas volume was calculated as the initial headspace plus the volume of the gas lines plus the volume of the gas syringe. The total liquid volume removed did not need to be included in subsequent gaseous volume calculation during tests, since the gas syringe volume adjusted with changes in the total system volume. The gas syringe was an inverted syringe cylinder connected on the inlet side to the gas lines and submerged on the other end in high viscosity silicone oil. The vapour pressure of high viscosity silicone oil was determined to be negligible and would not affect the test results.

3.3.2. Liquid Analysis Methods

Liquid concentrations were measured as parts per million using high performance liquid chromatography, HPLC and Total Carbon, TC, tests at the UQ Analytical Sciences Laboratory. Small liquid samples were taken from the sampling bottle using a sterile syringe plunger and a 0.22 micron sterile filter. The filter eliminated any cellular material that would otherwise damage the HPLC machine. The liquid samples for HPLC were 0.9 mL filtered liquid and 0.1 mL formic acid. Separate tests were available to measure the presence of formic acid, lactic acid, succinate and glycerol. Those tests were only used to occasionally measure for the presence of such materials in order to rule them out as products. The TC tests measured for organic carbon and inorganic carbon in mg/L. These two tests characterised the products formed and tracked the movement of products and HCO_3^- through the anion exchange membrane into the anode.

The total liquid volume was calculated as the total volume in the packed bed plus the full line volumes plus the initial volume in the sample bottle minus the total volume removed to that point. The loss of graphite due to degradation of the packed bed on either side was not included in volume calculations. The calculation of the degradation rate of the bed was beyond the scope of this project.

The concentration of dissolved gases in the liquid was measured with by gas chromatography using Execu-tainer evacuated tubes. A known amount of liquid was injected into the 12 mL evacuated tube. The vacuum caused the dissolved gases to volatilize at equilibrium into the headspace. The headspace was then sampled using GC and the concentration was used to calculate the equilibrium amount of gas in the liquid.

The total moles of gas present were then the amount of gas originally dissolved in the liquid.

3.3.3. Efficiency Analysis Methods

The efficiency was calculated as the coulombic efficiency, which is the ratio of the total number of coulombs in the products divided by the total number of coulombs pushed into the system, times 100. The efficiency was measured during testing and at the end of tests. The coulombic efficiency is dependent upon accurate volume measurements and susceptible to errors in volume reporting. A continuous operation system using mass spectroscopy at the outlet of the system would be preferable for future testing.

3.3.4. Biofilm growth and electroactivity Analysis Methods: CV and community identification

Biofilm growth cannot be meaningfully analyzed with COD or opacity for cells, since the electrode retains the biofilm. CV is used to look at the reactions happening on the electrode surface. It measures how electroactive the microbes are and see if there is a shift in the overpotential (hydrogen peak). Voltage is cycled between two points at a constant rate and the current is measured. Many constants voltage rates (steps) are used. CV tests were performed between the potential of 0.2 V and 1.0 V SHE, at the voltage rates of 0.5 mV/s, 1 mV/s, 5 mV/s, 10 mV/s, 20 mV/s, and 50 mV/s.

A CV curve has a seed shape. The lower curve is the movement from right to left indicating reduction. The upper curve is the movement from left to right, indicating oxidation. Electrochemical reactions on the electrode, such as microbial consumption,

looks like a dip or a peak (all are called peaks) protruding out of the general seed shape. The redox of a product will have a dip going down and a peak going up. If the film is very thin or there is not an abundance of food available at the time of the CV test, then the dip/peak will not be very pronounced. The biofilm on cathodes tend to be thinner than anode biofilms. Widening and lengthening of the curve indicates a thicker biofilm. The hydrogen evolution tail shows where the limit of microbial bio-electroactivity ends and there is only electrochemical activity, meaning there is just hydrogen bubbling off (or being absorbed on until the concentration depletes).

To measure the microbe type we would use FISH probes and pyrotag, to elucidate species. To be meaningful, FISH and pyrotag need to be done regularly to see how the community changes during the course of the experiment (time only) and in response to changes to the environment. It was difficult to check bacteria population on the cathode and unfortunately no viable cell samples could be extracted. Two possible reasons for this are that cathode biofilms are usually quite thin, and the system was not designed for sampling, so all cell samples had to come from centrifuging the cathode effluent at the of tests.

Bibliography

Mieke, C., Van Eerten-Jansen, A., Heijne, A., Buisman, C., Hamelers, H. (2011) Microbial electrolysis cells for production of methane from CO_2: long-term performance and perspectives. Int. J. Energy Res. doi: 10.1002/er.1954

Villano, M., Aulenta, F., Ciucci, C., Ferri, T., Giuliano, A., Majone, M. Bioelectrochemical reduction of CO_2 to CH_4 via direct and indirect extracellular electron transfer by a hydrogenophilic methanogenic culture. Bioresource Technology 101 p.3085-3090.

4. RESULTS AND DISCUSSION

The anode and cathode of the MFC were to both be biofilm catalysed. The anode would run acetate with nutrient salts (synthetic wastewater) and the cathode would run first on bicarbonate and then on CO_2 and electrolyte salts, both solutions with nutrient salts.

4.1. Anode operation

The anode was inoculated with a known acetate oxidizing bacteria capable of EET. The inoculum was taken from another healthy reactor in the centre. The anode was supplied with acetate feed and the potential was progressively lowered until the potential came down to -300 mV. The bacteria performed as expected and the bioanode with acetate feed was used for the first several experiments. It was soon found that the concentration gradient of acetate was causing diffusion of acetate across the anion exchange membrane, against the current flow, into the cathode. To remedy this, the acetate feed was ceased and the anode ran on the electrolysis of water and the carbon electrode for the remainder of the experiments. The tests results reported here were conducted with the resulting MEC. Restoring MFC functioning would require reactor design changes, but would not negate the applicability of the findings here. The anode was run with continuous flow for the nearly all of the experiments. For the final third of the experiment sets the anode was closed to observe the changes to production (see further discussion in Closing the Anode).

One possible solution to restore the reactor function as an MFC, instead of a MEC, would be to use a three-chamber system. Using the same nutrient salt concentrations in all three chambers, the cathode would retain its separation with an anion exchange membrane and the anode would be separated with a cation exchange membrane. The negative charge from the cathode would be carried to the centre chamber. The cations from the anode would continue to be attracted towards the centre chamber and cathode as well.

4.2. Cathode operation with CO_2 gas

The overpotential for hydrogen production on the un-colonized cathode was 904 mV SHE.

The current will dictate the rate at which CO_2 will absorb in an electrochemical system. The best rate for CO_2 absorption for the system was determined to be 5mA. Bicarbonate was used for testing purposes and the headspace was not doped with CO_2 gas, but used pure N_2 instead. The reason was due to the electrochemical behaviour of the gas in the cathode.

With CO_2 as gas in the headspace and the liquid concentration at 20mM, just below the saturation level, the CO_2 will initially desorb slightly to the equilibrium concentration and then absorb due to current flow. Since the current is carried through the membrane by the CO_2, the sooner the current is turned on the less CO_2 will initially desorb. By this same logic, when the gas phase has a large CO_2 headspace then this gas will be absorbed to carry the charge through the membrane. The absorption of the gas created a negative pressure. It risked, and occasionally resulted in, air leaks into the gas headspace of the sampling bottle through the gas outlet. Once

microbes were added the rate of absorption increased to include the rate of microbe consumption, further increasing the risk for air leaks with this system.

If a much larger syringe were available, along with more liquid and a more lenient range in liquid uptake, then this adjustable volume could have allowed for tests that started with bicarbonate concentrations above the saturation point and/or significantly increased currents.

As the cathode runs the increase of current into the system causes the pH to become more basic. This behaviour is characteristic of all liquid based cathodes and can be explained as the production of Lewis bases. Various mechanisms can occur inside a cathode to achieve the increase in pH given the salts present. To counter the increase in pH, the plan had been to use the continuous addition of CO_2 gas, which acts as a weak acid. This would additionally require the addition of salts to maintain the conductivity. Adding bicarbonate ($NaHCO_3$) continuously would have the same effect as adding CO_2 gas and salts. When adding CO_2 gas, or N_2 gas, directly into the bottom of the packed bed, the resistance of the cathode became very high as gas bubbles increased the resistance by nucleating on the rough graphite granule surfaces while traveling through the bed. This phenomena is commonly referred to as bubble resistance. For these two reasons, bicarbonate was used for all tests. When additional carbon was needed, purging through the sampling bottle was done with pure CO_2 gas using a flow controller and timing the duration of flow.

From the graph below (Figure 14) you can see where the resistance increased as CO_2 gas was introduced into the bed, at points 1, 2 & 3. The potential became more negative, meaning more power was needed to force the -5mA of current into the

electrode. The sparge was at the bottom of the bed. The increase (lower potential) at the end, point 4, was after the CO2 sparge was done. This means the presence of protons as food was able to reduce the potential, while the act of sparging in the bed increased the potential.

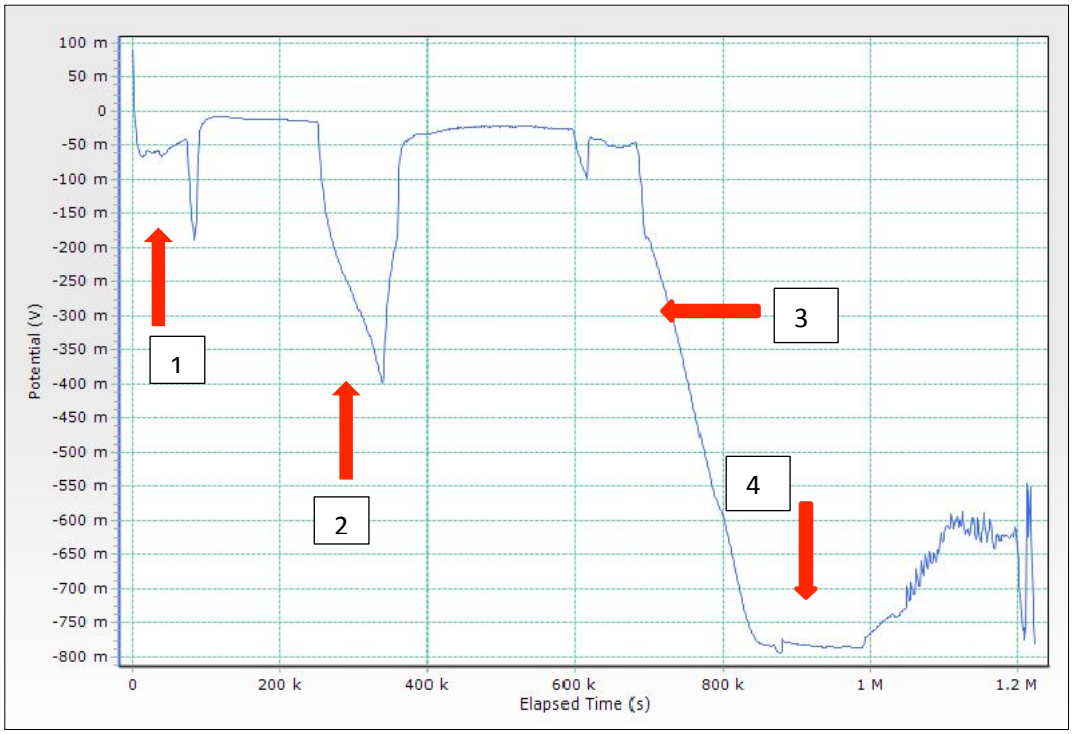


Figure 14: Resistance increasing (potential increasing) for constant current when sparging CO_2 through the packed bed, potential is versus Ag/AgCl

Sparging to the bed would best be done by placing the sparge below the bed in a liquid only area, see Figure 15 below. A sparge placed below the bed has a lower pressure to overcome, which means smaller bubbles. Smaller bubbles allow more gas to dissolve before the liquid enters the bed. This would be the ideal method for proton delivery as close to the bed as possible.

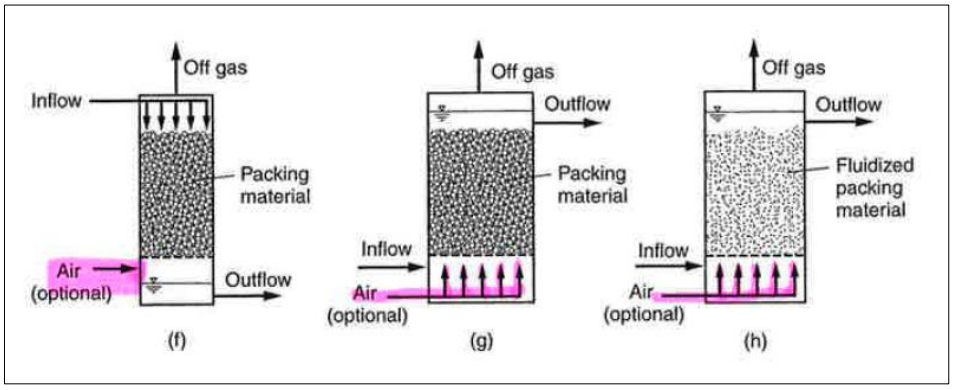


Figure 15: Packed bed designs with gas flow. Image referenced from Tchobanoglous et al.

4.3. Cathode operation with bicarbonate

Initial production of methane at the cathode followed the expected behaviour. The concentration of hydrogen was characteristically high in the first 5 mA test, remained steady for the length of time CO_2 was readily available in the liquid phase, and then peaked once the CO_2 ran out, Figure 16.

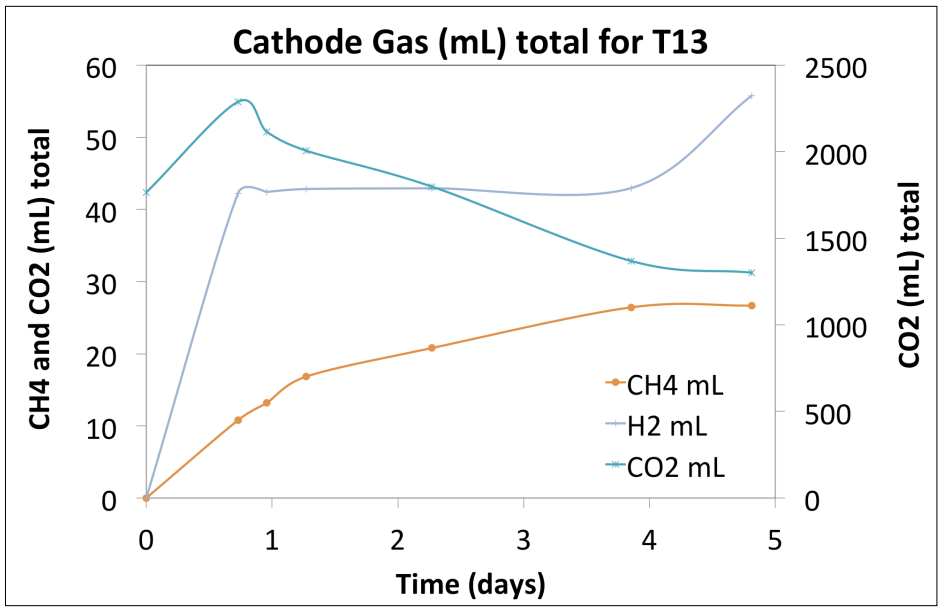


Figure 16: Initial methane production

As the biofilm grows the concentration of hydrogen in the gas phase should decrease significantly. The hydrogen concentration however did not get to as low a concentration as expected with progressive tests and the production of methane was not well correlated with the changes in hydrogen concentration. Calculation of the methane efficiency, Figure 17, found that the efficiency was also not well correlated with the charge to the cathode, sometime even increasing past 100%.

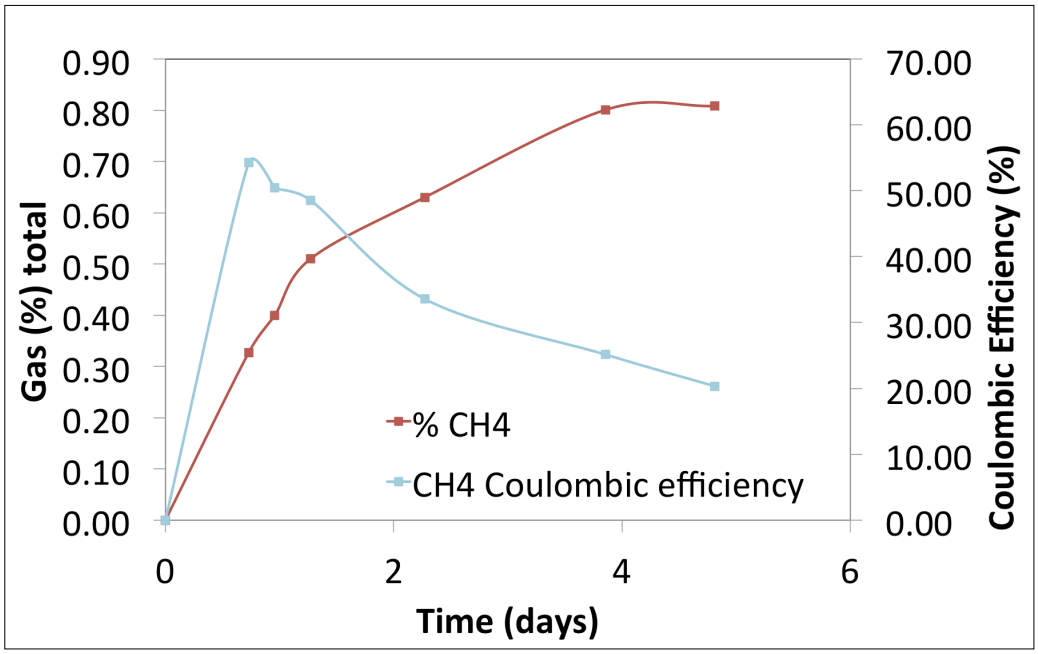


Figure 17: Coulombic efficiency of methane production versus methane production percentage

Subsequent VFA testing, Figure 18, verified that methane concentration was well correlated to the duration of the test and the flux of acetate through the anion exchange membrane, against the current flow, and into the cathode. The oxidation the acetate, instead of using the CO_2 and the cathode, decreased the current to the cathode and produced methane, giving a false efficiency that was high.

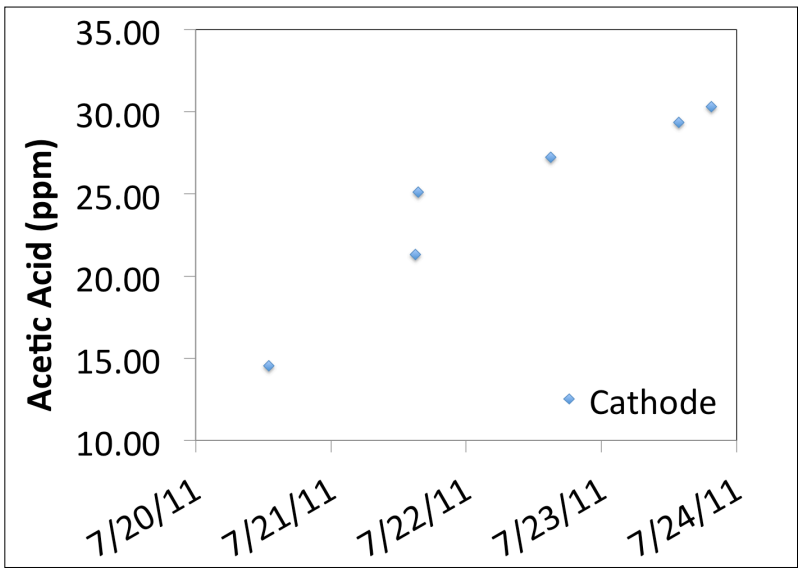


Figure 18: Diffusion of acetate measurement: concentration of acetate in the cathode with time

Unable to find a suitable feed source for the given system, the anode side ran by oxidizing carbon and water (i.e. electrolysis) for the test, meaning it was converted to an electrolytic cell and required energy input. A complete flush of the anode and cathode was done to remove all the acetate. Chemical analysis of the effluent showed acetate removal after >24 hours of flushing.

4.3.1. Adjusting the Potential

Cyclic Voltammetry, CV, verified there was an electroactive biofilm, as shown by the blue lines in Figure 19.

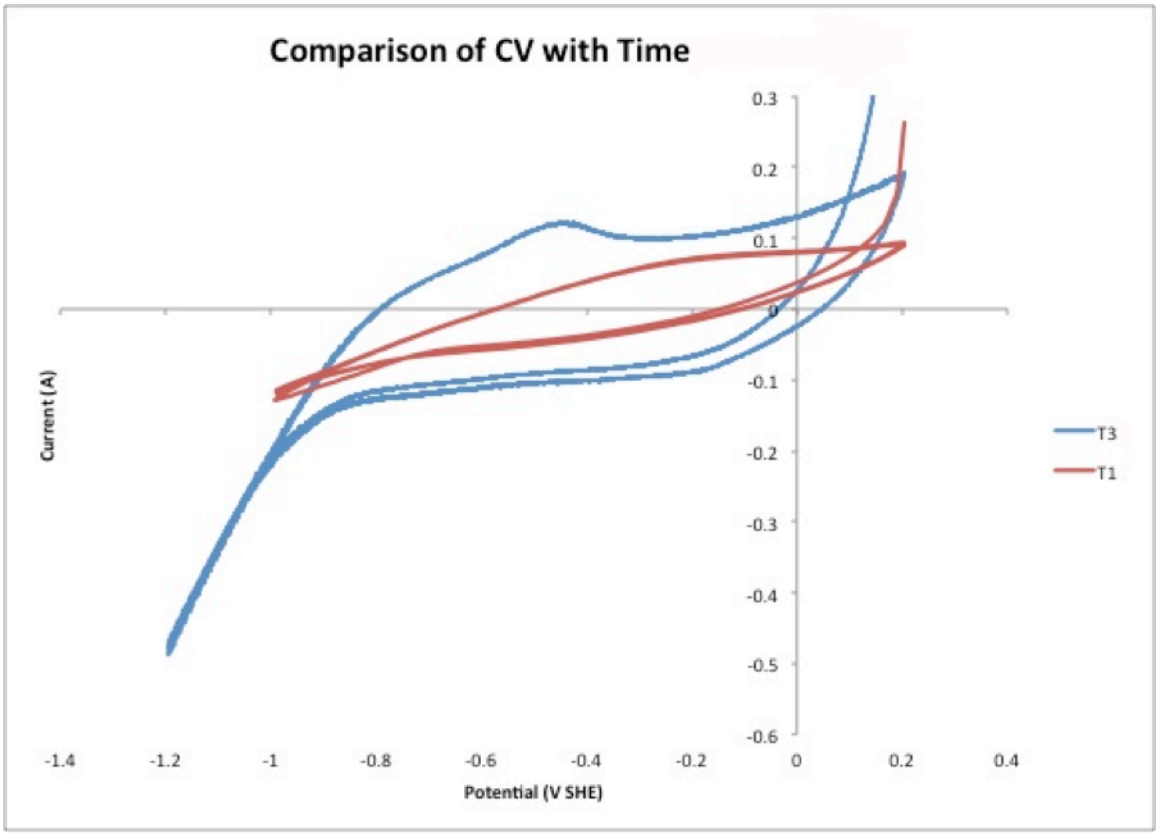


Figure 19: CV for the cathode

The methane production efficiency was then tested sequentially against potential, Figure 20. The cathode was batch; the anode was in continuous flow mode.

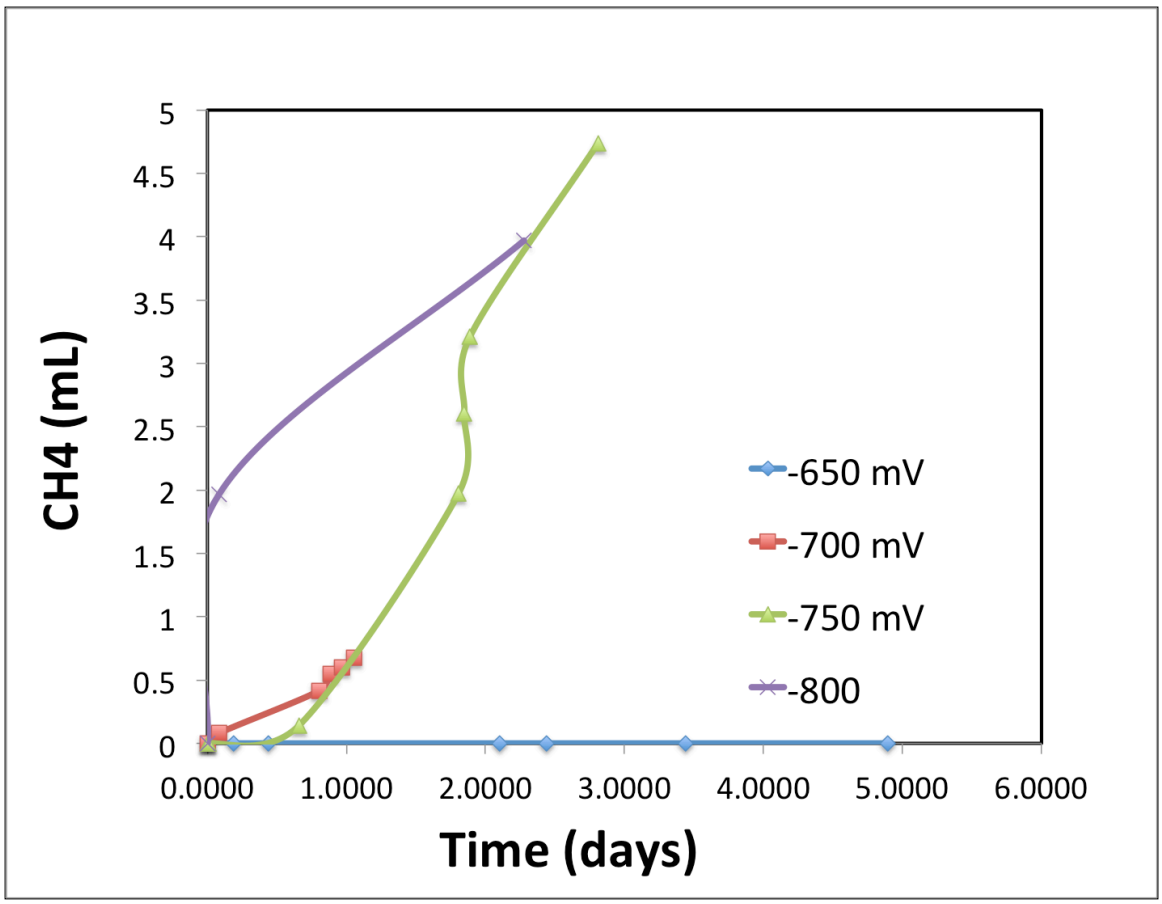


Figure 20: Methane production with potential (SHE)

The methane generation efficiencies were:

-650 mV Methane Efficiency: 0%

-700 mV Methane Efficiency: 45%

-750 mV Methane Efficiency: 56%

-800 mV Methane Efficiency: 59%

The potential at -650 SHE produced no methane and no hydrogen. The rest of the tests evolved measurable amounts of H_2. Based on the increase in efficiency with potential this further confirms the methanogens (at that time) were hydrogen limited.

After -750 SHE the rate of efficiency increase slows, this makes -750 appear to be the optimum potential for methane production efficiency. Using more potential would not give as much of a return on production. This hypothesis was evaluate by switching to constant current to observe the methane efficiency and related potential.

4.3.2. Adjusting the Current

Methane production was measure versus current and the efficiency was calculated. The number sequence for each test for each current setting, Figure 21, were the order in which the test done with that increased current setting. The cathode was batch; the anode was in continuous flow mode.

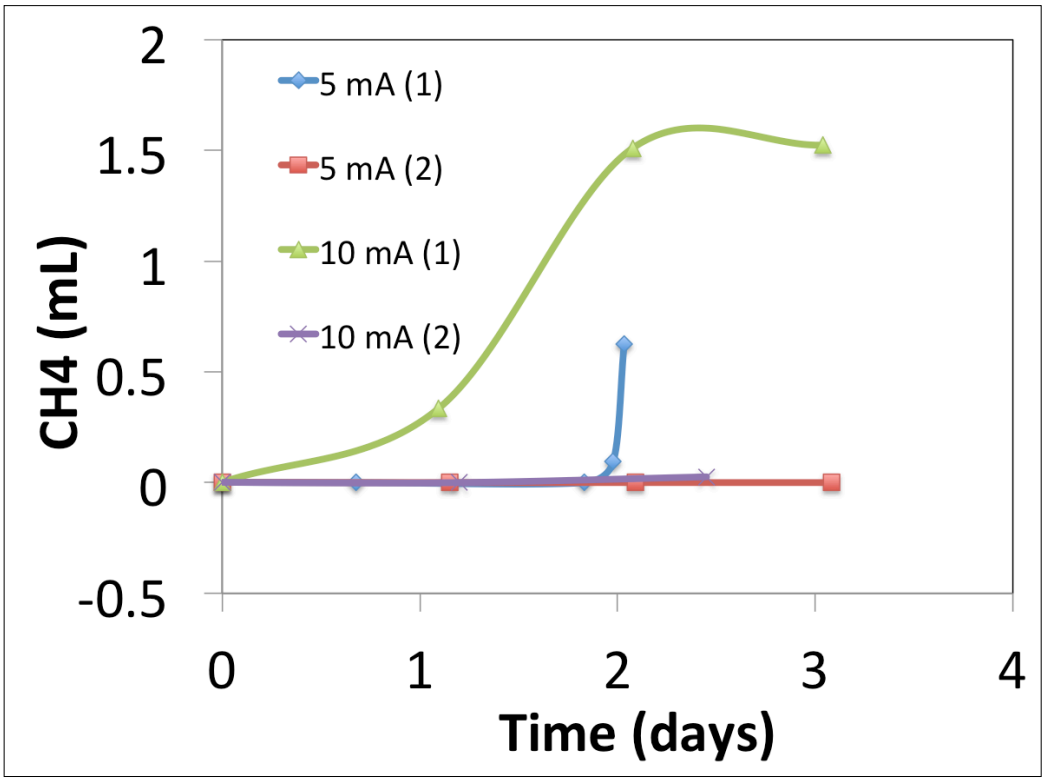


Figure 21: Methane production with current

For the first 5 mA test and the first 10 mA test, the initial test with 5 mA and 10 mA, a large amount of hydrogen was evolved at the same time – which was well

correlated to the methane production, as seen here. The methanogens (present at this time) were therefore likely hydrogen limited and were probably planktonic phase microbes responding to the availability of hydrogen gas.

At this point, VFAs were being produced as well. It was found that the efficiency for VFA products went up with time too.

Methane Efficiency (calculated assuming CH_4 is the only value product):

4.4%, 0% [5 mA]; 2%, 0.8% [10 mA]

Combined Efficiency (calculated for CH_4 and all other products):

45%, 75% [5 mA]; 58%, 54% [10 mA]

The efficiency of the VFAs increased with current overall, however, based on the methane and hydrogen increase during the first 10 mA test, these initial tests are on a biofilm that has not yet adjusted to receiving 10 mA. It would appear that the methane efficiency goes down with the increase in current over time. An explanation for this could be that the push of more current pushes the potential down (or up) too and so the potential shifts from what the microbes were accustomed to using. Another explanation could be that the hydrogen partial pressure was inhibitory to them. With time the combined efficiency at a set current went up, as shown in the case of using 5 mA.

In the open anode set up, the hydrogenotrophic methanogens were limited by hydrogen gas. Similarly, the system did not have high enough VFA concentrations to feed the acetotrophic methanogens. Figure 22 shows the gas evolution of two tests, where test 1 and 2 are separated by 3 months of continuous culture in the open anode

set up. Logically, the VFA concentration was low was because the VFAs were either not being produced in high enough concentrations, or the concentration was being kept artificially low by removal through the anion exchange membrane. Short tests ran 3 to 3.5 days each time. If very long tests had been possible, as they had been preformed in the associated literature, this may have allowed the VFAs to build up and lead to methane (or hydrogen) production without indicating the syntrophy. However, the increase in VFA efficiency over time indicated the flow rate out of the anode was helping to keep the concentration low.

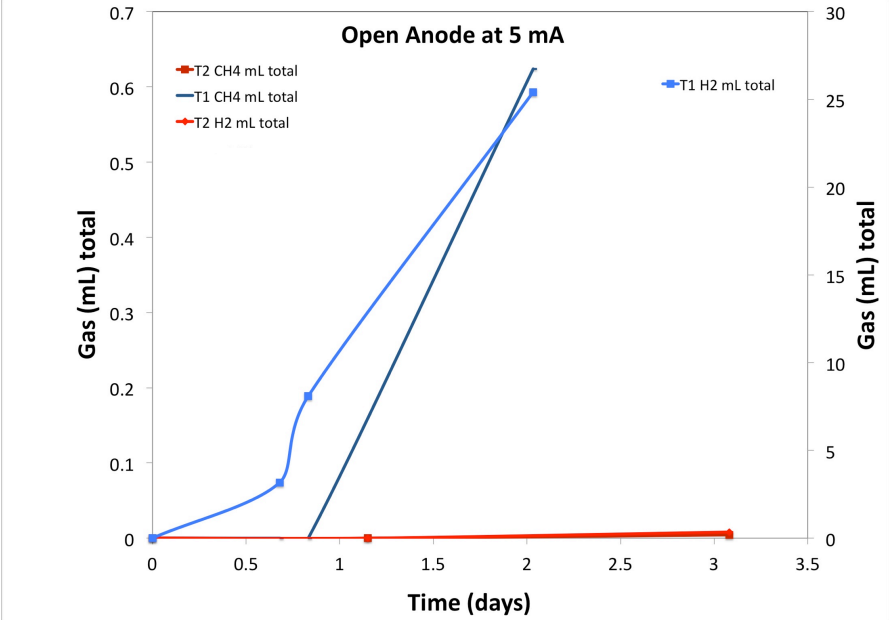


Figure 22: Gas production in the cathode with an open anode system

The anode was closed (see Chapter 3 for methods) and methane was produced from the beginning of testing, Figure 23. Only trace hydrogen gas evolved, until the end of the test when the bicarbonate ran out. This indicated the methanogens were able to feed on the VFAs.

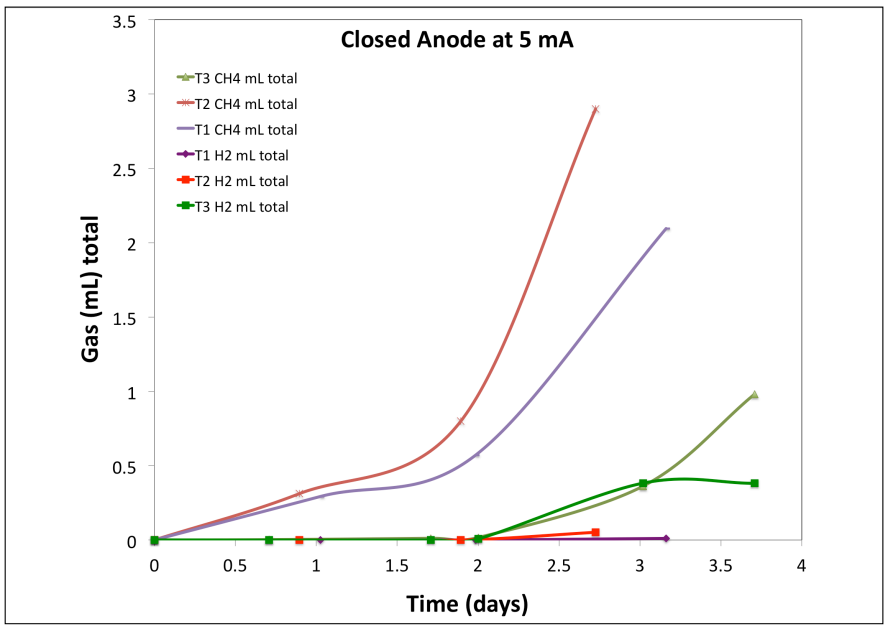


Figure 23: Gas production in the cathode with a closed anode system

The methanogen response to the flux change could be explained in two ways. One assumes a sufficient population already existed. Because the liquid flow has stopped, the flux of anions has slowed down to the rate of natural migration (plus purging on anode). The VFAs, which are anions in the cathode environment, were allowed to accumulate and the threshold for food was met. The second assumes a lack of population. Because the overall flux of anions has slowed down for this system (compared to the open system), there was a longer HRT and now the acetotrophic methanogens could grow. Previously, the threshold of food concentration had kept their numbers low, so it took longer for them to consume the same amount of (sparse) food. With food and time, the population increased and was able to respond faster, even to the same low concentration of food. Autotrophic methanogenesis is unlikely, since it did not occur in the open flow set up and the methane response to the closed system was immediate. Continued reduction by acetotrophs using EET is possible.

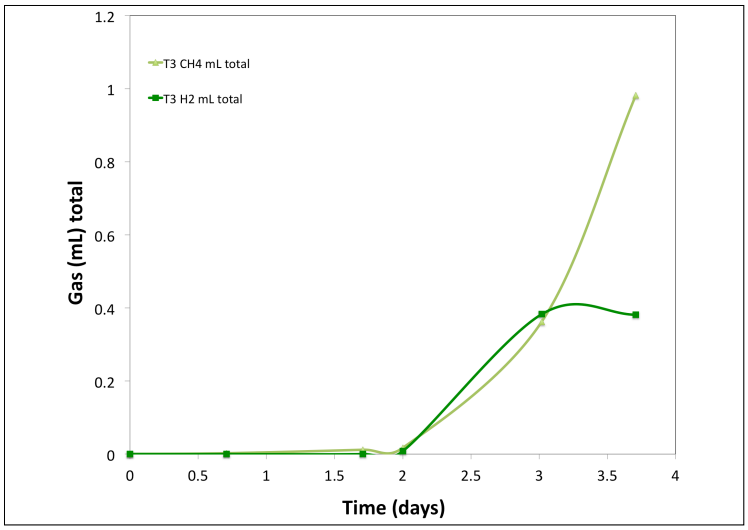
Closing the anode improved the methane efficiency. The methane efficiency for tests one and two were 9.0%, 9.4 % respectively. The combined efficiency was 20.1%, 19.3 % respectively. They each had a constant current at 5mA and they each had a gentle N2 sparge in the anode to maintain the flux of CO_2 through the system. The methane efficiency was much higher, at 9%, compared to the decreasing efficiencies of 4.4% to 0% in previous constant current test run in the open anode system. The methane efficiency was half of the overall efficiency, however the overall efficiency had gone down drastically to 20%. The potential on test 1 got to -698 mV when the CO2 ran out and H_2 started evolving. This is the lowest the hydrogen overpotential was for any test. Previous tests had consistent hydrogen overpotentials at -750 mV SHE. The potential on test 2 got to between -700 and -720 mV when the CO_2 ran out rapidly and H_2 evolution point was reached.

In each test methane production began immediately, at an approximately steady rate, without the presence of hydrogen. The methane production rate increased dramatically as soon as hydrogen was present. This implied the methane was being made by both the acetotrophic methanogens, who were consuming the VFAs, and the autotrophic methanogens, who were generally hydrogen limited until the end of the test.

The lowered efficiency overall however was very likely due to the purge running in the anode liquid. The anode becomes acidic with time. If we assume the VFAs are not reviving an anodic biofilm, then if makes sense that anything that was an anion and could pass through the anion exchange membrane would have it's charge balanced (and no longer be an anion) in the acidified anode compartment.

Materials with neutral charge can volatilize and be purged from solution, thus the reduction in combined efficiency was likely due to the N_2 purge in the anode.

Increasing the purge and then turning the purge off and allowing the system to ferment any VFAs tested the effect of the N_2 purge in the anode on the cathode production efficiency, test 3 of Figure 23 above and detailed in Figure 24 below.



Methane Efficiency: 0.88 %

Combined Efficiency: 1.75%

Methane Efficiency: 2.62 %*

(1.75 + 0.88 = 2.63)

* Included H_2 efficiency in this calculation

Figure 24: Graph and detail of the methane and combined efficiencies

In the first part of the test the rapid N_2 purge kept the VFA concentration very low. Methane was produced and the ratio of methane to VFAs is still about 50%. Once the CO_2 in the liquid phase was depleted, hydrogen (and more methane)

evolved. The current and N_2 purge in the anode were turned off, with left the pumps recirculating.

The result was that more methane was produced, even though there was no more current. The methanogens consumed the VFAs in the liquid, at the concentration at the end of the test, at 99% efficiency. This confirmed that the rate of consumption by the acetotrophic methanogens was limiting the production of methane to 50%.

Interestingly the concentration of hydrogen remained steady. In previous tests the methane went up in response to the hydrogen and methanogens would eventually consume all the hydrogen and CO_2 (in the gas) phase to the limit of the amount of hydrogen available. In this test the hydrogen appeared to be inert at the end of the test. Overall the percentage of hydrogen here is still only 0.027%, which is small, however it could imply, among many things, that the methanogens present are able to function as both acetotroph and autotroph, with a preference for VFAs, acetate. Further, any strictly autotrophic methanogens would have had a smaller population by this point, after approximately four weeks of near starving conditions, and would have been slower in response. The point is the hydrogen was probably on its way down. The CO_2 was acting as the limiting factor now. Any CO_2 produced by acetotrophic methanogenesis would be slowly evolved and should be consumed by the autotrophic methanogens. This is not ideal since this hydrogen was produced electrochemically, but it does tell us that reaching the limit of carbon availability is possible with this system and can lead to higher purity methane gas, including in sequenced batches.

Hydrogen evolution aside, the population of methanogens was not large enough to consume the VFAs as fast as they were produced in a continuous flux set

up, even at the moderate N_2 purge used in closed tests one and two. There did exist a big enough population to consume the VFA products. It is quite possible that a sufficient population of acetotrophic methanogens were living in the sampling bottle in the planktonic phase, as well as in the reactor. Sequencing could include opening and closing the carbonate flux and turning it up and down. Turning the anode purge down very low (temporarily, with no current) could transfer any CO_2 produced by acetotrophic methanogenesis to the anode, without risking transferring significant amounts the VFAs. Many variations of batch sequencing could be tried. Continuous flow with multiple reactors and the cathodes in series should be examined for comparison.

The methane ratio for the first part of the test (50:50) maintained the pattern of production in previous tests. This implies there were also methanogens living in the reactor either in or closely associated to the biofilm. These methanogens would have had continuous and higher food concentrations, since the volume of liquid in the reactor is significantly smaller than the volume of liquid in the bottle and total system. A double cathode with gas passed to the outside cathode, through a second cathode and out the anode may be worth trying, if the resistance is kept low.

This test was replicated, with similar graphical results, at a lower N_2 purge and with methane testing in the anode compartment as well. The final methane efficiency improved to 65%.

Bibliography

Tchobanoglous, G., Burton, F., Stensel, H. (2003) Wastewater Engineering: treatment and reuse. Metcalf & Eddy. McGraw-Hill.

CONCLUSIONS

Biogas can have value added (i.e. upgrading) using the BES described in this thesis, by electrochemical and bioelectrochemical means. The absorption of CO_2 from the gas phase, due to the transfer of charge by the dissolved CO_2, will removed some fraction of the CO_2 from the biogas mixture. This would require a sustained input of energy to drive absorption and ion transfer, so electrochemical separation alone is not an economic solution to upgrading. The absorption rate is increased by the presence of microbes, performing a cacophony of autotrophic reduction reactions. The anion exchange membrane can similarly remove the products from these reactions. The reported coulombic efficiencies for VFAs in many tests were proven to be lower than reality. The anion exchange membrane removed these negatively charged, non-gaseous products.

The microbes were shown to have successfully colonized the cathode and performed some (unspecified) version of extracellular electron transfer. This was evidenced by CV tests and by the decrease in the hydrogen overpotential of the electrode. The decrease in the hydrogen overpotential of the electrode, from -904 mV SHE to approximately -700 mV SHE, was not below the theoretical hydrogen evolution potential, so it does not clearly support the use of direct extracellular electron transfer. The biofilm did successfully absorb all hydrogen gas to below any measurable limit for the majority of the tests, until the CO_2 ran out at the end, which evidenced

indirect extracellular electron transfer at the minimum. The potential of -700 mV SHE was within reason of the best performance for the tested range of potentials, however hydrogen gas evolved in those tests and said more about the need for hydrogen at the time than the true microbe potential preference. Without further testing of the resistance of the granular electrode, it cannot be known how close the final potential was to the theoretical potential for the production reactions.

The rate of VFA product removal, by transfer to the anode chamber as anions, is controlled by the flux, which can be controlled by a continuous liquid flow or inert gas purge in the anode. In open anode flow mode the efficiency strongly favours VFA production by reducing the residence time of possible methane substrates. The highest production efficiencies, where little to no hydrogen gas evolved, occurred in constant current tests with open flow mode. The H_2 gas concentrations continually decreased with progressive tests and remained below the measurable limit so long as sufficient CO_2 was available in the liquid. The biofilm adapted to changes in the current in about a week of sufficient CO_2 supply. Given enough growth time, the trend in biofilm efficiency reached 70% to 75% coulombic efficiency, with favour towards VFA production based on the flux. Continued investigation would evaluate a 3-chamber system, successfully capturing the VFAs by gas purge and condensation, or placing methanogens in either another cathode chamber or the anode.

Methane generation efficiency favours closed anode flow mode, with efficiencies equal to or less than that of the VFAs. The rate of decrease in CO_2 concentration slows in closed anode mode, separate to the use of the gas flow rate to maintain the flux, because

the acetotrophic methanogens consume VFA product, release methane and CO_2, and the CO_2 is available again to be reduced by the autotrophic biofilm. Further investigation might evaluate if the rate decreases by half and if control of the flux to the anode can minimize this.

Sequencing Batch Reactor methods achieve a total efficiency of methane close to the total efficiency of VFAs present, at the end of an open anode test. This was done when high flux rates were coupled with shut off and fermentation. Further work needs to be done to replicate these results and ensure the complete conversion or transfer of CO_2 is occurring and not just the residual absorption into the cathode liquid. The extension of this work, if successful, would compare SBR with continuous reactors in series.

The methane production rate was highly dependent upon the VFA concentration. The biofilm in the reactor has a community of its own, which likely includes some methanogens. It is most likely that acetotrophic methanogens are living in both the bottle and in or close to the biofilm in the reactor. Hydrogenotrophic methanogenesis was also reliably measured. Hydrogenotrophic methanogens are most likely present only in the planktonic phase, which presents an important point: the microbe species in the biofilm and in the planktonic phase must be measured. This will tell us which microbes make up the biofilm and which microbes are simply benefited from the biofilm.

The production rate of hydrogenotrophic methanogens is longer than most of the microbes that proceed it in anaerobic digestion. Originally, continuous tests were tried; however the production rates were too low to accurately measure against the abundance of CO_2. The brevity of the batch tests could be one factor that led to the production and

measurement of VFAs over methane. The production of VFAs, including acetate, should be investigated to determine that all the carbon used came from CO_2 and was not dissolved from the graphite electrode. If it did come from the electrode, this could make an interesting application for the conversion of coalfields.

6. Bibliography

Aeltermann, P., Rabaey, K., De Schamphelaire, L., Clauwaert, P., Boon, N. and Verstraete, W. (2008) Bioenergy. Wall, J.D., Harwood, C.S. and Demain, A.L. (eds).

Cambridge Museum of Technology, http://www.museumoftechnology.com/, accessed Feb. 24, 2012.

Clauwaert, P., Toledo, R., Van Der Ha, D., Crab, R., Verstaete, W., Hu, H., Udert, K.M. and Rabaey, K. (2008) Combining biocatalyzed electrolysis with anaerobic digestion. Water Sci. Technol. 57 (4), 575-579.

Food and Agricultural Organization of the United Nations. (1997) Renewable biological systems for alternative sustainable energy production (FAO Agricultural Services Bulletin - 128). Miyamoto, K. (ed.). FAO Corporate Document Repository.

Freguia, S., Rabaey, K., Yaun, Z.G. and Keller, J. (2008) Syntrophic processes drive the conversion of glucose in microbial fuel cell anodes. Environ. Sci. Technol. 42 (21), 7937-7943.

Habermann, W. and Pommer, E.-H. (1991) Biological fuel cells with sulphide storage capacity. Appl. Microbiol. Biotechnol. 35, 128-133.

Heijnen, J.J. (1999) Bioprocess Technology: fermentation, biocatalysis and bioseparation. Flickinger, M.C. and Drew, S.W. (eds). John Wiley & Sons, Inc., New York, pp. 267-291.

IWA Task Group for Mathematical Modelling of Anaerobic Digestion Processes (2002) Anaerobic Digestion Model No. 1 (ADM1). IWA Publishing.

Logan, B.E., Call, D., Cheng, S., Hamelers, H.V.M., Sleutels, T., Jeremiasse, A.W. and Rozendal, R.A. (2008) Microbial electrolysis cells for high yield hydrogen gas production from organic matter. Environ. Sci. Technol. 42(23), 8630-8640.

McCarty, P.L. (1981) Anaerobic Digestion. Hughes, D.E. et.al. (eds.). p3-22. Elsevier Biomedical Press, Amsterdam, New York, Oxford.

Mieke, C., Van Eerten-Jansen, A., Heijne, A., Buisman, C., Hamelers, H. (2011) Microbial electrolysis cells for production of methane from CO_2: long-term performance and perspectives. Int. J. Energy Res. doi: 10.1002/er.1954

Pham, T.H., Boon, N., Aeltermann, P., Clauwaert, P., De Schamphelaire, L., Vanhaecke, L., De Maeyer, K., Hofte, M., Verstraete, W. and Rabaey, K. (2008) Metabolites produced by Pseudomonas sp. Enable a Gram-positive bacterium to achieve extracellular electron transfer. Appl. Microbiol. Biotechnol. 77(5), 1119-1129.

Rabaey, K., Rozendal, R. (2010) Microbial electrosynthesis – revisiting the electrical route for microbial production. Nature Reviews Microbiology. 8, 706-716.

Rabaey, K., Rodriguez, J., Blackall, L., Keller, J., Gross, P., Batstone, D., Verstraete, W., Nealson, K. (2007) Microbial ecology meets electrochemistry: electricity-driven and driving communities. ISME J 1(1) 9-18.

Roche Applied Science Biochemical Pathways wall chart. http://web.expasy.org/pathways/, accessed Feb. 24, 2012.

Tchobanoglous, G., Burton, F., Stensel, H. (2003) Wastewater Engineering: treatment and reuse. Metcalf & Eddy. McGraw-Hill.

Villano, M., Aulenta, F., Ciucci, C., Ferri, T., Giuliano, A., Majone, M. Bioelectrochemical reduction of CO_2 to CH_4 via direct and indirect extracellular electron transfer by a hydrogenophilic methanogenic culture. Bioresource Technology 101 p.3085-3090.

Voet, D., Voet, J., Pratt, C. (2002). *Principles of Biochemistry*. Wiley.

APPENDIX I: NOMENCLATURE AND DEFINITIONS

Wastewater Treatment Terms

Primary and Preliminary Treatment: Prepare wastewater for biological treatment using physical and chemical treatment. Solids removal can include settling, floatation for oil and grease, and filtration. Chemical and flow homogenizing can include equalization tanks for flow and concentration loads, neutralization tanks for pH (can be included as part of an available equalization tank).

Secondary Treatment: Biological Treatment including both aerobic and anaerobic biodegradation of organic (carbon) compounds. Aerobic treatment of organics can use activated sludge process, aerated ponds, and trickling filters. Anaerobic treatment of organics can use anaerobic biogas process, high rate biogas process, non-aerated ponds.

Tertiary Treatment: specific pollutant removal using any combinations of physical, chemical or biological treatment method. Includes phosphate precipitation and flocculation; organic absorption with activated carbon; nitrogen removal with bacteria (biological); Disinfection with chemicals (Cl, O_3) or energy (UV, heat).

Anaerobic Digestion: Anaerobic Digestion, AD, is the term to describe the treatment process to convert the carbon in wastewater to a mixture of methane and carbon dioxide, jointly referred to as biogas, and residual solids. The hydrogen and electrons naturally available in the wastewater limit the conversion of carbon to methane, instead resulting in carbon dioxide.

Biosolids: Biosolids is the term used to describe the solid byproducts left by sewage treatment processes. Biosolids are mainly cell debris and are an expensive burden on wastewater treatment operation when normally disposed of in landfills, as required by the government for sanitary disposal. Biosolids can alternatively be treated as a high volume, renewable carbon and energy source.

CAMBI Thermal Hydrolysis Process: A major problem in using Biosolids as a substrate for resource production is that 55% to 80% is non-degradable. The CAMBI thermal hydrolysis process reduces the fraction of non-degradable material to 25% to 40%. The CAMBI process is being applied world wide.

Fermentation: Fermentation is a term generally used to describe the conversion of organic substrates to alcohols and organic acids. In fermentation the ratios and types of volatile fatty acids produced can be predicted based on the composition of the substrate, regardless of operating changes. The mixture of volatile fatty acids produced in fermentation is ideally suited for use in a continuous microbial fuel cell process.

Microbial Fuel Cell: A microbial fuel cell has the ability to continue processing wastewater beyond the concentration range of fermentation and anaerobic digestion. Fermentation and anaerobic digestion produce volatile fatty acids and methane to the limit of available compounds present in the wastewater feed. A microbial fuel cell, integrated into the wastewater treatment process, can use the same bacteria to continue to produce resources by supplying to the bacteria the previously limited compounds without creating the need to remove large organic fractions (dead cells). The bacteria develop niche abilities and produce continuously in either an oxidative or reductive capacity.

Lewis Acids and Bases

Lewis Acid: The definition of Lewis acid, as given by IUPAC is:

a molecular entity (and the corresponding chemical species) that is an electron-pair acceptor and therefore able to react with a Lewis base to form a Lewis adduct, by sharing the electron pair furnished by the Lewis base.

Lewis base: any species that donates an electron pair to a Lewis acid to form a Lewis adduct. Both OH^- and NH_3 are Lewis bases since they can donate a lone pair of electrons. The donated or shared electron pair need not be a lone pair. It could be the pair of electrons in a π bond.

The reaction must create an adduct and not simply be a displacement reaction. Some compounds, such as H_2O, are both Lewis acids and Lewis bases, because they can both accept a pair of electrons and donate a pair of electrons, depending upon the reaction.

Electrochemical Terms

Voltage (V) or Potential difference (V) or electro-motive force (EMF or E_{emf}): A quantity measured as a signed difference between two points in an electrical circuit which, when divided by the resistance between those points (in Ohms), gives the current flowing between those points (in Amperes), according to Ohm's Law. Voltage is expressed as a signed number of Volts (V). The voltage gradient in Volts per metre (say along a wire) is proportional to the force on a charge. Voltages (the difference between two potentials) are often given relative to "earth" or "ground" which is taken to be at zero Volts. Voltages are assumed to be in reference to the earth unless explicitly stated otherwise. A circuit's earth may or may not be electrically connected to the actual earth. The voltage between two points is also given by the charge present between those points (in Coulombs) divided by the capacitance (in Farads). The capacitance in turn depends on the dielectric constant of the insulators present. Yet another law gives the voltage across a piece of circuit as its inductance (in Henries) multiplied by the rate of change of current flow through it in Amperes per second. A simple analogy likens voltage to the pressure of water in a pipe. Current is likened to the amount of water (charge) flowing per unit time.

Watts/ wattage: Watts is a measure of the amount of electricity being used - a rate of electrical power consumption. Most people use a very simple mathematical formula to determine how many watts an electrical circuit can carry or how many watts an electrical device will require: Watts = Volts x Amps.

Ampere: In practical terms, the ampere is a measure of the amount of electric charge passing a point per unit time. Around 6.241 × 1018 electrons passing a given point each second constitutes one ampere.

Voltage, watts and amperes are all electrochemical terms that deal with electrons. A good analogy is to define all the above in terms of water, where the electrons are the water. Voltage is the same as the water pressure in a garden hose. Even if the spigot is shut off, there is pressure/voltage available once the spigot is opened (circuit completed, including accidental electrocution). Amperes would be the number of gallons/electrons that flow per second. Watts would be the number of gallons/electrons that flow per second, times the water pressure/voltage continuously available.

Standard Redox Potential: The thermodynamically determined reduction/oxidation potential is called the redox potential, the ORP, or the E_h. Reduction and oxidation reactions happen reversibly, therefore the point (potential) at which the oxidation reaction happens is the same as the point at which reduction happens. The numeric value of the potential is the same; the sign of the value is switched. Oxidation reactions happening on the anode have a positive sign. Reduction reactions happening on the cathode have a positive sign. Redox potentials are reported as compared to a standard reference point, which is arbitrary. The reduction/oxidation potential of hydrogen is the universal standard of reference (set as 0.0 Volts), unless otherwise stated. It is called the standard hydrogen electrode (SHE).

- **Standard Reduction Potential:** The thermodynamically determined measure of the potential at which a chemical species will acquire electrons and be reduced. When quoting the standard potential at which a reaction happens electrochemists default to the potential for the reduction reaction, called the Standard Reduction Potential, E_0.

- **Oxidation Potential:** The thermodynamically determined measure of the potential at which a chemical species will lose electrons and be oxidized. The oxidation potential is the same (comparative) numeric value as the reduction potential, however with the opposite sign.

Table A1: Standard Electrode Reduction and Oxidation Potential Values

Anodic - exhibits greater tendency to lose electrons compared to the other chemical species in the table			
Reduction Reaction	E^o (V)	Oxidation Reaction	E^o (V)
$Li^+ + e^- \rightarrow Li$	-3.04	$Li \rightarrow Li^+ + e^-$	3.04
$K^+ + e^- \rightarrow K$	-2.92	$K \rightarrow K^+ + e^-$	2.92
$Ba^{2+} + 2e^- \rightarrow Ba$	-2.90	$Ba \rightarrow Ba^{2+} + 2e^-$	2.90
$Ca^{2+} + 2e^- \rightarrow Ca$	-2.87	$Ca \rightarrow Ca^{2+} + 2e^-$	2.87
$Na^+ + e^- \rightarrow Na$	-2.71	$Na \rightarrow Na^+ + e^-$	2.71
$Mg^{2+} + 2e^- \rightarrow Mg$	-2.37	$Mg \rightarrow Mg^{2+} + 2e^-$	2.37
$Al^{3+} + 3e^- \rightarrow Al$	-1.66	$Al \rightarrow Al^{3+} + 3e^-$	1.66
$Mn^{2+} + 2e^- \rightarrow Mn$	-1.18	$Mn \rightarrow Mn^{2+} + 2e^-$	1.18
$2H_2O + 2e^- \rightarrow H_2 + 2\ OH^-$	-0.83	$H_2 + 2\ OH^- \rightarrow 2H_2O + 2e^-$	0.83
$Zn^{2+} + 2e^- \rightarrow Zn$	-0.76	$Zn \rightarrow Zn^{2+} + 2e^-$	0.76
$Cr^{2+} + 2e^- \rightarrow Cr$	-0.74	$Cr \rightarrow Cr^{2+} + 2e^-$	0.74
$Fe^{2+} + 2e^- \rightarrow Fe$	-0.44	$Fe \rightarrow Fe^{2+} + 2e^-$	0.44
$Cr^{3+} + 3e^- \rightarrow Cr$	-0.41	$Cr \rightarrow Cr^{3+} + 3e^-$	0.41
$Cd^{2+} + 2e^- \rightarrow Cd$	-0.40	$Cd \rightarrow Cd^{2+} + 2e^-$	0.40
$Co^{2+} + 2e^- \rightarrow Co$	-0.28	$Co \rightarrow Co^{2+} + 2e^-$	0.28
$Ni^{2+} + 2e^- \rightarrow Ni$	-0.25	$Ni \rightarrow Ni^{2+} + 2e^-$	0.25
$Sn^{2+} + 2e^- \rightarrow Sn$	-0.14	$Sn \rightarrow Sn^{2+} + 2e^-$	0.14

$Pb^{2+} + 2e^- \rightarrow Pb$	-0.13	$Pb \rightarrow Pb^{2+} + 2e^-$	0.13
$Fe^{3+} + 3e^- \rightarrow Fe$	-0.04	$Fe \rightarrow Fe^{3+} + 3e^-$	0.04
Arbitrary Neutral: H_2			
Reduction Reaction	E^o (V)	Oxidation Reaction	E^o (V)
$2H^+ + 2e^- \rightarrow H_2$	0.00	$H_2 \rightarrow 2H^+ + 2e^-$	0.00
Cathodic - exhibits greater tendency to gain electrons compared to the chemical species in the table			
Reduction Reaction	E^o (V)	Oxidation Reaction	E^o (V)
$S + 2H^+ + 2e^- \rightarrow H_2S$	0.14	$H_2S \rightarrow S + 2H^+ + 2e^-$	-0.14
$Sn^{4+} + 2e^- \rightarrow Sn^{2+}$	0.15	$Sn^{2+} \rightarrow Sn^{4+} + 2e^-$	-0.15
$Cu^{2+} + e^- \rightarrow Cu^+$	0.16	$Cu^+ \rightarrow Cu^{2+} + e^-$	-0.16
$SO_4^{2+} + 4H^+ + 2e^- \rightarrow SO_2 + 2H_2O$	0.17	$SO_2 + 2H_2O \rightarrow SO_4^{2+} + 4H^+ + 2e^-$	-0.17
$AgCl + e^- \rightarrow Ag + Cl^-$	0.22	$Ag + Cl^- \rightarrow AgCl + e^-$	-0.22
$Cu^{2+} + 2e^- \rightarrow Cu$	0.34	$Cu \rightarrow Cu^{2+} + 2e^-$	-0.34
$ClO_3^- + H_2O + 2e^- \rightarrow ClO_2^- + 2OH^-$	0.35	$ClO_2^- + 2OH^- \rightarrow ClO_3^- + H_2O + 2e^-$	-0.35
$2H_2O + O_2 + 4e^- \rightarrow 4OH^-$	0.40	$4OH^- \rightarrow 2H_2O + O_2 + 4e^-$	-0.40
$Cu^+ + e^- \rightarrow Cu$	0.52	$Cu \rightarrow Cu^+ + e^-$	-0.52
$I_2 + 2e^- \rightarrow 2I^-$	0.54	$2I^- \rightarrow I_2 + 2e^-$	-0.54
$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$	0.68	$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$	-0.68
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	0.77	$Fe^{2+} \rightarrow Fe^{3+} + e^-$	-0.77

$NO_3^- + 2H^+ + e^- \rightarrow NO_2 + H_2O$	0.78	$NO_2 + H_2O \rightarrow NO_3^- + 2H^+ + e^-$	-0.78
$Hg^{2+} + 2e^- \rightarrow Hg$	0.78	$Hg \rightarrow Hg^{2+} + 2e^-$	-0.78
$Ag^+ + e^- \rightarrow Ag$	0.80	$Ag \rightarrow Ag^+ + e^-$	-0.80
$NO_3^- + 4H^+ + 3\ e^- \rightarrow NO + 2H_2O$	0.96	$NO + 2H_2O \rightarrow NO_3^- + 4H^+ + 3\ e^-$	-0.96
$Br_2 + 2e^- \rightarrow 2Br^-$	1.06	$2Br^- \rightarrow Br_2 + 2e^-$	-1.06
$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$	1.23	$2H_2O \rightarrow O_2 + 4H^+ + 4e^-$	-1.23
$MnO_2 + 4H^+ + 2e^- \rightarrow Mn^{2+} + 2H_2O$	1.28	$Mn^{2+} + 2H_2O \rightarrow MnO_2 + 4H^+ + 2e^-$	-1.28
$Cr_2O_7^{2-} + 14H^+ + 6e^- \rightarrow 2Cr^{3+} + 7H_2O$	1.33	$2Cr^{3+} + 7H_2O \rightarrow Cr_2O_7^{2-} + 14H^+ + 6e^-$	-1.33
$Cl_2 + 2e^- \rightarrow 2Cl^-$	1.36	$2Cl^- \rightarrow Cl_2 + 2e^-$	-1.36
$Ce^{4+} + e^- \rightarrow Ce^{3+}$	1.44	$Ce^{3+} \rightarrow Ce^{4+} + e^-$	-1.44
$Au^{3+} + 3e^- \rightarrow Au$	1.50	$Au \rightarrow Au^{3+} + 3e^-$	-1.50
$MnO_4^- + 8H^+ + 5e^- \rightarrow Mn^{2+} + 4H_2O$	1.52	$Mn^{2+} + 4H_2O \rightarrow MnO_4^- + 8H^+ + 5e^-$	-1.52
$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O$	1.78	$2H_2O \rightarrow H_2O_2 + 2H^+ + 2e^-$	-1.78
$Co^{3+} + e^- \rightarrow Co^{2+}$	1.82	$Co^{2+} \rightarrow Co^{3+} + e^-$	-1.82
$S_2O_8^{2-} + 2e^- \rightarrow 2SO_4^{2-}$	2.01	$2SO_4^{2-} \rightarrow S_2O_8^{2-} + 2e^-$	-2.01
$O_3 + 2H^+ + 2e^- \rightarrow O_2 + H_2O$	2.07	$O_2 + H_2O \rightarrow O_3 + 2H^+ + 2e^-$	-2.07
$F_2 + 2e^- \rightarrow 2F^-$	2.87	$2F^- \rightarrow F_2 + 2e^-$	-2.87

Table references

1) http://www.physchem.co.za

2) http://hyperphysics.phy-astr.gsu.edu

Enzymes: Enzymes are proteins that catalyze (increase the rates of) chemical reactions, specifically biochemical reactions.

Catabolism: when living things break down food to provide energy

Catabolic pathways: used to break down the food to provide energy

Anabolic pathways: used to make specific components for the cell, using basic molecules and energy.

VFA: volatile fatty acids, are carboxylic acids with 6 or less carbons in their carbon chain. Acetate is one VFA. They are organic acids produced by acidogens, fermenters, in the fermentation process, which is stage 1 of methane fermentation/Anaerobic Digestion. When in basic solution they remain charged, with a single negative charge. When in acidic solution they can become neutrally charged and then volatilized given either the correct pH or physical removal by inert gas sparging.

Overpotential: is the potential difference (voltage) between a reduction or oxidation reaction's thermodynamically determined potential and the potential at which the reduction/oxidation event is experimentally observed. Overpotential directly affects a cell's voltage efficiency. Power is voltage times amps. The greater the overpotential for a reaction, the greater the total potential (voltage) needed to drive the reaction and therefore the greater the power needed for the same reaction (and the lower the efficiency). In an electrolytic cell, where we are supplying energy to drive a chemical reaction, the overpotential means more energy is required than thermodynamically expected to drive a reaction. In a galvanic cell, where we are gaining energy from a chemical reaction, the

overpotential means less energy is gained than thermodynamically expected from the reaction. This work deals with an electrolytic cell and focuses on the cathode, so the cathode is more negative due to the overpotential meaning more energy is used than thermodynamically expected to drive the reaction. Overpotentials come from various sources of resistance in the cell and from resistance to the electrochemical reaction. Minimizing the overpotential is one of the goals of all electrochemical designs and one of the reasons for investigating catalysts for electrochemical reactions.

- **Activation overpotential:** The potential difference above the equilibrium value required to produce a current, which depends on the activation energy of the redox event. It often refers exclusively to the activation energy needed to transfer an electron from an electrode to the anolyte. This is also called electron transfer overpotential, a component of polarization overpotential, and is observed in cyclic voltammetry. It is related to the physical materials used and the system's mass transfer abilities.
    - **Reaction overpotential:** is an activation overpotential referring to the overpotential for any chemical reactions that precede electron transfer. The electrochemical reaction rate and related current density are dictated by the kinetics of the electrocatalyst and substrate concentration. It can be reduced or eliminated using homogeneous or heterogeneous electrocatalysts. For example, platinum electrodes are common in electrochemistry. They are non-inert for many electrocatalytic reactions. At the platinum surface of a standard hydrogen electrode in aqueous solution, hydrogen is oxidized and protons are reduced readily and

reversibly. Irreversible reduction and oxidation peaks with large overpotentials would result if an electrocatalytically inert glassy carbon electrode was used in its place.

- **Concentration overpotential:** includes a variety of overpotentials, all involving the depletion of charge-carriers at the electrode surface.
    - Bubble overpotential is a form of concentration overpotential where the concentration of charge-carriers is depleted on the electrode due to the nucleation of a bubble. This reduces the surface area for current and increases the local current density.
    - Diffusion overpotential can refer to a concentration overpotential created by slow diffusion rates, as well as polarization overpotential, whose overpotential is derived mostly from activation overpotential but peak current is limited by diffusion of anolyte. It can happen when an electrochemical reaction is fast enough to lower the surface concentration of the charge-carriers below that of bulk solution. The potential difference between the electrode and the liquid is the result of concentration differences between the charge-carriers in the bulk solution and on the electrode surface. The rate of reaction is then dependent on the ability of the charge-carriers to reach the electrode surface.
- **Resistance overpotentials**: involve the cell design. It includes all junction overpotentials, overpotentials occurring at electrode surfaces, electrolyte interfaces and membranes, aspects of electrolyte diffusion, and surface polarization, called capacitance.

Aerobic Nitrification: $NH_4^+ + O_2$ (bio) $\rightarrow NO_2^- + O_2$ (bio) $\rightarrow NO_3^-$

Anoxic Denitrification: $NO_3^- + COD$ (bio) $\rightarrow NO_2^- + COD$ (bio) $\rightarrow N_2$

HRT = $V_{reactor}$/Q **OLR = C_{in}/HRT**

OLR = BOD (load needed as food) = F:M * MLSS = (kg/m^3*d) food in need for bugs = (conc./day)*BOD

G = solids loading rate to the surface (of clarifier)

G = $\frac{Q(1+a)}{A} * X_a$ $\frac{Q}{A}(1+a)X_a$

In and recycle flow to the surface **Solids concentration in incoming flow, X_a = MLSS**

SRT = 1/μ

WAS = βQ using average ADWF

SRT = $\frac{HRT}{\beta}$ if the sludge is take directly from the tank as concentration or if there are no solids left in the effluent

SRT = $\frac{biomass\ (kg)}{biomass\ removal\ (\frac{kg}{d})} = \frac{biomass\ (kg) at\ the\ beginning\ of\ clarification}{biomass\ (\frac{kg}{d}) removed\ from\ the\ system}$

SRT is a balance of the solids done as a ratio of $\frac{in}{out}$ around the clarifier as though the aerobic tank and clarifier were one and the same. The volume of the aerobic tank is used or the volume of the aerator since that is where the reaction is and the volume of retention.

SRT = $\frac{V_{reactor} * X_a}{(1-\beta)QX_e + \beta QX_r}$

When sludge is directly removed from the tank: $\frac{V_{reactor} * X_a}{\beta * Q * X_r} = \frac{V_{reactor} * MLSS}{\beta * Q * X_r}$