A Bioelectrochemical Sensing System for Measuring Acidic Bioprocess Fermentation End Products

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PhD Thesis

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Dedicated To my Parents (Harnek Singh and Sukhinder Kaur)

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Abstract

Monitoring is an important tool in the management of bioprocesses such as anaerobic digesters, wastewater treatment plants and bioelectrochemical systems. There are several important parameters which are considered for regular monitoring, including pH, temperature, conductivity, gas composition and volatile fatty acids (VFAs) concentration. To date the most reliable methods for offline VFA measurement are gas chromatography (GC) and high performance liquid chromatography (HPLC). Attempts have been made to develop *in situ* enzyme based sensor for online VFA measurements but the long-term stability and speciation is not yet satisfactory.

In this study microbial fuel cell (MFC) technology has been tested for its capabilities to measure VFAs and the method explored for its stability, repeatability, recovery and implementation on real wastewater samples. The amperometric signals derived from MFCs were correlated to corresponding, cross VFA and sucrose concentration that ranged up to 40 mg/l using electrochemical methods such as coulombic efficiency (CE) and cyclic voltammetry (CV); with response time of >20 hours (for 20 mg/l) and <3-4 minutes respectively. The stability of the sensor responses were studied further enhanced by fabricating anode electrodes with various natural and functionalised conductive polymers. Poly (alkyl) ammonium showed improved response, shortened start up and recovery times for sensor arrays. The range of the MFC sensors was also improved to reach up to 200 mg/l, by poising the enriched anodes at specific potentials. The individual VFA enriched MFC sensor array was calibrated offline to measure VFA concentrations in the samples collected from a sucrose fed scaled up MFC and hydrogen fermentation reactor with electrodialysis cell. Acetate and propionate enriched MFCs showed a satisfactory response when compared with GC analysis and could be further improved. However, the poor response from the butyrate enriched MFCs required more study with regards to calibration, degradation pathways and electron transfer mechanisms. This study also revealed various operating strategies to inhibit methanogenesis in MFCs enriched with individual VFA and enhanced degradation of recalcitrant fermentation end products, which could contribute to further increasing the overall performance of the proposed sensor array.

The study presented here is the first attempt to use MFC as a sensing system to detect VFAs and is an important contribution in the field of bioprocess monitoring and will provide a platform for further improvements in MFC based VFA sensing.

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Abbreviations

Ac	Acetate
AD	Anaerobic Digester
AEM	Anion Exchange Membrane
AMFC	Acetate enriched microbial fuel cell
AMFC-Ppy^{+}	AMFC with Ppy+ modified electrode
ARB	Anode Respiring Bacteria
BES	Bioelectrochemical systems
BOD	Biological Oxygen Demand
Bu	Butyrate
CC	Closed Circuit
CE	Coulombic efficiency
CEM	Cation Exchange Membrane
COD	Chemical Oxygen Demand
CV	Cyclic Voltammetry
EAB	Electro Active Bacteria
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
MEA	Membrane Electrode Assembly
MFC	Microbial Fuel Cell
c-MFC	cubic MFC
h-MFC	H-type MFC
MPPT	Maximum Power Point Tracking
MS	Mass Spectroscopy
NIRS	Near Infra Red Spectroscopy
OCV	Open Circuit Voltage

OC	Open Circuit
ORP	Oxidation Reduction Potential
ORR	Oxidation Reduction Reaction
PEM	Proton Exchange Membrane
Ppy	Polypyrrole
Ppy^{+}	Poly alkyl (ammonium) modified electrode
Ppy^{-}	Poly propionic acid modified electrode
Pr	Propionate
PTFE	Polytretrafluoroethylene
SCFA	Short Chain Fatty Acids
TVFA	Total VFA
VFA	Volatile Fatty Acids

1 Introduction

1.1 Overview

Volatile fatty acids (VFAs) also known as short chain fatty acids (SCFA), are widely present in activated sludge, landfill leachates and wastewater for example, and are important products and contaminants seen in anaerobic processes. These are predominantly acetate (ethanoate), propionate (propanoate) and butyrate (butanoate) with the chemical structures as shown in Figure 1.1, are low molecular mass carboxylic acids and are important metabolic intermediate compounds in many biological processes.

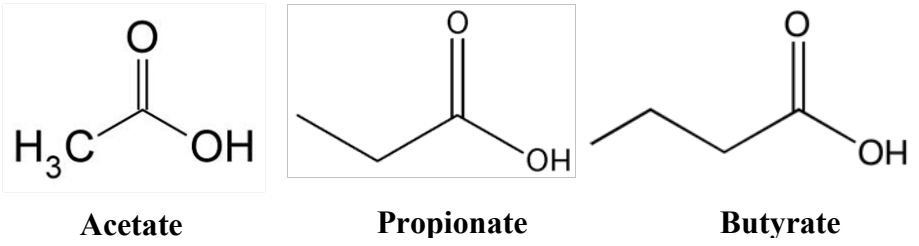

Figure 1.1: Chemical structure of three major volatile fatty acids evaluated in this study

VFAs are also evident in more dilute environments such as still waters, sewer systems and primary wastewaters and are also frequently used as substrates in a number of biological processes such as denitrification as well as bioelectrochemical systems (BES) (Kim et al., 2010). These compounds are found typically within the range of 1-5,000 mg/l. The presence of VFAs in a sample matrix is often indicative of bacterial activity that can originate from biodegradation of organic matter. As shown in Figure 1.2 the complex polymers first hydrolysed to simpler monomers like monosaccharides, long chain fatty acids and amino acids which then degraded to acetate, propionate and butyrate. VFAs can be inhibitory and indicative of microbial stress if present in high concentrations (Ahring et al., 1995, Siegert and Banks, 2005). Their accumulation results in a decrease of pH; and can ultimately lead to a failure of such biochemical processes. VFA concentrations can be indicative of chemical oxygen demand (COD) removal performance in aerobic, BES or other effluent polishing processes. Therefore the monitoring of these compounds would be a useful optimization and controlling tool. In

addition to determining total VFA (TVFA) concentrations, the measurement of individual VFAs have often been proposed as a important variables in the control of Anaerobic Digester (AD) (Di Lorenzo et al., 2009a, Steyer et al., 2002, Pind et al., 2002, Perrier and Dochain, 1993) and other wastewater treatment bioprocesses. Off-line monitoring of VFAs has been widely used to monitor these processes; however due to the significant lag in measuring VFA and inputting the data into a feedback control loop might have difficulties in preventing a rapid system failure.

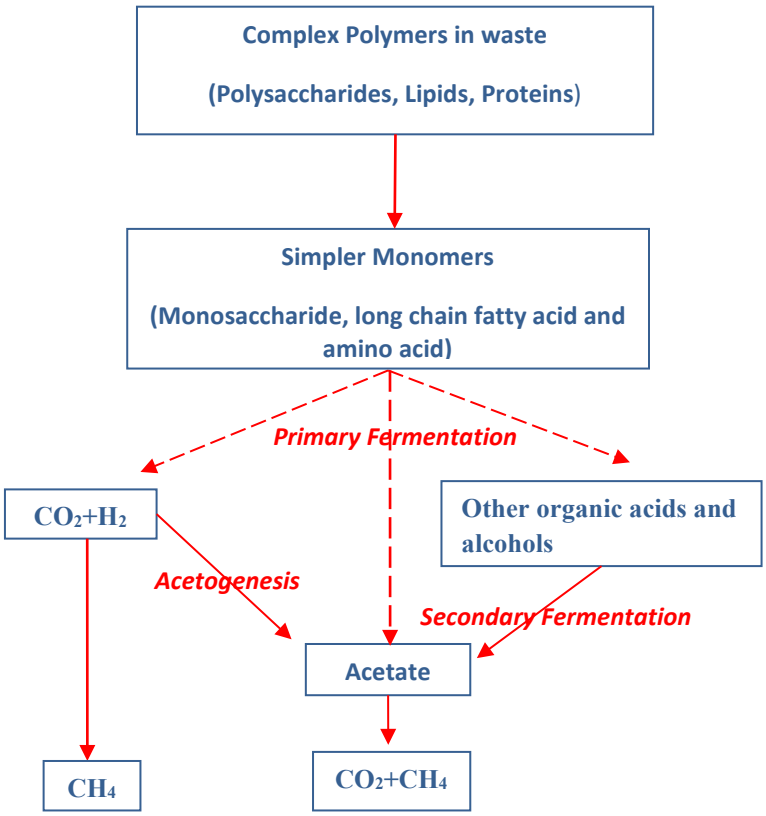

Figure 1.2: Production of volatile fatty acids from complex waste

Only a few reports exist on the development of on-line VFA monitoring systems for individual VFA species, typically by Gas Chromatography (GC) (Slater et al., 1990) or High Performance Liquid Chromatography (HPLC) (Zumbusch et al., 1994). In addition to traditional methods of VFA measurement, only a limited number of biosensors and other sensors for VFAs have been reported in the literature to date (Yano et al., 1997, Rajashekhara et al., 2006).

In recent years, biosensors have demonstrated a great potential as an alternative to conventional analytical methods for monitoring anaerobic bioprocesses. The main advantages of using biosensors over conventional analytical techniques are the possibility of portability, miniaturization, and the ability of working online; furthermore they may not require any additional processing steps such as reagent additions (Rodreguez-Mozez et al., 2006). Low cost measurement of the concentration of key VFAs would be a very useful approach to improve the operation of a number of important bioprocesses, through computer based monitoring and control. The use of MFCs could be a new addition to the commercial biosensor market and is receiving increasing attention from researchers due to their flexible sensing capabilities. *In situ* MFC based VFA sensors could replace the current generation of relatively complicated and expensive techniques for on-line VFA analysis. MFCs convert the energy in a bio-convertible substrate, directly to electricity. This is achieved when bacteria switch from the natural electron acceptor, such as oxygen or nitrate to an insoluble electron acceptor such as the MFC anode (see Figure 1.3).

A schematic representation of the functions of a MFC is shown in Figure 1.3. In the MFC the organic molecules are utilized by the anode respiring bacteria (ARB) and results in the generation of electrons, protons and carbon dioxide. The typical anodic and cathodic reactions with acetate as a model substrate are shown in equation 1.1 and 1.2.

Anode: $CH_3COO^- + 2\ H_2O \rightarrow 2CO_2 + 7\ H^+ + 8\ e^-$ ***Equation 1.1***

Cathode: $4H^+ + 4e^- + O_2 \rightarrow 2H_2O$ ***Equation 1.2***

The electrons are then transferred to the electrode. This transfer can occur either via membrane associated components, or soluble electron shuttles. The electrons then flow through a resistor to a cathode, and to compensate for the charge differential that would otherwise result, protons pass through a cation exchange membrane (CEM) to the cathode chamber. Electrons and protons react with and reduce terminal electron acceptor oxygen to form water in what is known as the Oxygen Reduction Reaction (ORR) (Rabaey and Verstraete 2005) as shown in Figure 1.3.

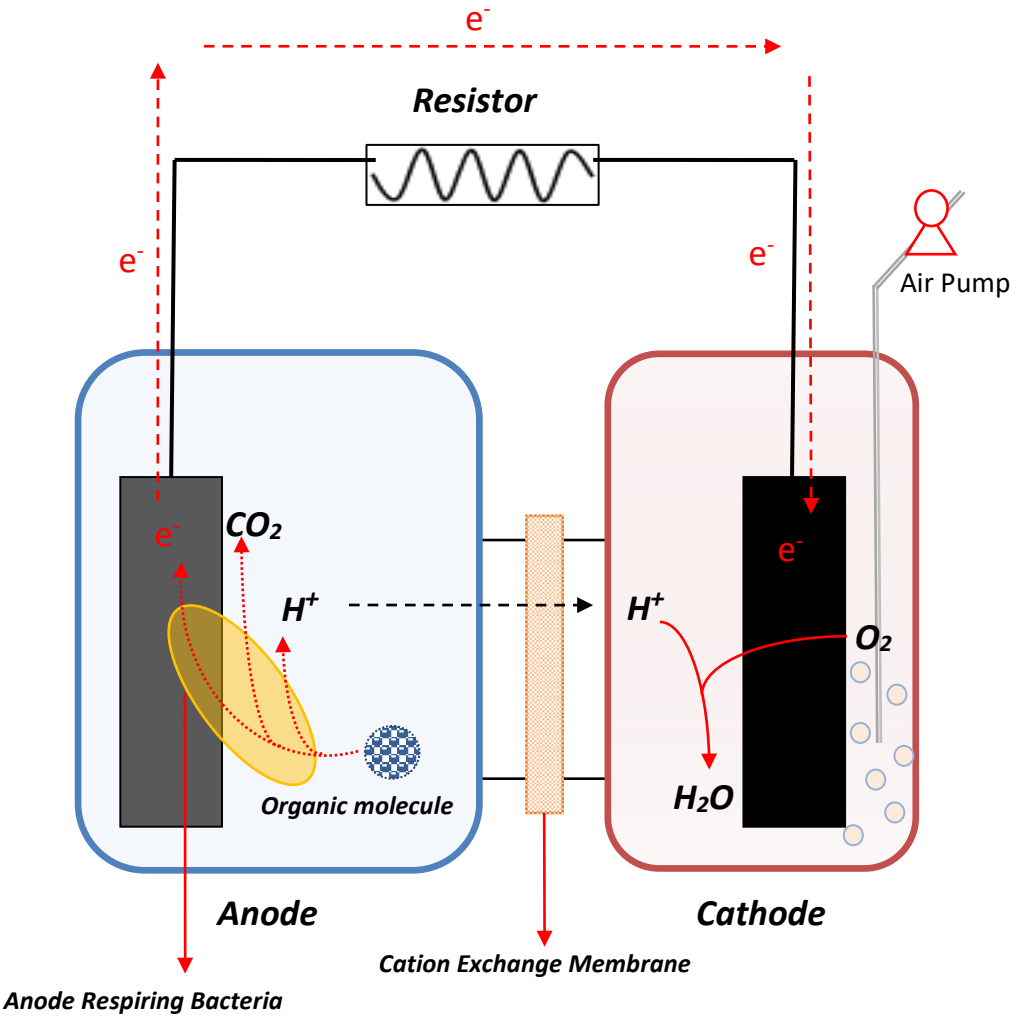

Figure 1.3: Schematic representation of working of typical Microbial Fuel Cell

MFCs have been extensively studied as a Biological Oxygen Demand (BOD) sensor, based on the fact that the coulombic energy generated from the MFC is proportional to the concentration of substrate used. As compared to other methods like enzymatic biosensors and chromatography currently being used, MFCs have received increasing attention due to their long term stability and repeatability with minimum maintenance due to the microbial growth and cost effectiveness. Microbial fuel cells have been used for the construction of a wide variety of biosensors (Racek. J, 1995). As VFAs can result in electricity production *via* MFC technology (Teng et al., 2010), the study presented in this thesis aims to use the MFC principle for detecting, quantifying and speciating VFAs that are commonly found in wastewater streams.

1.2 The Use of VFA Sensor Response as a Control Signal:

The importance of VFA concentration in the control of anaerobic digesters has been established for some time (Ahring et al., 1995, Feitkenhauer et al., 2002). The information obtained from a automated VFA sensors would help in managing these waste treatment and energy producing systems, by facilitating their stable operation and optimisation. In anaerobic digestion, a reactor could become less effective for the production of energy and the effective treatment of wastes when an organic overload occurs. This is when the amount of organic matter fed to the digester exceeds the total degradation capacity of the microbes to produce biogas. This organic overload can lead to an accumulation of VFAs which can lead to a reduction in pH and bicarbonate alkalinity as well as reduced gas yields and reduced substrate treatment efficiency (Guwy et al., 1994, Williams et al., 2013, Dinsdale et al., 1996) . Knowing the instantaneous reactor concentration of VFAs, it is possible in AD systems to reduce the organic loading rate (OLR) in response to an increase in VFA concentration. This would enable the removal of the excess VFA via methanogenesis to occur and therefore improve the overall system efficiency in terms of methane yield and the overall process stability in terms of pH and bicarbonate alkalinity levels.

This organic overload to the anaerobic digester could be due to a number of reasons including, a compositional change in the feedstock, an increase in the biodegradability or volatile solids composition of the feedstock, the presence of inhibitory compounds (which preferentially reduces effectiveness of the methanogens), an increase in feedstock flow rate, lack of essential micronutrients reducing microbial growth or an equipment failure such as temperature control. The main advantage of VFA monitoring compared to other parameters is that it provides an insight into a major metabolic activity of the anaerobic digester and can be used to support other simpler monitoring parameters such as gas flow and composition, temperature as well as pH and alkalinity in managing anaerobic digestion performance. The use of VFA monitoring could be used as a part of a robust automated digester control and optimisation strategy which is supported by the measurement of the other parameters such as gas flow and reactor temperature

A similar approach can be beneficial for the stable operation of the industrial deployment of bioelectrochemical systems which are being postulated as a future technology for treating wastewaters (Fradler, 2014, Huang and Logan, 2008). In scaled up MECs and MFCs for waste treatment, the monitoring of VFA levels in the influent and in effluent from the process would give the information of the degree VFA removal and also facilitate the calculation of the coulombic efficiencies which would aid in understanding the contribution of the wastewater substrate to electricity. This would also be helpful in maintaining the optimal pH for the anodic biofilm and enhance the COD removal and ultimately the power output of the process (Behera and Ghangrekar, 2009).

The presence of VFAs in effluent liquors is also extremely odorous so the close monitoring and control of these compounds would increase the public acceptability of these processes in waste treatment and energy production.

1.3 Aims and Objectives

The overarching aim of this research was to investigate the measurement of fermentation end products; specifically short chain VFA (homologous carboxylic) acids (C_2-C_4), which are typically present in anaerobic processes and indicative of the state of the process. Acetic, butyric and propionic acids in particular, were identified as the principle target analytes to be measured using MFCs. The primary ambition of the research was to replace more sophisticated and costly techniques required for VFA measurement, with mobile deployable and cost effective MFC based biosensors. Several specific objectives were identified to indicate the scope of this study:

1. To determine the correlation between the MFC signals and concentrations of VFAs, specifically acetate, propionate and butyrate
2. To stabilize the microbial community on an electrode by using different natural and carbon/conductive polymers
3. To improve the applicability of such sensors by increasing their range, using poised potentials in conjunction with cyclic voltammetric analyses of oxidation peaks.

4. To investigate implementation of the sensor by measuring samples from scaled up MFCs or low concentration VFA containing wastewater streams for offline/online measurement.
5. To study the syntropism and thermodynamic instability between acetogens and methanogens (possibly electrode respiring species) and consider if electrode reactions can replace syntropism to enhance the degradation of propionate and butyrate in a MFC sensor system. To further analyse any effects on the suppression of methanogens or differential selection of electrogens over methanogens by using different operating strategy.

1.4 Structure of the Thesis

The thesis includes a total of 9 chapters. Chapter 1 introduces the motivation of the study, gives an overview of the drivers for monitoring of VFAs in bioprocessing and about the novelty of the work by using MFC technology as a VFA sensor. The overarching aim and objectives of the study are also highlighted in this chapter. Chapter 2 reviews the current state of art associated with the individual objective set for this study and are discussed in detail. The common and general materials and methods used in this study are summarized in Chapter 3 in addition to details of more specific reactor operations considered for individual experiment.

Chapter 4 considers the findings on the correlation between the MFC signals and the VFA concentrations. The results obtained from cyclic voltammetry showed linear correlation and are presented in this chapter along with an evaluation of the potential cross interferences to other VFAs and sucrose. Chapter 5 presents the steps taken towards improving the signal responses and their stability over time by using anode fabrication with different natural and carbon/conductive polymers (Polypyrrole). The sensor was then tested for the offline monitoring of real samples obtained from two different sources to show the implementation of the MFC sensor for mainly low VFA containing water streams in Chapter 6. Enhanced degradation of recalcitrant fermentation end products was also studied. Various side reactions in the MFC reduce Coulombic efficiency (CE) and considering it's important for MFC based sensing, a novel method for suppressing methanogenic activity by operating MFCs on different OC/CC

regimes is presented in Chapter 7. The effect of starvation on methanogenesis was also considered to completely inhibit the methanogens and hence methane in MFCs.

Chapter 8 summarises the whole thesis while conclusions and future work is compiled in Chapter 9.

In addition to the above mentioned chapters Appendix 1, contains the description of pure cultures considered at the start of the project but subsequently discontinued due to the restricted growth observed. Appendix 2 contains a table summarizing the calibration concentrations used for TVFA in BMFC. Appendix 3 highlights the outputs from the PhD in terms of papers, poster and oral presentation.

2 Literature Review:

This chapter presents a review of the various methods and techniques that have been reported in the literature for the analysis of VFAs in the aquatic environment. The chapter also reviews the relevant details of MFC technology; particularly progress toward their use as biosensors over the last few decades; and advantages and limitations that MFCs present over other methods.

2.1 Volatile Fatty Acid Monitoring

The monitoring of anaerobic bioprocesses is difficult and complex, as it is a multi-variable process with few reliable on-line sensors for the measurement of these important parameters. Ideal monitoring methods should be online, robust and give early indications of imbalance in the microbial activity in the system under observation. Monitoring of the anaerobic processes for example, requires access to a suitable parameter or parameters reflecting the metabolic state of the process (Ahring et al., 1995). Various commonly used indicators include gas production and composition, pH, oxidation and reduction potential (ORP) and bicarbonate alkalinity. Previous studies in the literature have shown that VFAs can be one of the most suitable parameters for the monitoring of anaerobic bioprocesses, mainly in AD (Perrier and Dochain, 1993, Pind et al., 2002, Steyer et al., 2002). VFAs are important intermediate compounds in the metabolic pathways of anaerobic processes and both cause and indicate microbial stress if they are accumulated within the system in high concentrations. VFAs accumulation during process imbalance directly reflects a kinetic uncoupling between the acid producers and the VFA consumers (Switzenbaum et al., 1990). It is well recognized that monitoring of specific concentration of VFA can give vital information about the status of the AD process (Hill and Holmberg, 1988, Hickey and Switzenbaum, 1991, Anderson and Yang, 1992, Lahav et al., 2002). Acetic acid is usually present in higher concentrations and indicate imbalance when >13 mM than the other acids during anaerobic digestion, but propionic (> 0.12 mM) and butyric acids are also common and are not beneficial to the methanogens. The level of isobutyrate, isovalerate and n-butyrate has also been suggested as indicators of stress level (0.06-0.17 mM), or as a measure of the degree of process imbalance. During process overload or stressed

conditions, increases in hydrogen partial pressure can thermodynamically affect the degradation of propionate before other VFAs (Pauss and Guiot, 1993). Thus some reports have proposed propionic acid as a sole process indicator (Renard et al., 1991, Hansson et al., 2002) while others have suggested using the variation in propionic : acetic acid ratio as an indicator for impending failure (Hill and Holmberg, 1988, Marchaim and Carsten, 1998). With the increase in the full-scale application of anaerobic processes in future BES, there is a need to develop reliable, simpler and lower cost methods for their evaluation and control. Process instability can be avoided by proper monitoring and control in order to allow substrate at higher rates of loading. Therefore monitoring is very important to prevent systems failure due to inappropriate operation resulting in high concentration of VFAs. The present technologies for VFA measurement require either time consuming assays or are expensive and measurement has generally only been possible offline for e.g. GC (Slater et al., 1990) HPLC (Zumbusch et al., 1994), with very few exceptions. There are various methods available for off line monitoring of VFAs such as straight distillation, steam distillation, colorimetric techniques, gas and liquid chromatography, titration techniques and electromigration methods. The methods mainly used for the measurement of VFAs are discussed as follows.

2.1.1 Titrimetric and Spectrophotometric Methods

Different methods have been developed in the literature to simplify measurement of VFAs in anaerobic processes. For total VFA determination titration and spectrophotometric techniques are well known. The most cost effective analysis of VFA and alkalinity with acceptable accuracy is the titrimetric method. Determination of VFAs by titrimetric methods is based on the equilibrium of VFAs in their dissociated and non dissociated forms. Many titrimetric methods have been developed consequently for determination of total VFA have been proposed, e.g. a simple titration (Anderson and Yang, 1992), a 5-point titration (Moosbrugger et al., 2009), and an 8-point titration (Lahav et al., 2002). Both online and offline testing have been demonstrated by various researchers (Feitkenhauer et al., 2002). Although titrimetric methods are very simple, they have several disadvantages such as the typically cumbersome set up required for on-line monitoring, and the inability to speciate between different VFAs. Also during the

titration, other chemical components in the sample consume acid/base. These may include bicarbonate, phosphate, ammonium and sulphate and interferences from any other type of acids present in system. The titrimetrically measured TVFA concentration leads to the higher estimate due to the complexity of samples, although there have been studies and methods published to remove such factors (Purser et al., 2014). As a consequence, online titrimetric methods are largely no longer in use. Optical methods have also been reported in the literature. Robert-Peillard et al., (1991) and Palacio et al., (2010) developed an online spectro fluorometric system for rapid VFA analyses. The near infrared spectroscopy (NIRS) monitoring also seems to be suitable for on-line applications (Jacobi et al., 2009). In fact very good correlation between on-line monitoring and reference samples were obtained for all the tested compounds. Issues with spectral changes due to bulb aging, and temperature effects need to be solved before long term on-line monitoring would be possible.

2.1.2 Chromatographic Methods

For the determination of individual VFAs in a sample, separation techniques such as HPLC, ionic chromatography (IC), Capillary zone electrophoresis (CZE) and GC are widely used. GC is a technique which separates the mixture of volatiles by passing them through a heated packed column. The flow of the mixture is facilitated by a heated inert gas, usually helium or argon. The individual separated species are then transferred to detectors such as; FID or mass spectrometer for detection and quantification. HPLC is another chromatographic technique to quantify metabolic intermediates such as VFAs or other acids such as lactate. This technique is an improved type of column chromatography where the mobile phase solvent is forced through under pressure. It also enables the use of very small particle size for the column packing material which provides a much greater surface area for contact between the stationary phase and the molecules flowing through them. This leads to improved separation of the components in the sample. However, this method suffers from a poor detector response in common HPLC sensors such as UV Vis detectors. The fact that carboxylic acids undergo dissociation and when the pH is sufficiently high they are mainly in an ionized form and can be separated as carboxylate anions by anion exchange chromatography (AEC). When in ionic form they can also be measured by electrophoretic techniques. Low

molecular mass carboxylic acids can be monitored in aqueous samples of different origins.

Only a few reports exist on the development of on-line VFA monitoring instruments based on chromatographic systems (Xu and Mitra, 1994, Boe et al., 2007, Diamantis et al., 2006). Slater et al., (1990) placed a filter in the recirculation loop of a fluidized-bed reactor and transferred the final permeate to a GC with a modified injection port allowing on-line analysis every 12 minutes. However, no data from these measurements and no validation of the suitability of this procedure were published. Ryhiner et al., (1993) used a 0.45m filter on a similar recirculation loop acidifying permeate with 1% formic acid. Zumbusch et al., (1994) used a membrane with a normal molecular weight cut-off (NMWC) of 20,000 (equal to 20 kDa) for sampling in a UASB reactor. The permeate was pumped through a gas separator and then analyzed in an HPLC using an injection valve. The accuracy of the HPLC was much lower than normally achieved by GC; however, the HPLC was not specifically optimized for this purpose. Maricou and colleagues have shown that VFAs can be detected in the headspace of liquid samples in the range of ppmv (Maricou et al., 1998).

Out of all the mentioned methods above, the most widely used analytical techniques for the determination and quantification of VFA is GC and Liquid Chromatography like HPLC in some cases linked to Mass Spectroscopy (MS) and other detectors such as FID. Although these methods are very reliable, sensitive and capable of giving information on individual VFA, they are also expensive and in most cases samples have to be sent to specialist laboratories for testing. Hence, these methods are not compatible with on-line and/or on-site monitoring, which are essential to process management in the anaerobic process. Off-line monitoring for VFA has been widely used; however the delay in gaining a measured value results in difficulties for feedback control, therefore off-line monitoring cannot be readily utilized to prevent a short term system failure. FID and GC-MS systems are relatively expensive and time consuming for measuring VFAs in bioprocess samples. In addition online monitoring is not possible with such conventional methods and to overcome this problem simple online sensors are required and MFC based sensors are a promising online monitoring approach for improving anaerobic process control, or to monitor VFA levels in various aquatic systems.

2.1.3 Sensors and Biosensors

Currently most digestate sample analyses are conducted by collecting and sending the samples to specialist laboratories and facilities with appropriate instruments for analysis, a process which can takes up to two weeks for the results to be received. This can be too long a time delay for corrective action to be taken when VFA levels are high and affecting the bioprocess system, e.g. reducing the digester's efficiency. Even if the instrumentation were locally placed, the burden of frequent and persistent sampling would be prohibitive. A very limited number of biosensors/sensors for VFAs have been reported in the literature to date. Compared with conventional chromatographic methodologies such as GC or HPLC, which are technically onerous and not considered portable, the use of biosensors is gaining more attention as they offer a rapid and potentially an on-line monitoring system for even trace level of target species (Liu et al., 2011a, Su et al., 2011, Tothill et al., 2011).

The most widely accepted definition of a biosensor is "a self-contained analytical device that incorporates a biologically active material (bioreceptor) in contact with an appropriate transduction element for the purpose of detecting the concentration or the activity of chemical species in any type of sample" (Arnold and Meyerhoff, 1988). The classification of biosensors is based on the transduction element, and bioreceptor used. The bioreceptor can use organisms, tissues, cells, enzymes, antibodies, nucleic acids, etc. and would detect the target analyte. The transducer is used to convert any biological changes to measurable signals, (which can be electrochemical, optical, thermal or mechanical in nature) and converts the recognition event into a measurable signal. For the whole biosensor to work, different disciplines including materials science, electrical engineering and physics, along with biology/biochemistry are often required to come together to deliver meaningful solutions. Biosensors generally, determine the concentration of various constituents and other parameters of biological interest and contribute to a broad spectrum of applications. Biosensors have a expansive range of applications in medicine, agriculture, environmental monitoring and many bioprocessing areas (Turner, 2013, Krishnamurthy et al., 2010). In all bioprocessing industries there are various parameters which need to be monitored on-line, for example pH, conductivity, VFAs, BOD, COD etc. Most of these parameters are measured

off-line and as with VFAs they can take a significant time to conduct the measurements. Some of these measurements also require sample processing in advance, in addition to the actual sample analysis time, which makes the measurement even more difficult to implement for control purposes. Also, in most cases accurate analyses of biological materials are expensive and need to be performed at an appropriate laboratory equipped with sophisticated instrumentation and procedural controls. The area of biosensors has been studied extensively, with a number of research and development articles publishing proof of concept studies. Environmental sensors are an emerging area of application, specifically for monitoring bioprocesses to prevent them from failing (Dennison and Turner, 1995, Lagarde and Jaffrezic, 2011, Rodriguez-Mozez, 2006, Rawson et al., 1989). Biosensors also play a significant role in other major fields of science, such as the monitoring of natural and disease processes (Bobby et al., 2006, Joseph, 2008). Whole cells are a good alternative to enzymes since they have the benefit of low cost and improved stability (self replication) compared to enzymes or other proteins. Moreover, microbes are easy to manipulate and have better stability under harsh environments (Su et al., 2011, Lagarde and Jaffrezic, 2011). A few groups have tried to identify the denitrifying bacteria which are only capable of using VFA as a carbon source. Constanin et al., (1996) have attempted to find a bacterial system to allow competitive denitrification for industrial wastewaters. Various studies have been reported where denitrifying bacteria were used as a catalyst in a biosensor for VFA measurement (Meyer et al., 2002, Lorenzen et al., 1998). Yano et al., (1997) have developed a piezoelectric quartz crystal sensor using lipids with various properties to detect chemical vapours. They successfully showed a response to isovaleric acid. Electronic noses are also very suited to and commonly used in qualitative analysis; several examples of quantification of individual VFAs and other compounds are present in the literature (Tothill et al., 2011). Maricou et al., (1998) showed that VFAs can be detected in the headspace of a liquid sample in the ppmv range. Various enzyme based sensor have also been developed to detect either total VFA concentrations or individual VFA concentration. For example, Rajashekhara et al., (2006) developed an onsite enzyme sensor for monitoring VFA (propionate). A much simpler enzyme biosensor for VFA has been reported by Zeravik et al., (2010) for detection of VFAs in wine. In addition to electronic nose technology Tothill et al., (2011), have also tried to develop an enzyme

sensor. Their focus was the detection of VFAs in samples from AD. The enzyme used was sarcosine oxidase and its inhibition on addition of VFAs was measured as a means of detection. The biosensor showed good results with pure VFA samples but gave random response with complex samples.

2.2 Microbial Fuel Cell Based Sensor

In addition to all the above mentioned types of sensor technologies, over recent years MFCs have emerged as a new technology which can be used as biosensors. MFC based sensors have been attracting limited, but increasing attention from researchers over the past decade, with 2011-2020 on track to show an further decade on decade increase as there have been 23 publications in 2011-2014 compared to the 18 publications in 2001-2010.

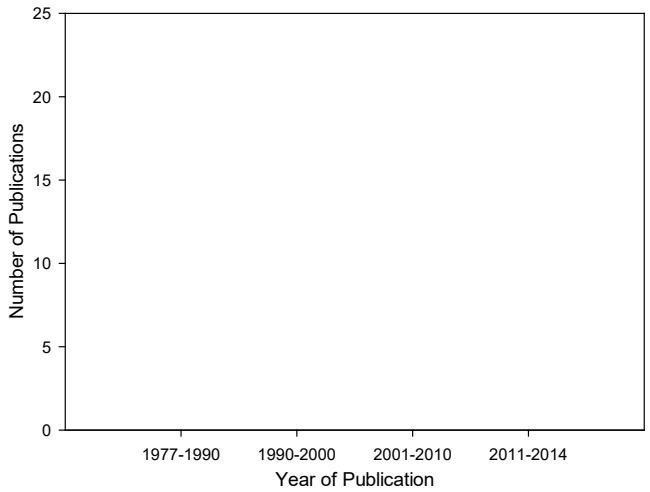

Figure2.1: Development in the research on Microbial fuel cell based sensor since 1977 (Karube et al., 1977)

MFCs represent an emerging technology which in addition to wastewater treatment and generating electricity may have various other useful applications. Arguably the most promising application of MFC technology in the short term is in the development of biosensors. There are significant potential advantages in using MFCs as sensors over other more conventional sensors which derive mainly from their projected cost effectiveness and the direct nature of their electrical output, useful in online monitoring of bioprocessing and other systems. Several advances in the MFC based sensor reactor configuration have also been made in recent years. These include advances in electrode

and membrane materials and design, as well as cell configurations and understanding of the processes involved. MFC based sensor technology is steadily evolving on many fronts, including MFC design, material selection and renewable substrate utilization, moving it closer to realizing its full potential. The fact that bacteria can generate electric current was introduced by Potter in (1911). The basic principle behind MFCs is that the conversion of chemical energy available in a bio-convertible substrate to electricity is possible and practical (Rabaey and Verstraete, 2005, Logan et al., 2006, Logan, 2009, Logan and Regan, 2006). The biosensor based on MFC technology works on the same principle, where microorganisms in the anode compartment act as biocatalyst and the whole MFC works as a transducer when the signal is presented directly as an electrical response of a range and power appropriate for integration through signal conditioning to processing device.

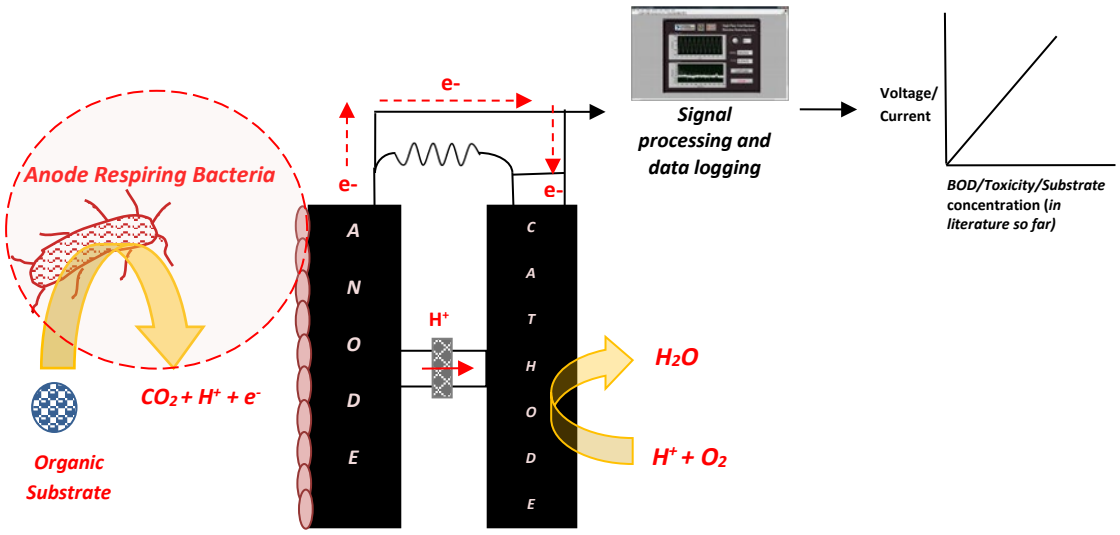

Figure 2.2: Schematic representation of working principle of microbial fuel cell based sensor

A typical MFC based sensor mainly consists of an anode, cathode and CEM and the electrodes are electrically connected via an external circuit. MFCs have been used in a various configurations, often governed by their intended application (Chang et al., 2004, Kim et al., 2003a, Gil et al., 2003, Moon et al., 2005, Tront et al., 2008a, Logan et al., 2006, Rabaey and Verstraete, 2005). Electrons travel via an external circuit from the anode to the cathode where the terminal electron acceptor is reduced. The electrical activity can be recorded variously as the current produced or any associated derived

electrical measure. An illustration of the principle is shown in Figure 2.1. To achieve electrical power generation, bacteria act as a catalyst to convert substrate into electrons. The anode electrode and anode chamber are the main parts of the MFC. Microbes enriched by selection from an inoculum containing a diverse mixed population which includes electrogenic organisms, are immobilised on electrode (though not exclusively). By utilizing substrate in the anolyte, the electrogenically functional species derive energy by using the anode as an electron acceptor and so generate electricity. In the anode chamber many microbial bioprocesses may takes place which improves sensor flexibility but may reduce specificity. The anode chamber is inoculated typically with anaerobic sludge or wastewater and the electrogenic bacterial cells selectively acclimate in the anode chamber by virtue of their ability to take better advantage of the operational conditions.

The anode is often made up of carbon based materials for example carbon paper, carbon cloth, carbon veil or graphite with low resistance where the electrogenic anodophiles acclimate. For cation exchange membrane most MFC studies so far have applied Nafion™ (Dupont; http://www.dupont.com) however, these membranes are sensitive to (bio) fouling by ammonium. The overall best result so far have been obtained from (Membranes International; http://www.membranesinternational.com/) cation exchange membrane (Rabaey et al., 2004). The membrane was omitted by (Liu and Logan, 2004) , using pressed carbon paper as the separator in a membrane less MFC but to date all the MFC sensors studied used a membrane based MFC configuration. The cathode completes the circuit of the cell by transferring electrons to a high potential electron acceptor. The material for the cathode used to date for MFC based sensors are different forms of platinised carbon or graphite. Table 2.1 summarises the MFC based sensors reported so far and their operational set up details. The volume of the reactor and materials plays important role and a range of different reactor volumes from 1.6 ml to 1000 ml have been considered by various researchers compiled in Table 2.1. A section is included in this table for references to the descriptions of the experimental equipment and materials used. High anodic potential is desirable for increased energy generation, which can be achieved primarily by excluding oxygen from the anode chamber. The pH and buffering properties of the anodic chamber can be varied to maximize microbial

growth, energy production and electrical potential. MFCs can be inexpensive biosensors if operated with naturally occurring bacteria and constructed using common materials. Due to its conversion mechanism MFCs, has received considerable interests particularly in the environmental and energy fields (Kim et al., 2003a, Chang et al., 2004, Kaur et al., 2013). MFCs have been considered for use as biosensors in previous studies (Chang et al., 2005, Gil et al., 2003, Kumlanghan et al., 2007) and are discussed in more detail in Section 2.3.1.

Table 2.1: Summarizes the some of the MFC based sensors and their respective characteristics published in literature

MFC Type Sensor	Mediator-Mediator less	Microorganism	Anode	Cathode/Catholyte	External Resistance(Ω)	Volume(ml)	Reference
BOD	Mediator-less	Mixed Culture	Graphite felt	Graphite felt/Air cathode		20	(Karube et al., 1977)
Lactate	Mediator-less	Mixed Culture	Plate of Graphite felt	RVC*/$K_3Fe(CN)_6$	500	20	(Kim et al., 1999b)
BOD	Mediator –less	Mixed Culture	Graphite felt	Graphite felt	500	25	(Gil et al., 2003)
BOD	Mediator-less	Mixed Culture	Graphite felt	Graphite Felt with Platinum	10	25	(Kim et al., 2003a)
BOD	Mediator-less	Mixed Culture	Graphite felt	Graphite felt	10	17	(Kim et al., 2003b)
BOD	Mediator-less	Mixed Cultures	Graphite felt	Graphite felt with platinum	500	20	(Kang et al., 2003)
BOD	Mediator-less	Mixed Culture	Graphite felt	Graphite with platinum	10	25	(Chang et al., 2005)
BOD	Mediator-less	Mixed Culture	Graphite felt	Graphite felt	10	20	(Chang et al., 2004)
BOD	Mediator-less	Mixed Culture	Graphite felt	Graphite felt	10		(Moon et al., 2005)
BOD	Mediator-less	Mixed Culture	Graphite felt	Graphite with platinum	10	25	(Chang et al., 2005)
BOD	Mediator-less	Mixed Culture	Graphite roll	Graphite rod	0-2000	100:1000	(Kumlanghan et al., 2007)
BOD	Mediator-less	Mixed Culture	Graphite cloth	Platinum embedded Graphite cloth	100	36.4	(Tront et al., 2008b)
BOD	Mediator less	Mixed Culture	carbon cloth	Carbon with 0.3mg/cm^2platinum	500	-	(Di Lorenzo et al., 2009a)
Toxicity	Mediator-less	Mixed Culture	Graphite plate	Graphite plate	1000	33	(Stein et al., 2010)
BOD	Mediator-less	Mixed Culture	Carbon paper	Carbon paper with platinum	1000	-	(Peixoto et al., 2011)
AD monitoring	Mediator-less	Mixed Culture	Graphite roll	Graphite roll	800	1.6	(Liu et al., 2011b)
Toxicity	Mediator-less	Mixed Culture	Carbon paper	Carbon paper with platinum	1000	20	(Stein et al., 2012c)
Toxicity	Mediator-less	Mixed Culture	Carbon paper	Carbon paper with platinum	1000	20	(Stein et al., 2012b)
Volatile Fatty Acid	Mediator-less	Mixed Culture	Carbon paper	Carbon paper with platinum	1000	250	(Kaur et al., 2013)
Shear rate	Mediator-less	Mixed Culture	Carbon cloth	Carbon cloth with Platinum	5	28	
Neural network processing	Mediator-less	Mixed Culture	Carbon Fiber	Powdered activated carbon with platinum	470	40	(Feng et al., 2013a)
Smart Biosensing	Mediator-less	Mixed Culture	Carbon fiber	Powdered activated carbon with platinum	470	40	(Feng et al., 2013c)
Water quality	Mediator less	Mixed Culture	Carbon cloth	Carbon cloth	1000	3-164	(Di Lorenzo et al., 2014)
AD monitoring	Mediator less	Mixed Culture	Graphite roll	Graphite roll	200	1.6	(Liu et al., 2014)

2.2.1 Microbiology and Electron Transfer Mechanisms

The bacteria are small (µm scale) organisms which can utilize a variety of organic compounds. In an MFC the bacteria act as a catalyst and could be very useful for extracting electricity and exploiting them as a sensor. The possibilities to control oxidation and reduction reactions at electrodes has created an array of niche applications and targeted products. The electrical current produced by the MFC is virtually a direct measure of the metabolic activity of the bacteria and may be used as an indicator of the oxygen, inhibitory chemical species or substrate concentrations in the reactor. High reproducibility in the current output and flexible fuel utilization, including substrates of the macromolecular nature, are prerequisites for the implementation of the MFC as a sensor for BOD/Toxicity and/or environmental, metabolic-related water quality parameters. In comparison to other existing sensors (e.g. pH, temperature, gas flow meter) used in anaerobic processes, MFCs work as a tightly controlled mini-bioreactor with high selectivity. MFC based biosensors may have long term stability, which provides a prolonged life time for the sensing element. Furthermore, organic matter such as acetate could be directly converted to electricity and the MFC itself may be considered to represent an integration of signal generator and transducer, which reduces the cost of external conversion to useful electrical signals. Along with the many existing MFC architectures, there have also been many microorganisms and substrates studied.

Various mechanisms have been proposed in order to describe the pathway of electron transfer from bacteria to the electrode. To date, there have been three major mechanisms of microbial electron transfer in MFCs have been described (Schroder, 2007b, Logan and Regan, 2006). These are:

1. Direct transfer from outer membrane of microorganisms through cytochromes, (effectively an electron shuttles which stay anchored to the cell membrane). Electron transfer takes place directly from membrane bound redox active proteins to the electrode.
2. Self-produced or natural mediators (electron shuttles that leave the cell), and
3. Conductive pili (nanowires).

In direct electron transfer, the anode electrode must provide a favourable surface for the bacteria to attach, with a higher potential than other possible electron acceptors or they would be released to the solution. Direct electron transfer between a cell and electrode was discovered by Kim et al., (1999a) in an MFC using *Shewanella putrefaciens.* There are some electrogenically active bacterial species such as *Geobacter sp* and *Shewanella sp* which directly attach to the electrode and form a biofilm. These electrogens were observed to produce conductive nanowires and have been studied in both pure and mixed consortia by various researchers (Gorby et al., 2006, Logan, 2009). Cytochromes play an important role in direct electron transfer from bacterial cells to the electrode. OmcZ cytochromes play an interfacial role in the transfer of electrons and OmcS has been associated with exocellular electron transfer from pili (Inoue et al., 2011). *Shewanella oneidensis* also secretes flavins which act as an electron mediator (also considered a 'natural' mediator) but in other cases an artificial mediator is required to facilitate electron transfer and the other mechanism described may plays a role even when additional redox mediators are used for transfer (Schroder, 2007b). Early investigations of MFCs employed redox active mediators (electron carriers) to facilitate the transfer of electrons from the microbes to the anode surface. The selection of mediator depends on its redox potential which should be suitable for reduction at the organism and oxidation at the electrode, i.e. as negative as possible, but higher than the potential of substrate and sufficiently lower than that of the anode electrode. The mediator should be able to be readily reoxidized on the anode surface under the operating conditions of the MFC (Schroder, 2007b). Artificial mediators such as thionine, methylene blue and neutral red have been used to assist anodic electron transfer in MFCs (Bennetto et al., 1983). These mediators penetrate the bacterial cell and scavenge electrons from the reducing agents such as NADH (Bennetto, 1987).

The biocatalyst can be selected with reference to the application to which the MFC technology is directed. Systems based on the *Shewanella* showed promising performance as sensors for quantifying BOD in sewage (Kim et al., 2003a, Moon et al., 2004). This concept might readily be expanded to detect other compounds such as VFAs that can act as electron donors for electricity production. It is generally more difficult to work with and maintain a pure culture, primarily due to the ease of contamination and

the synergistically facilitated degradation of some biodegradable materials which requires at least two species or taxa. Some of the benefits of using a pure culture include the possibility that the culture has been fully sequenced, the ability to compare the work with previous studies of the same culture and also to focus on increasing power production via MFC architecture/substrates while keeping the microbial genotype constant. However, the application will frequently determine whether mixed or pure culture can be used.

2.2.2 Oxidation of Different Substrates

A variety of substrates, such as acetate, butyrate, starch, glucose, cellulose, lactate, sulphite and more generally wastewater, have been considered as electron donors in MFC to investigate their potential to generate electricity (Deepak Pant et al., 2010, Lee et al., 2009, Sun et al., 2009). Owing to the diverse nature of the biofilms in MFC, many substrates have been reported to generate electricity, but the power output varies from substrate to substrate. The oxidation of mixed components as in wastewaters requires the anode microbial communities to degrade a range of complex organic compounds and a number complex waste streams such as domestic wastewaters, landfill leachates, brewery wastewaters, paper recycling wastewater and AD effluent have successfully been used in MFCs (Catal et al., 2008).

While complex substrate degradation is necessary for wastewater treatment, pure substrate can initially be studied to determine the applicability of MFCs as biosensors, as in this study which considers VFAs. There are reports in the literature related to electric current generation from VFAs and these studies can may be used as a basis for further studies into the implications of using such devices as VFA sensors (Teng et al., 2010, Freguia et al., 2010, Cárcer et al., 2011, Liu et al., 2004a). One molecule of acetate is stoichiometrically converted to 8 electrons, 8 protons and carbon dioxide, and this can be considered as a theoretically ideal model for sensing this VFA species. Propionate and butyrate are also important intermediates in the degradation pathways of complex organic matter, where they may be fermented to acetate and hydrogen or may be oxidised directly when a suitable metabolic pathway and electron acceptor (for example

a anode in MFCs) are available and would ideally generate 12 and 20 electrons per molecule respectively.

2.3 MFC Response and Electrochemical Detection Methods

The ability to achieve *in situ* monitoring, useful information about microbial respiration can be obtained by using electroactive biofilms coated on the electrodes to establish a quantitative sensor. Information in the form of useable voltage or current signals can easily be generated for conditions such as the concentration of a substance of interest or the presence a toxic chemical species. The signals can be made available and manipulated in real time because the respiratory chain of the microorganisms involves electron transfer directly to the anode.

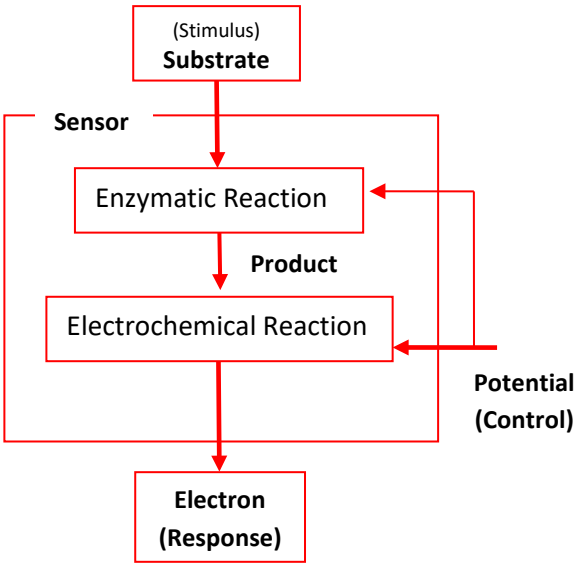

Figure 2.3: Conversion of substrate concentration to measureable response

This metabolic activity can therefore be easily measured and the only temporal delays are associated with the hydraulics and mass transfer within the cell and the kinetics of the biochemical and electrochemical reactions. All respiratory processes involve stoichiometric coupling of electrons transferred to the electron acceptor, but notwithstanding some parasitic reactions, the current generated is also a measure of available substrate (Figure 2.2).

Several bioanalytical parameters, such as BOD and toxicity, substrate concentration amongst others can be related to or can be inferred by the measurement of metabolic

respiratory activity or changes in the culture media associated with the half-cell. The convenient signal generated may be directly integrated to signal conditioning and data processing devices as necessary. In comparison with other existing sensors (i.e. pH, temperature, and gas flow metres) used in AD, MFC biosensors work as small bioreactors which may present high selectivity (Spanjers and van Lier, 2006) and exhibit long term stability (Gil et al., 2003). Thus MFCs might be designed so not to need catalyst replacement. Transducers are essential to convert a particular biological and chemical transformation into electrical data which can identify different biochemical components of a complex compound, possibly to isolate the desired biochemical compounds or for many other purposes. However, MFCs do not need any extra transducer as they work as a small bioreactor converting biological activity to voltage/current/power signals directly. There is widespread usage of conventional methods for measuring BOD, toxicity and concentrations of various substrates/components in wastewater, but the BOD_5 test for example has a number of limitations, such as questionable accuracy, irreproducibility and its labour intensive and time consuming practicalities and similarly some of the issues arise for the measurement of different organic acids.

2.3.1 Coulombic Efficiency – MFC based BOD sensing

Since Karube and co-investigators (Karube et al., 1977) first reported on a BOD sensor that used immobilized microorganisms with an oxygen probe, a number of studies on MFC-based sensors have been published (Chang et al., 2004, Kim et al., 2003a, Gil et al., 2003). The CE has been proposed as an accurate measure of the BOD values, as a linear relationship was observed between the BOD value and the coulombs produced in MFCs. Various studies have been published on further improvements of overall performance of the MFC based sensors (Kang et al., 2003, Moon et al., 2004, Chang et al., 2004). There are many advantages in MFC based BOD sensors over other types of sensor; particularly because of their perceived cost effectiveness and excellent operational stability. An MFC-type sensor constructed and initiated with microbes enriched appropriately, can be kept operational for many years without significant maintenance (Kim et al., 2003b). However, high BOD concentration requires a longer response time as it is not possible to calculate CE until substrate/BOD depletes completely. The samples could be initially diluted to achieve the shortest possible measurement time; however this may not be

easily achieved in situ. The measurement time of such MFC-type BOD sensors varies significantly, from 1 hour up to several hours (Chang et al., 2004, Kang et al., 2003). Therefore, the analysis of concentration based on charge recovered delays measurement and would not be the most applicable approach above a certain particular concentration for *in-situ* monitoring. High strength samples may therefore have to be diluted, prior to being introduced to a small chambered MFC based sensor, in order to analyse the sample within a reasonable time. Also, higher CE values are important with this approach, but there are many parameters which adversely affect CE in MFCs. These may include the presence of dissolved oxygen and alternative electron acceptors such as nitrate and sulphate, or the presence of metal reducing bacteria and methanogens (Logan et al., 2006, Schroder, 2007b, He et al., 2005, Chae et al., 2010b).

The overall performance of the MFC type sensor can be further influenced by other and numerous factors such as the rate of fuel oxidation and electron transfer to the electrode by the microbes, the external ohmic load, membrane proton exchange effectiveness, oxygen diffusion to the anode through the membrane or other barriers. The oxygen supply and subsequent reduction in the cathode may also affect sensor performance. Improvements may increase the applicability of MFC based sensors and the technology has increased the available possibilities for online determination of various monitoring parameters. The literature on biosensors based on MFC has been summarized in Table 2.1 and 2.2, indicating their respective characteristics and configuration details. Both offline and online testing of the sensors using different substrate and source of inoculums have been considered with the response time varied from 3 minutes to 10 hours. It can be seen in Table 2.2 that in addition to BOD and related measurements, the MFC signals have been used in determining toxic components in wastewater.

Table 2.2: Microbial fuel cell based sensor and their respective characteristics

MFC Type Sensor	Substrate/Source	Range (mg/l)	Response Time	Online/ Offline	Reference
BOD	Glucose and Glutamic acid	10-200	10-15min	offline	(Karube et al., 1977)
Lactate	Lactic acid	3000	-	offline	(Kim et al., 1999b)
BOD	Wastewater	400	NA	offline	(Gil et al., 2003)
BOD	Wastewater	2.6-206.4-	30min-10hrs-	offline	(Kim et al., 2003a)
BOD	Wastewater	10-150	45min	online	(Kim et al., 2003b)
BOD	Wastewater	<10	≈120min	offline	(Kang et al., 2003)
BOD	Artificial waste with glucose and glutamic acid	20-200	36 ±2min	online	(Chang et al., 2005)
BOD	Wastewater	100	≈60min	online	(Chang et al., 2004)
BOD	Artificial waste with glucose and glutamic acid	2-10	≈60min	offline	(Moon et al., 2005)
BOD	Anaerobic Digester effluent	1-2500	3-5min	online	(Kumlanghan et al., 2007)
BOD	Acetate	0-2300	60-240min	offline	(Tront et al., 2008b)
BOD	Artificial and treated wastewater	350	40min	online	(Di Lorenzo et al., 2009a)
Toxicity	Active microbial fuel cell	30-Ni 3-Bentazon 50-SDS	12-46hr	offline	(Stein et al., 2010) (Stein et al., 2012b)
BOD	Domestic wastewater	17-78	30min-10hr-	offline	(Peixoto et al., 2011)

The basic principle of a MFC can, when used in a slightly modified way, present a new kind of sensor i.e. toxicity sensor for the detection and quantification of toxic compounds. When a toxic compound comes into contact with a microbial community, it adversely affects the respiration rate of the bacterial biofilm which has been acclimated in the anode. This result in a decreased current output from MFC and so can be detected electrochemically as a signal related to toxicity. In other words, microbes will need more time to utilize the carbon source if toxic compounds such as phenols or chromium inhibit them (Davila et al., 2011, Kim et al., 2007b).

MFCs have consequently been proposed for toxicity sensing, monitoring the presence of chemical toxicants in various environments (Stein et al., 2012a). Patil et al., (2010) investigated the effect of selected biocides on wastewater-derived electroactive microbial biofilms and found that the current generation of a mediator-based MFC enriched with planktonic cells from wastewater was affected by the presence of the antimicrobial agents. To obtain a simple, compact and planar device for toxicity monitoring, Davila et al., (2011) developed a novel silicon-based MFC. The device

consisted of a proton exchange membrane placed between two micro fabricated silicon plates that act as current collectors. The sensor is capable of detecting the variation of current produced when toxic compounds (e.g., formaldehyde) are present in the medium. Stein et al., (2011) modified a bioelectrochemical model combined with enzyme inhibition kinetics, to describe the polarization curve of an MFC-based biosensor. This allowed the identification of four types of toxicity. They discovered that the overpotential should also be controlled in order to get a stable and sensitive biosensor. Further to this, Stein et al., (2012b) experimentally investigated the sensitivity of a MFC-based biosensor for nickel and they also found that the effect of four types of ion exchange membranes (cation exchange, anion exchange, monovalent cation exchange and bipolar membranes) on the sensitivity was not significant (Stein et al., 2012c). The sensor had a higher response at high overpotentials, even if the nickel concentration was low. Although there are some positive directions to measure toxicity using MFC systems, the recovery time for the bacterial biofilm after exposure of toxicant, makes it difficult for online implementation. Although BOD/toxicity sensors using MFCs have been developed, whole cell based biosensor for individual and/or total VFAs and specific target organic chemicals had not been previously investigated. This study aims to design and test a whole cell MFC-based biosensor for its ability to detect and quantifying individual VFAs such as acetate, butyrate and propionate.

2.3.2 Cyclic Voltammetry

Voltammetry is a technique in which a varying potential is applied to an electrode in an electrochemical system while the resulting current is measured. One form of voltammetry is cyclic voltammetry (CV) which is used in initial electrochemical studies due to its ability to provide details of complex electrode reactions. The fact that certain bacteria can direct electrons to their outer surface, or transfer electrons from their outer surface to their inner compartments, makes it possible to measure the rate of electron flux from living organisms. In the simplest cases of irreversible chemical reactions occurring at an electrode, observed reaction rates are a function of diffusion coefficient of the donor, the standard rate constant of the reaction and the electrode potential.

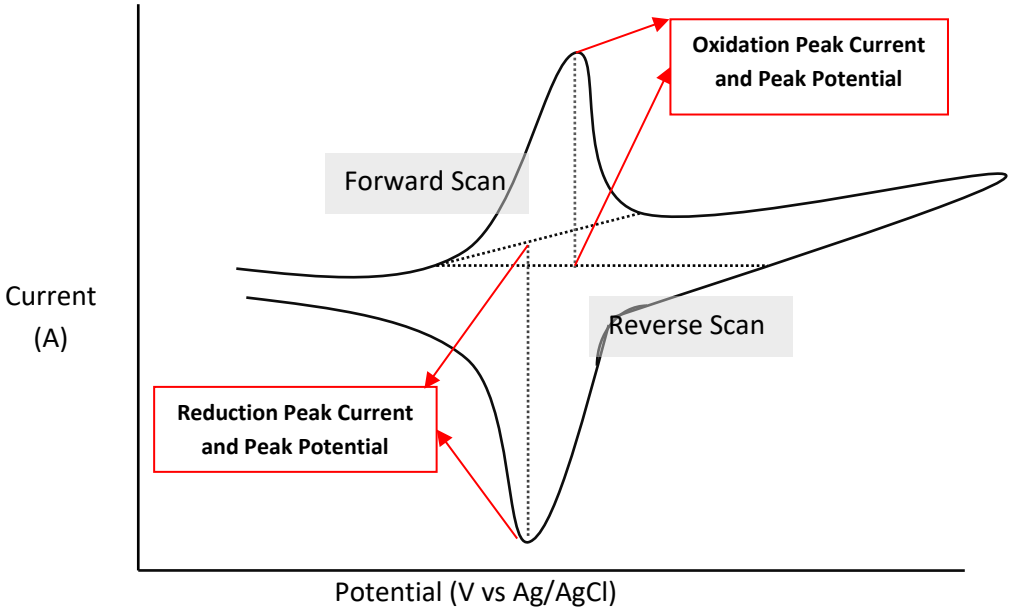

Figure 2.4: Typical cyclic voltammogram showing oxidation and reduction peak from reversible reaction

It is proposed by Fricke et al., (2008) that the scan rate dependence is indicative of diffusion control and that the complexity of the microbial and bioelectrochemical processes makes the voltammogram interpretation challenging. The diffusion layer at the surface of the macroelectrode is linear and the CV recorded for a reversible electrochemical reaction using macroelectrodes can be characterised as shown in Figure 2.4. The size of the diffusion layer at the electrode surface will be different depending on the voltage scan rate. In a slow voltage scan, the diffusion layer will grow much further from the electrode in comparison to a fast scan. Consequently, the flux to the electrode surface is considerably smaller at slow scan rates than it is at faster ones. As the current is proportional to the flux towards the electrode, the magnitude of the current will be lower at slow scan rates and higher at high rates.

A starting potential during CV measurement is defined along with potential boundaries at which point the sweep is to reverse its direction. A single cycle is complete when the sweep hits the potential and bounds back to its initial voltage. In MFCs, the range over which the potential is varied, should be limited in order to prevent harmful oxidizing or reducing conditions and the scan rate should be carefully selected to minimise sampling time while maintaining accuracy. CV can induce the oxidation or reduction of various

species on and around the working electrode by giving the electrode surface a sufficiently positive or negative potential. When the voltage at the electrode surface reaches an analyte's standard reduction or oxidation potential, the resulting increase in current can be seen as peaks in the voltammogram (see Figure 2.4). Depending on whether the electrode is being reduced or oxidized, electrons will be transferred either to or from the electrode surface, respectively. This flow of electrons will show up on the voltammogram as an increase in current, in either the positive or negative direction, depending on the direction of electron flow. The current will increase as the species on the surface of the electrode is oxidized (anodic current) or reduced (cathodic current) until its concentration has been depleted.

Matsunaga and Namba in (1984) used CV to understand the electron transfer mechanism between *Saccharomyces cerevisiae* and a graphite electrode. They also used the technique to detect the bacteria in the suspension. Many researchers have used CV for various purposes, including electron transfer mechanisms, and the investigation of fabricated electrode performances. This phenomenon can also be used to detect various substrates in the solution. There are various enzyme based glucose detectors which have been established using CV methods (Narang et al., 1994, Sirkar et al., 2000). A number of other substrates, apart from glucose, such as lactate, cholesterol, chlorinated hydrocarbons, have also been investigated by voltammetric detection (Sirkar et al., 2000, Shumyantseva et al., 2004, Aulenta et al., 2011). Although there is little published work on the impact that CV has on the microbial community, biofilm structure, electrode materials, planktonic cells and overall MFC performance, it seems clear that such scanning would affect the biocatalyst in particular. This is a simple and well-known technique that can quickly provide information about redox reactions as they occur in electrochemical systems. It is through these redox reactions that fuel cells produce electrical current, and which is why this method is widely used in the analysis of MFC systems. There are many studies on electron transfer mechanisms in the literature, but no specific reports of detecting substrates in the MFCs by using this technique has been published. In this study CV was investigated and their changing characteristic with respect to the VFAs was considered.

2.3.3 Anode Poised Potential - Amperometry

Amperometry is based on the measurement of the current resulting from the electrochemical oxidation or reduction of electroactive species. It is usually performed by maintaining a constant potential at working electrodes with respect to reference electrode. The resulting current is directly correlated to the bulk concentration of the electroactive species or its production or its consumption rate within the adjacent layer. The anode potential is one of the important factors controlling the synergistic interactions between complex microbial communities as it can influence the electron liberating capacity of the biocatalysts (Schroder, 2007b, Aelterman et al., 2008, Boghani et al., 2013, Finkelstein et al., 2006). The effect of anode poised potential has been studied for its effects on start up and the performance of EABs (Srikanth et al., 2010, Wang et al., 2009b). However, these two cited studies presented conflicting results with respect to the start-up times. Wang et al., (2009b) reported shortened start-up times of more than 20days with the anode potential poised at +0.2V (vs Ag/AgCl) in a dual chamber reactors for control under a 1000 ohm loading. However, Aelterman et al., (2008) also reported that anode potential did not have an effect on start-up time, but this was attributed to the inoculum being sourced from an active MFC. Another implication of poised potentials was studied on selecting anode respiring bacteria by Torres et al., (2009) which would be useful for a MFC based sensor to enhance utilization of the substrate and quicker electron transfer. Poised potential has also been studied for enhanced oxidation of substrates and can be used for sensing specific substrates. Aulenta et al., (2011) studied the degradation of chlorinated hydrocarbons at -400mV anode potential. The potential was selected on the basis of the oxidation peak of the chlorinated hydrocarbons in the CV at -400mV. Furthermore, there are many reports on non-MFC poised potential based sensors in the literature (Tripathi et al., 2006, Li et al., 2008, Vianello et al., 2006). The question therefore arise if a similar approach could be used for the detection of VFAs by using poise potential obtained from CVs and has been explored in this thesis in Chapter 4 and 6.

2.4 MFC Sensor Performance Improvement

2.4.1 Anode Fabrication

In an attempt to use modified electrodes to improve MFC based sensors, it is useful to think of the biofilm-anode as a tri-composite material. The electrode and catalyst form the initial composite and the anode respiring biofilm, which add the biological material, acts as a final extension of the electrode. The electrode fabrication might significantly influence electron transfer kinetics and biofilm colonization. Immobilization of the microbial community has been considered in biosensors. Various natural and conductive polymers have been extensively studied for their application in the field of biosensors. The selection of the polymers is an important issue in the performance of any biosensor in which they are used. There are several types of immobilization matrices used for whole cells studied. Similar methods could maintain a more consistent and predictable performance from an MFC sensor by either encapsulating the biofilm within a thin layer of protective but permeable material, or by promoting tight and stable adherence and electronic communication between the bacterial community and the electrode for a sufficiently extended time period commensurate with cost effective devices. It has been shown that immobilization not only facilitates the necessary close proximity between the biofilm and the electrode, but also helps in stabilizing the biocatalyst for reuse (D'Souza, 2001). The anode material and its structure can directly affect the bacterial attachment, electron transfer and substrate oxidation. Various nanostructured materials such as carbon nanotubes (Ci et al., 2012, Liang et al., 2011), PANI (Polyaniline, a polymer) (Qiao et al., 2007), ruthenium oxide (Lv et al., 2012) and immobilization of redox mediators (Feng et al., 2010, Adachi et al., 2008, Popov et al., 2012) have been used to modify the MFC electrodes. In addition to these, many applications of conducting polymers, including in analytical chemical and biosensing devices, have been reviewed by several researchers (Trojanowicz et al., 1995, Situmorang et al., 1998, Schuhmann, 1995, Wring and Hart, 1992). Polypyrrole is one of the most extensively used conducting polymers applied in the design of bio-analytical sensors, because of its good electrical properties and long term stability of conductivity. Polypyrrole has been used in MFCs to improve the performance by various researchers (Zhou et al., 2011, Zou

et al., 2010, Zou et al., 2008) and all of these studies have found positive results in terms of current output from the MFCs.

2.4.2 Eliminating Side Reactions

The performance of MFCs is affected by several non-electrogenic reactions considered to be parasitic energy consuming factors and numerous studies elaborating these factors have previously been published (Liu et al., 2005, Oh et al., 2004, Zhao et al., 2009, Kim et al., 2011). The loss of chemical energy to methanogenesis is one such inefficiency, hence improving performance by suppressing methanogenesis is an ongoing issue in the area of BES; of which, MFCs are a subset. During methanogenesis carbon acts as a terminal electron acceptor leading to the production of methane (Chynoweth and Isaacson, 1987), as opposed to oxygen in aerobic respiration shown in equation 2.1 and 2.2.

Methanogenesis:

Acetoclastic

$$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2 \qquad \Delta G = (-36kJ/mol) \qquad \text{Equation 2.1}$$

Hydrogenotrophic

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad \Delta G = (-131kJ/mol) \qquad \text{Equation 2.2}$$

Methanogens live close to the thermodynamic limits for a limited range of organic substrates i.e. CO_2 and acetate but have evolved specific mechanisms for energy conservation which can ultimately compete against electrogenic activity (Gerardi, 2003), thus, methanogenesis acts to divert coulombic energy from electrogenesis in MFCs by adversely affecting the conversion efficiency from biodegradable material to electricity (Freguia et al., 2007, Kim et al., 2005a, He et al., 2005). There are many other parameters which adversely affect CE in MFCs, including the presence of dissolved oxygen (Liu et al., 2005, Logan et al., 2006), which favours aerobic respiration in all but anaerobic conditions. Furthermore, with other alternative electron acceptors such as nitrate-, sulphate- or through metal reducing bacteria unconnected to the electrode (He et al., 2005, Logan et al., 2006, Schroder, 2007a), a lower CE may result when such redox

components are directly and chemically oxidized and losses caused by methanogenic archeae are accepted as having considerable importance (Chae et al., 2010b, Ishii et al., 2008a). To increase the overall performance of MFCs it is advantageous to reduce substrate oxidation by metabolic processes other than those leading to electrogenesis; thus a preponderance of electrogens is advantageous. This bias toward electrogenesis can be assisted by the elimination or suppression methanogens in the anode compartment.

Methane is also known to be a green house gas and its emission is of great environmental concern (Wahlen, 1993, Minamikawa et al., 2006). Mechanisms capable of inhibiting methanogenesis are therefore of considerable interest, particularly in the study of methane production by ruminants. In BES or MFC applications, which may include scaled-up systems for treating industrial or municipal wastewaters; or small devices used as power sources of sensing elements, methane inhibition may have environmental and/or performance benefits. (Chae et al., 2010b) showed that injection of 2-bromoethanesulfonate (concentration of 0.1 – 0.27 mM) increased CE from 35% to 70% by suppressing methanogens and introduction of oxygen in anodic chamber also suppressed methanogens whilst only slightly suppressing exoelectrogens. 2-bromoethanesulfonate is an expensive chemical and would be prohibitively costly so, to use for large scale operations (Chae et al., 2010b). These inhibitors suppress methanogens but also affect electrogens on the anode electrode (Chiu and Lee, 2001, Liu and Logan, 2004). Other studies have shown that anodic conditions such as redox potential, pH, low pulses of oxygen, timing of batch feeding, temperature and applied voltage can be used to modulate methane production (Freguia et al., 2007, Wang et al., 2009a). Hence to enhance the overall performance and efficiency, there is a need to suppress methanogenic activity without affecting the electrogenic population in MFCs. Similar growth conditions for both methanogens and electrogens make suppression of methanogens by altering operating condition difficult. There remains a lack of published information relating to the ecological make-up of methanogenic archaea on the anode electrode and in planktonic suspension in MFC anodes. Most studies to date have focussed on the bacterial domain, (Chae et al., 2010b, Jung and Regan, 2007, Kim et al., 2007a). The suppression of methanogens affects the CE of MFCs although its importance

is application dependent, i.e., avoiding methanogenesis may be essential for MFC based sensors in order to increase accuracy but methanogenic activity may be less critical in scaled-up MFCs designed to remove COD from waste streams. From the perspective of its use in MFC VFA sensing, a high CE is necessary to discriminate the real substrate concentration.

3 Materials and Methods

This chapter describes the common electrochemical, analytical and molecular methods along with the experimental set-ups and their specific operation modes considered for individual study.

3.1 Reactor Design

Two MFC reactor types, cubic air cathode reactors and H-type reactors, were used as part of the experimentations in this study. The configuration of each reactor type is described below in detail:

3.1.1 Cubic MFCs Reactors (c-MFCs)

The c-MFC was constructed from Perspex material with external dimensions (50 x 50 mm) to have a single cylindrical chamber with internal diameter of 25 mm as shown in Figure 2.1 (A,B and C), and was manufactured at the University of South Wales. The thickness of the front and outer cover was 6 mm and the middle chamber 14.20 mm thick.

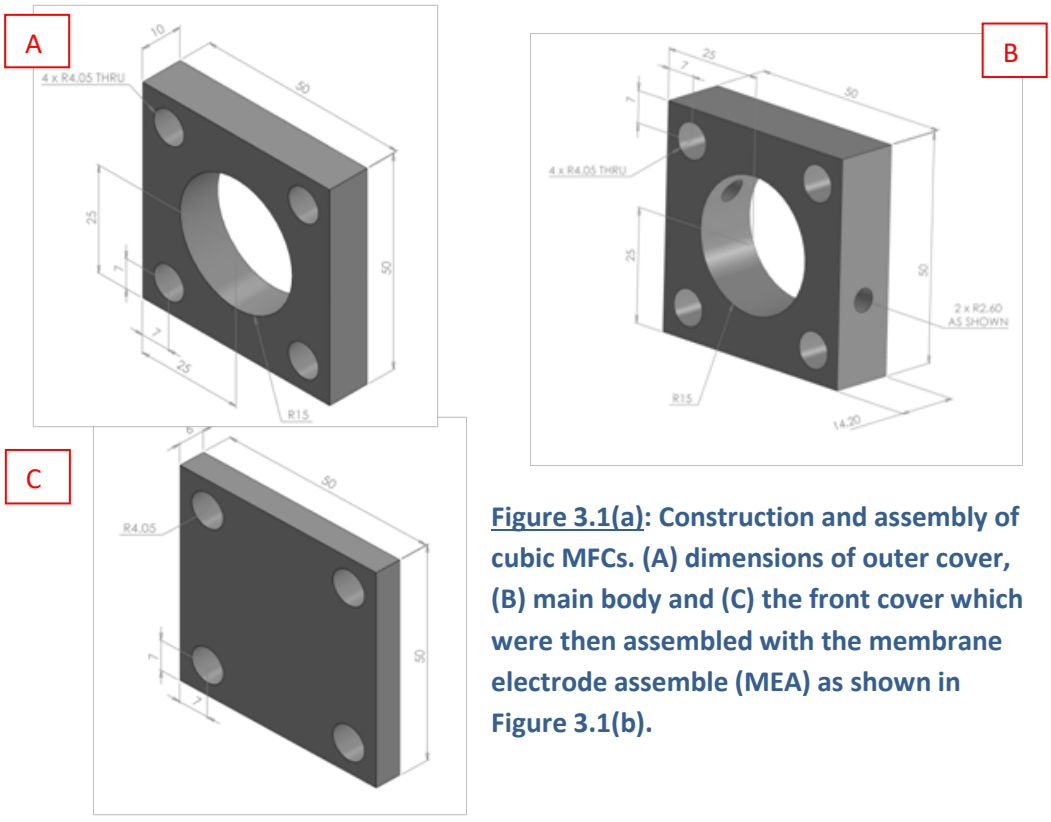

Figure 3.1(a): Construction and assembly of cubic MFCs. (A) dimensions of outer cover, (B) main body and (C) the front cover which were then assembled with the membrane electrode assemble (MEA) as shown in Figure 3.1(b).

The three individual parts were joined together using aluminium screws to hold the sandwiched assembly, including the membrane electrode assemble (MEA) of anode, cation exchange membrane (CMI-7000, Membrane International Inc. NJ, USA) and cathode. The system was sealed to make it water secure, giving a total liquid reactor volume in the anode chamber of 10 ml. The c-MFC assembly was used in Chapter 6 for off-line measurement of samples collected from scaled up MFC and ED cell as described in Section 6.1.2.3.

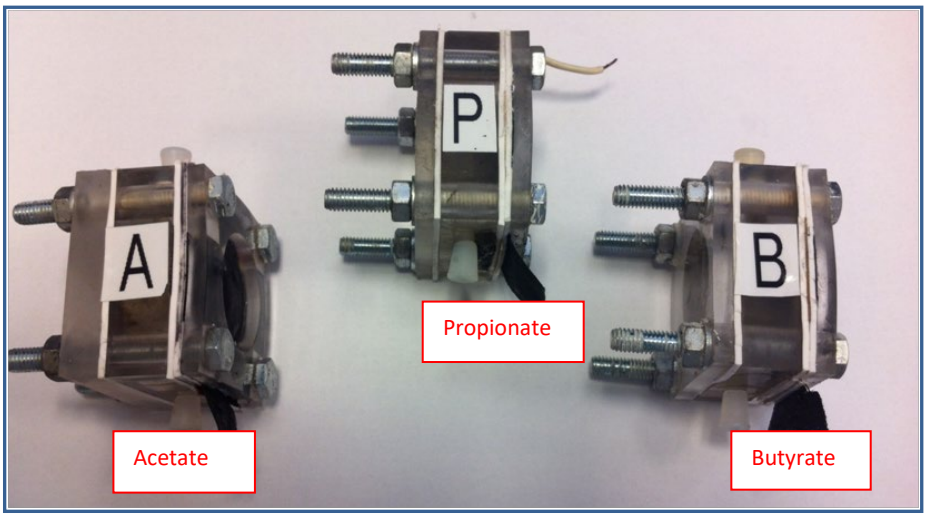

Figure 3.1(b): Complete assembly of c-MFC. The labels on reactors (A, P, B) represents the substrate i.e. acetate, propionate and butyrate.

3.1.2 H-type/Dual Chamber MFC Reactors (h-MFCs)

The H-type MFCs (h-MFCs) were constructed by combining two modified glass bottles (320ml) with clamp connections between them as shown in Figure 3.2. The cathode compartment, which was filled with 250 ml of phosphate buffer (50mM,pH7.0) prepared as described in Section 3.5, was continuously aerated using an air pump. The anode compartment was inoculated as described in Section 3.4. Both compartments were separated by a cation exchange membrane (CMI-7000, Membrane International Inc.) and anode and cathode electrodes were connected to an external resistance 1000Ω. The anode chamber was placed on a magnetic stirrer for vigorous mixing and to maintain uniform conditions in the anode chamber further to ensure that there was no affect resulting from the proximity of the anode to the cathode/ion exchange membrane.

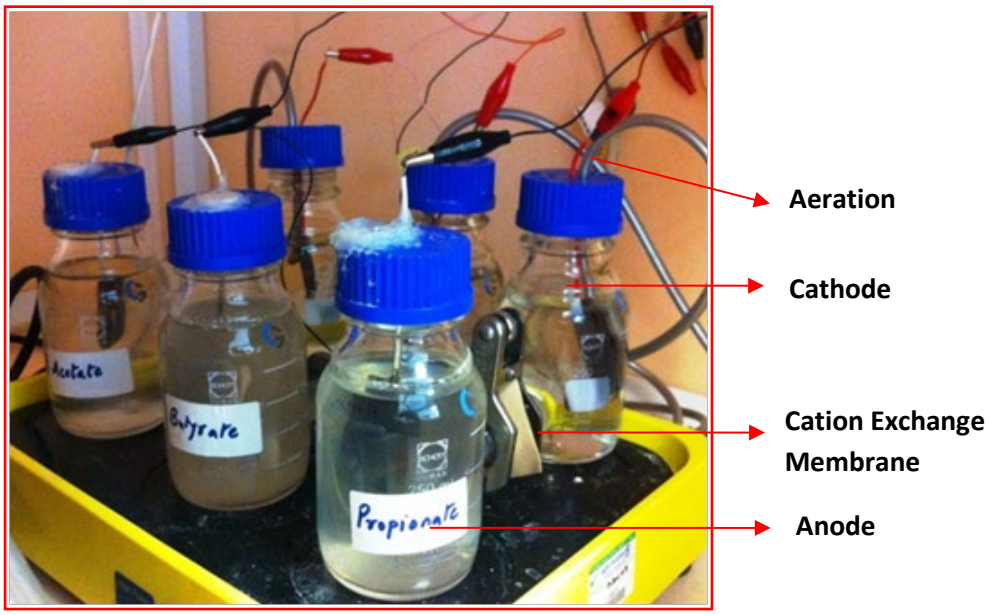

Figure 3.2: Dual chamber 'H' type MFC reactors with 250 ml of anode volume

Three VFAs, sodium acetate, sodium butyrate and sodium propionate (Sigma-Aldrich, MO), were used as the sole carbon and energy source for bacteria to enrich both reactor configurations.

3.2 Electrode Materials:

3.2.1 c-MFC Reactor:

For the anode material, a plain carbon cloth (BASF Fuel Cell Inc., NJ, USA) was used, with an exposed surface area of 4.9 cm^2. The cathode was prepared on plain carbon cloth (CCP40, Fuel Cell Earth LLC, MA, USA) as described by Cheng et al., (2006). The carbon base layer i.e. a paste made up of 2.5 mg/cm^2 of carbon black, (Vulcan XC-72) and 10 µl/mg of carbon black, of Polytetrafluoroethylene, PTFE (30% weight dispersion in water, SIGMA-ALDRICH®, UK) was applied on one side of carbon cloth. The carbon cloth with base layer was then left to air dry for 2 hours and then heated at 370 °C in a pre-heated furnace for 30 minutes. The cloth was allowed to cool and then 60% PTFE solution was applied evenly on the carbon black layer with the help of brush and left to dry for 15 minutes. Again the carbon cloth was heated to 370 °C for 15 minutes. A platinum catalyst with 0.5 mg/cm^2 loading was applied to the solution facing side of the carbon cloth. This was allowed to dry for at least 24 hours before the use.

3.2.2 h-MFC Reactor:

The anode electrode was made of carbon paper (TGPH-120Toray carbon paper, E-tek, 2.5 cm × 4.5 cm). Cathode electrodes were made of plain porous carbon paper (TGPH-120, Toray carbon paper, E-TEC, 2.5 cm × 4.5 cm, projected area of 22.5 cm^{-2}) with an incorporated Pt catalyst (0.35 mg cm^{-2}; 10% Pt; E-Tek, NJ. In Chapter 6 the electrodes were fabricated using different natural and conductive polymers. These fabricated electrodes were then implemented in the anode chamber of the h-MFCs for further analysis. The process of anode fabrication with both natural polymer and polypyrrole was done as described below:

3.2.2.1 Natural Polymers:

Microbial electrodes using immobilized whole cells have been developed for the determination of BOD (Karube et al., 1977). In these cases, bacteria were active for a long time in a natural polymeric gel. The natural polymers considered for this study were agarose, polyacrylamide, polyvinyl alcohol, calcium alginate as these polymers are not toxic towards living microorganisms. A mediator, neutral red (NR), was also added to the polymers to increase the electron transfer rate from the substrate to the electrode. Three different methods were investigated to increase the stability of the sensor array.

3.2.2.1.1 Agarose and Mediator (AG+NR)

A solution of 1% agarose was prepared by adding 25mg of agarose powder to 25 ml of the phosphate buffer solution and the mixture was then brought to the boil in a microwave. Once the polymer was completely dissolved it was allowed to cool down and 5 mg of neural red mediator was added to it before gel formation. Before the gel started solidifying, the acclimated electrode with bacterial biofilm attached, was submerged in the solution and coated with a gel layer and dried for 15-20 minutes. The electrode was then replaced in the anode chamber after proper solidification of the gel layer over the electrode and the MFC was connected to the external circuit. The voltage was subsequently monitored for 5 days.

3.2.2.1.2 Polyacrylamide and Mediator (PA+NR)

In the procedure to prepare Polyacrylamide gel with integral mediator, 25 ml of phosphate buffer containing 5 mg neutral red mediator was prepared. To 25 ml of

neutral red mediator solution, 20 ml of 25% acrylamide monomer solution and 52 ml DI was added. Polymerization was initiated by the addition of 2 ml of 5% ammonium persulfate (APS) and 1.0 ml of 5% N, N, N, N-tetramethylenediamine (TEMED) and allowed to stirred. To this solution 5 mg of neutral red mediator was added. The electrodes with acclimated bacterial communities were then coated with polyacrylamide gel and allowed to dry in an anaerobic glove box for 10 minutes. Electrodes were then reinserted in the anode chamber and connected to the external circuit in readiness for experimentation.

3.2.2.1.3 Polyvinyl alcohol (PVA) + Calcium alginate + Carbon powder (PVA+CA+C)

Polyvinyl alcohol is considered a good immobilization matrix as it is non-toxic to the bacterial cells (Zhang et al., 2007, Bai et al., 2010). As PVA is water soluble and the gel formed did not remain stable in water, calcium alginate and carbon powder were added to the PVA in the ratio of 7:2:1 (PVA: calcium alginate: carbon). The gel mixture was allowed to cool and electrodes were coated by dipping in this gel mixture before solidification occurred. The electrodes were left as previously described, in order to encapsulate the bacterial community. Subsequently, the electrodes were reinserted in the anode chamber and the MFC was connected to an external circuit.

To achieve immobilization of enriched biofilm with natural polymers, the anode electrodes were temporarily removed from the reactors, anaerobically under a nitrogen environment in a glove box. Once the natural polymer was coated onto the electrode it was placed back in the anode chamber. The effectiveness of the bacteria immobilized on the anode by the natural polymer materials was examined by comparing the voltages before and after immobilization. The effects of immobilization on the process stability and repeatability were studied by periodically measuring CV responses and results obtained are presented in Section 5.2.2.

3.2.2.2 *Polypyrrole Modified Electrodes* [1]

[1] Polypyrrole modified electrodes were fabricated in collaboration with UEA, Norwich

The conducting polymers, often termed 'organic metals', are generally conjugated materials such as polypyrrole, polyacetylene, polythiophene and polyaniline. Among the conducting polymers, polypyrrole remains one of the most extensively studied owing to its great stability in its conducting state and the facile functionalisation of the monomer units. In this study the electropolymerization of pyrrole monomers was carried out using (a Hi-Tek type Dt2101/ waveform generator type PPR1 and Autolab PGSTAT 30N) potentiostat-galvanostat in a single compartment three-electrode electrochemical cell containing 0.1 M $[Bu_4N][BF_4]$-MeCN supporting electrolyte and 10 mM of monomer. The working electrode was a carbon paper electrode (projected area 4.5 X 2.5 cm^2) and a silver wire was employed as a pseudo-reference electrode (potentials are quoted versus SCE).

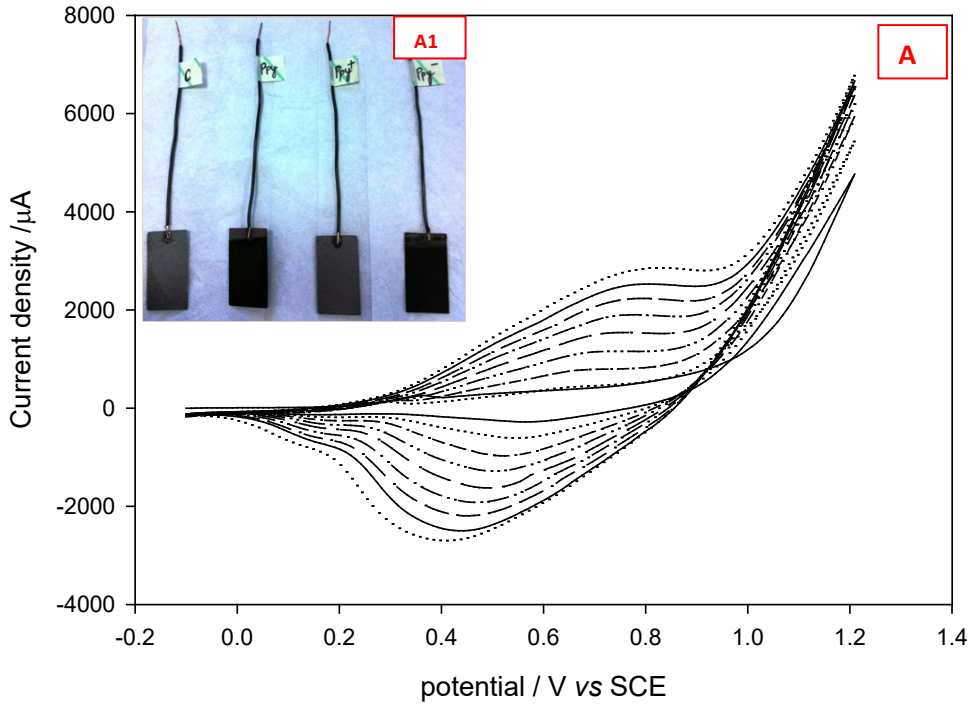

Figure 3.3: Growth of the propanoic acid polypyrrole derivative (PPy^-). Produced by repetitive scanning at the surface of a carbon paper electrode (4.5X2.5 cm2), scan rate 100 mV s^{-1} in 0.1 M $[Bu_4N][BF_4]$-MeCN with 10 mM of monomer.

The counter electrode was constructed of stainless steel of almost the same size as that of working electrode. The potential of the working electrode was positioned at the foot of the voltammetric wave corresponding to onset of oxidation of the pyrrole monomer

and repetitively scanned between this potential and that for reduction of the (growing) electropolymer as is shown by Figure 3.3.

The three carbon paper electrodes prepared by polymerisation are shown in Figure 3.3 (A1). Three different monomer units were electropolymerised on the carbon paper electrodes in this way, the structures of which are shown in Figure 3.4 pyrrole (Ppy), pyrrole propanoic acid (Ppy^-) (Pickett and Ryder, 1994) and pyrrole alkylammonium (Ppy^+) (Moutet and Pickett, 1989). After deposition of the polymers the electrodes were washed repetitively in MeCN and water before evaluation as MFC sensor electrodes in the appropriate media.

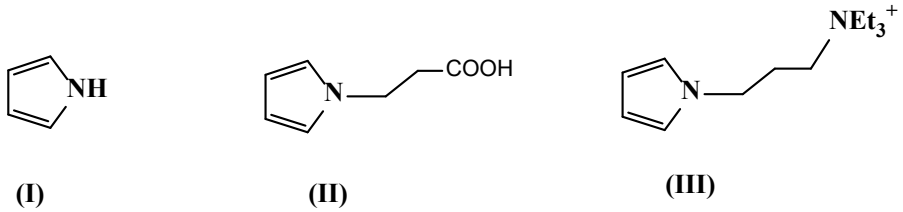

Figure 3.4: Chemical structure of the functional polypyrrole used in fabricated anodes

3.3 Reactor Operations

To further an understanding of the experiments carried out as part of this study, the experimental set-ups and reactor operations during individual study are summarized below:

3.3.1 MFC Responses to VFAs

Three h-MFCs were constructed as explained in Section 3.1.2 and were inoculated with the anaerobic sludge using acetate, propionate and butyrate as sole electron donor for acclimation. Both electrodes were connected to static external load (1000 Ω). MFCs were operated in fed batch mode by conducting a batch mode test until the voltage decreased to a low value (<10 mV) and then replacing the feed solution in the anode chamber with the respective VFA as substrate. After enrichment and establishment of stable voltage generation, the individual VFA enriched reactors were then tested for their response to respective and cross fed VFAs. For CE measurements the reactors were fed with standard concentrations of respective VFA and were then allowed to deplete in the anode chamber. After complete depletion of the substrate the CE was calculated as described in Section 3.9.1. The contents of the anode chambers were then replaced

with fresh media containing higher concentrations of the substrate, to check the correlation between CE and substrate concentration when ranging 2-40 mM of VFA concentrations. For CV measurements, the experimental set-up is shown in Section 4.1.3. Further to this the pure sucrose standard concentrations range from 20-200 mg/l were also tested in each reactor to check the response. The results obtained from this study are presented in detail in Chapter 4.

3.3.2 Stability, Sensitivity and Recovery of the Sensor Response:

Two sets of experiments were conducted to study the effects of the immobilization of microbial biocatalyst with respect to stability and recovery response of the sensors; using either natural polymer (PA+NR, AG+NR, PVA+CA+C) or polypyrrole modified electrodes (Ppy, Ppy^{+}, Ppy^{-}).

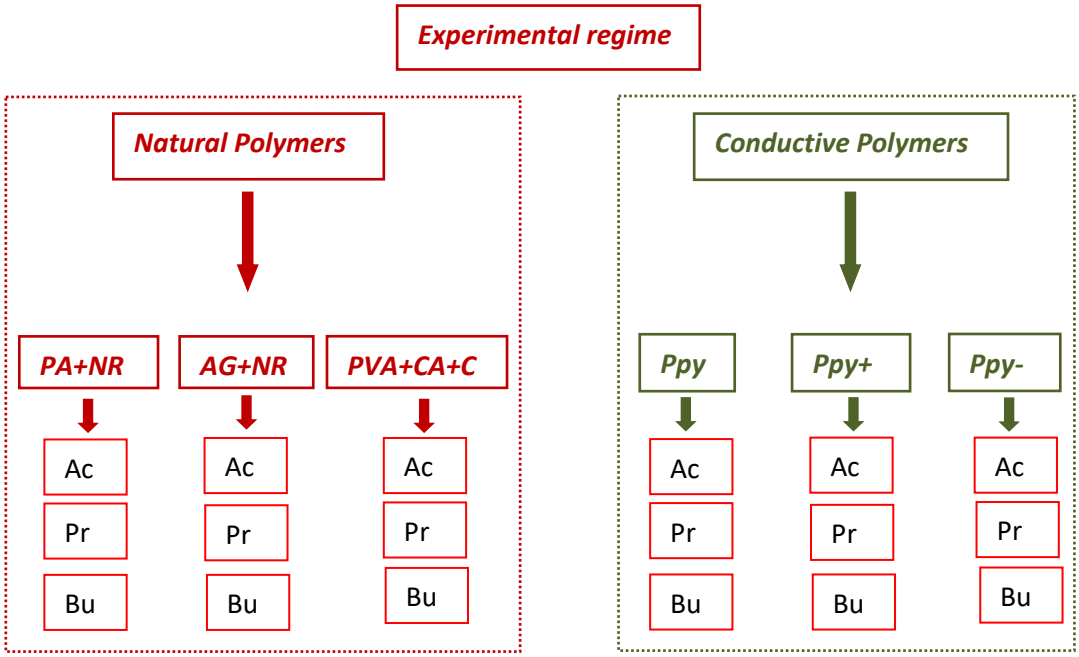

Figure 3.5: Experimental regime with total number of MFCs inoculated and maintained on individual VFA (Ac=acetate, Pr=propionate and Bu=butyrate)

Three sets of MFCs with a further three reactors in each, as shown in Figure 3.5, were inoculated with 20 ml anaerobic digester sludge and 230 ml phosphate buffer, with 20 mM concentration only one of the VFAs species (acetate (Ac), butyrate (Bu), and propionate (Pr) . Immobilization of the biofilm was carried out by using three natural polymers. Another three sets of reactors were inoculated after fabrication of the carbon

paper electrode with functionalised pyrrole using electro-polymerisation. Each modified electrode was then installed and tested for individual VFA concentrations. One control set of MFCs for each VFA with carbon paper electrode but without any modification, operated on pure VFAs, was also inoculated and acclimated to develop an electrogenic biofilm on its surface.

Figure 3.5 shows the simplified complete experimental set-up for the electrode modification experiments, with the two different set of experiments, the total number of MFCs inoculated and sequentially studied during this investigation. All modified electrodes were analysed for their response to VFAs by using CV techniques and results obtained are discussed in Chapter 5.

3.3.3 Improved Range and Offline Measurement of Samples

Three h-MFCs and three 10 ml c-MFC reactors were inoculated with the same inoculums to keep the microbial community consistent in both reactor configurations. 20 mM of individual VFA was separately added to start the enrichment procedure. The electrodes were connected via external loads of 1000 and 500 Ω to h-MFCs and c-MFCs respectively to measure current flowing through the system. During enrichment period MFCs were operated in fed batch mode by conducting a batch mode test until the voltage decreased to a low value (<10 mV) and then replacing the feed solution in the anode chamber. The anode compartment was kept anoxic and all the media replacements were done in an anaerobic chamber to avoid oxygen intrusion particularly for the more sensitive propionate and butyrate reactors. Once the enrichment was achieved, h-MFCs, which were running for over 6 months were used for preliminary examination of the hypothesis which sought to check the effect of poising oxidation potentials obtained from the CV for individual VFA species. The experimental set-up for h-MFC is presented in Chapter 4. After preliminary examination the volume of the reactor was decreased to 10 ml as described in Section 3.1.1, Figure 3.1 (a&b). The experimental set-up for c-MFC has been described in Figure 6.1. The cation exchange membrane was sandwiched between anode and cathode, where the anode was facing solution and the cathode was facing exterior of the chamber. The working, counter and reference electrodes of the potentiostat were connected to the anode, cathode and the silver-silver chloride reference electrode of the Individual VFA saturated MFC, respectively. The reference

electrode Ag/AgCl was kept as close as possible to the anode electrode. The electrode potential was ramped within the potential range of -750mv to +750mV at a scan rate of 50 mV s^{-1} to measure CV for each VFA sensor. The anaerobic environment and temperature for microbial consortia maintained during measurement by using anaerobic chamber. To assure quality of the results, all experimental data were validated for reproducibility by running for at least three repetitive cycles in each experimental test arrangement. In the case of poising potential on the anode electrode, the same experimental set-up was used with both configurations of MFC reactor. The current response obtained from pure standard VFA concentrations and samples from scaled-up reactors during the scans of biofilm at poised potential were recorded and plotted against concentrations, which are described in detail in Chapter 6.

3.3.4 Methanogenesis Suppression in MFCs

3.3.4.1 Substrate Utilization:

Three h-MFC reactors were individually enriched with acetate, propionate and butyrate. After enrichment the reactors were fed on 20 mM of the respective VFA and were then left for 5 days in open circuit (OC) operation. During this, liquid and gas samples were taken each day and were analysed to see the rate of utilisation of the substrate. The reactors were operated at 30°C temperature throughout the experiment. After 5 days of OC operation the anode chamber was refilled with fresh media and 20mM of respective VFA. The reactors were then left for 5 days in closed circuit (CC) operation and liquid and gas sampling was done as described in Section 3.8.2 and 3.11.1.

3.3.4.2 Open and Closed Circuit Operation

Six h-MFCs in three replicate sets of reactors were enriched individually with acetate, propionate and butyrate as the only source of carbon. After enrichment, media was replaced with 10 mM of substrate in the individual set of reactors. One set was continuously operated in CC mode for more than 1 year after initial open and closed circuit (OC/CC) operations. Another set was first operated in an OC mode with 10mM substrate for 10 days followed by 10 days CC mode (10 days OC/CC) with same substrate concentration. The same set of MFC was subsequently operated for 1 month in OC operation with 20 mM of substrate followed by 1 month in CC operation (1month

OC/CC) with 20 mM substrate concentration again. During OC operation gas and liquid sampling was done to measure gas composition in the headspace and biomass in the liquid. Media was replaced with fresh synthetic media with 20mM substrate before starting CC operation. Similarly, measurements were done in CC operation as well. Once 10 days OC/CC operation was completed, reactors were fed with 20 mM substrate again, for the growth of biofilm. After enrichment, reactors were first left for 1 month in OC operation with 20 mM of substrate and then media was replaced with same concentration of substrate to start circuit was closed. At the start as well as end of each open and closed circuit operation electrode biofilm samples and liquid samples were taken to study the effect of current flow on the biofilm composition in the anode chambers. The h-MFCs were continuously mixed using a magnetic stirrer in a temperature controlled chamber (30 °C).

3.3.4.3 Starvation of MFCs[2]

To study the effect of starvation on methane production in h-MFC reactors, they were first enriched on acetate under Maximum Power Point Tracking (MPPT) control operation as described by Boghani et al., (2013). Once a stable power generation was achieved, the initial inoculum charge was discarded completely and fresh media was added in the anode chamber. The enrichment to attain maximum power took 64 days prior to the CE measurements. Different concentrations (0.5 mM, 1mM and 2 mM) were introduced in the anode chamber individually with fresh media and were allowed to deplete completely to measure the CE for each instance. After that the MFC was provided no further substrate (starvation) once the 2mM acetate was depleted completely. The MFC was starved for 12 days and gas samples were taken before and after the starvation period. The MFC operation was then resumed by providing 1 mM and 5 mM sequentially and again and allowing substrate to deplete for CE measurement.

3.4 Media Composition

The 50 mM phosphate buffer was made up in 20 L batches and diluted as necessary. For 1 litre of buffer 12.5 ml of each vitamin and mineral solution was added. The detailed composition of media is given in Table 3.1.

[2] Starvation Experiment was conducted by Hitesh Boghani, PhD student, as one of the strategies considered in this study,

Table 3.1: Composition of 50 mM phosphate buffer with vitamins and minerals

Contents	Concentration (g L^{-1})
Buffer	
NH_4Cl	0.31
$NaH_2PO_4 \cdot H_2O$	2.69
Na_2HPO_4	4.33
KCl	0.13
Vitamins	
Biotin	0.002
Folic acid	0.002
Pyridoxine HCl	0.010
Riboflavin	0.005
Thiamin	0.005
Nicotinic acid	0.005
Pantothenic acid	0.005
B-12	0.0001
4-aminobenzoic acid	0.005
Lipoic acid	0.005
Minerals	
Nitrilotriacetic acid	1.5
$MgSO_4 \cdot 7H_2O$	6.2
$MnSO_4 \cdot H_2O$	0.5
$NaCl$	1.0
$FeSO_4 \cdot 7H_2O$	0.1
$CaCl_2 \cdot 2H_2O$	0.1
$CoCl_2 \cdot 6H_2O$	0.1
$ZnCl_2$	0.13
$CuSO_4 \cdot 5H_2O$	0.01
$AlCl_3 \cdot 6H_2O$	0.0052
H_3BO_3	0.01
$Na_2MoO_4 \cdot 2H_2O$	0.0261
$NiCl_2 \cdot 6H_2O$	0.024
$Na_2WO_4 \cdot 2H_2O$	0.025

The concentration of the individual substrate (acetate, propionate and butyrate) varied according to the requirement of each experiment.

3.5 Inoculum

The inoculum used was a mixed and undefined microbial population of anaerobic digester sludge. The anaerobic sludge was collected (Cog Moors wastewater treatment plant, Cardiff, Wales). During inoculation of the h-MFC reactors 20 ml sludge was used in 230 ml nutrient buffer media (Section 3.5) whereas 0.8 ml of sludge was used in 9.2 ml of media to keep the proportion same during enrichment. The sludge was stored in a sealed bottle at 4°C. The inoculation and cell transfers were conducted in an anaerobic glove box (Coy Scientific Products, MI).

3.6 Temperature Control

The temperature of all MFCs were maintained at 30°C in a temperature controlled chamber during enrichment and throughout all experiments, as the enrichments at room temperature in the cases of propionate and butyrate failed to establish an electrogenic biofilm.

3.7 Data Acquisition

A data acquisition unit was used with a desktop computer to capture data from MFC reactors. Voltages across the MFCs were recorded at 10 minute intervals using LabVIEW™ software and a NI 16-Bit, isolated M Series MIO DAQ, (National Instrument Corporation Ltd. Berkshire UK). A custom interface was written using LabVIEW™ in order to capture, collect and display data from this device. The software allowed the data to be stored in a separate file for subsequent analysis.

3.8 Chemical analysis

3.8.1 pH

The pH of fed-batch experiments and real samples collected from different sources as listed in Table 6.3 was measured using a Mettler-Toledo, Gmbh 8603 meter (Mettler-Toledo, UK). The pH meter was calibrated using fresh pH 4.0 and 7.0 buffers (Hanna). The probe was simply placed into the sample and the pH value was given after a few seconds.

3.8.2 Gas Sampling and Analysis

In Chapter 7 during substrate utilization, OC/CC and starvation experiments the gas samples were collected from MFC by using 1 ml gas tight syringe (Varian Ltd, Walton-upon-Thames, UK) and same syringe was then was used to inject the gas sample into the GC by activating the start option within the data handling package. Sample volume was not important since the GC was fitted with a sample loop to ensure consistent sample volume. A vacuum pump drew the gas sample through a loop (10 µl) and then the injector injected the gas sample from the sample loop into the carrier gas stream. The level of gaseous methane composition was checked using a Varian CP-4900 Gas Chromatograph (Varian, Walton on Thames, UK). The GC was equipped with two columns, a “molecular sieve 5A plot” (10m x 0.32mm) (Varian) running at 150°C and 30

psi for the detection of hydrogen, nitrogen and methane, and a "Porapack Q column" (10m x 0.15) (Varian) running at 60°C and 20 psi for the detection of carbon dioxide. The carrier gas in both columns was argon. This instrument was also able to analyze the methane, nitrogen, carbon dioxide, and hydrogen content of the gas samples simultaneously and with no need for multiple samples to be taken from the instrument. The instrument was calibrated every three months using gas mixtures of known proportions (Air Products, UK). A QC sample was run before each day of use and consisted of a gas mixture of known proportions (Scientific and Technical Gases Ltd., Newcastle-under-Lyme).

3.8.3 Volatile Fatty Acids

VFAs were measured according to the method of (Cruwys et al., 2002) using a Perkin Elmer headspace gas chromatograph (Model number HS40XL, Perkin Elmer, Beaconsfield, UK) in conjunction with a flame ionisation detector (FID) and a "Nukol" free fatty acid phase (FFAP) column (30 m x 0.32 mm) (Supelco Ltd, Poole, UK) running at 190°C and 14 psi. The carrier gas was nitrogen. The GC was connected to a headspace auto-sampling system (Perkin Elmer, Beaconsfield, UK). The machine was calibrated using standards of acetic, propionic, isobutyric, n-butyric, isovaleric and n-valeric acids with concentrations in the range of 0 mg/l to 1000 mg/l. In their paper (Cruwys et al., 2002) stated that the detection limit for these acids was below 4mg/l and analysis of replicates samples yielded a coefficient of variation between 0.039 and 0.065. Preparation of samples followed the method in (Cruwys et al., 2002). A 22.3 ml glass vial was used with a PTBE septum and a proprietary sealing system (Perkin-Elmer, Beaconsfield, UK). 1 ml of sample was pipetted into the vial together with 1ml of deionised water, 1 ml of $NaHSO_4$ and 0.1 ml of 2-ethylbutyric acid (1800 mg/l) as an internal standard.

3.9 Calculations

3.9.1 Coulombic Efficiency

The Coulombic efficiency (CE) in MFC is defined as the ratio of total coulombs actually transferred to the anode from the substrate to maximum possible coulombs, if all substrate removal produced current. The number of electrons produced varies

according to the substrates used. Where acetate generates 8 electrons per mole, propionate and butyrate generates 12 and 20 electrons respectively. In this study we have used acetate, propionate and butyrate as sole source of carbon and the CE for individual substrate was calculated as previously described by (Logan, 2008).

$$CE\ (\%) = (C_p/C_{Ti})\ x100, \qquad \textbf{Equation 3.1}$$

Where *Cp* is the total coulombs calculated by integrating the current over time. C_{Ti} is the theoretical accumulated charge (coulombs) that can be produced from each substrate respectively, calculated as,

$$C_{Ti}=F.b_i.S_i/M_i: \qquad \textbf{Equation 3.2}$$

Where F is Faraday's constant (96485 C/mol electrons), b_i is the number of moles of electrons produced per mole of substrate (b_a=8, b_p=12, b_b=20, for acetate, butyrate and propionate respectively), S_i the substrate concentration, and Mi the molecular weight of the substrate (M_a=82, M_p=96, and M_b=110).

3.9.2 Power, Current, Voltage

Current (I), power (*P=IV*) was calculated as described in the previous report (Min et al., 2005). The current (A) was calculated according to I=V/R, where V is the measured voltage and R is the resistance.

3.9.3 Percentage Error

The percentages error was calculated in Chapter 6 for comparing the MFC measured responses to the GC responses from real samples as follows:

$$Error= (C_{approx})-(C_{exact}) \qquad \textbf{Equation 3.3}$$

$$Error\ \%= C_{approx}\text{-}C_{exact}/C_{exact}\ *100 \qquad \textbf{Equation 3.4}$$

Where, C_{approx} is the approximate concentration from MFC sensor array and C_{exact} is the exact concentration from GC.

3.10 Electrochemical Analysis

The voltage and current across the load were monitored using a virtual instrument based data logging system (National Instruments, LabVIEW™) connected to an analogue input on the I/O card respectively. The power output measurements were determined

by potentiodynamic polarization (scan rate 10 mV s^{-1}) using a Solartron Electrochemical Interface (Farnborough, UK) controlled by dedicated software (CorrWare 2™, Scribner Associate Inc., NC) with an Ag/AgCl reference electrode. Polarisation curves were measured after 2 hours at OCV (open circuit potential). Each cell potential was measured after an interval of 10 minutes to allow stabilization of the current. The reference electrode consists of a silver wire, coated with silver chloride, which is immersed in a saturated potassium (or sodium) chloride solution. A porous frit is used for the junction between the reference electrode solution and the sample solution. The redox process for this electrode is:

$$AgCl_{(aq)} + e^- \rightarrow Ag_{(s)} + Cl^-_{(aq)} \qquad \textit{Potential vs. SHE, } E_0 = 0.22 \text{ V}$$

Equation 3.5

A potentiostatic method was used (CorrWare 2™, Scribner Associate Inc., NC), to apply a constant potential during poised potential study in Chapter 6 and then monitor current as a function of time. The current response from samples was measured for 10 minutes to achieve stable value. During experiments the voltage across a 1000 Ω resistor in the circuit in the MFC was sometimes also monitored using a multimeter (Keithley Instruments. Cleveland, OH, USA).

3.10.1 Cyclic Voltammetry

A conventional three-electrode system was employed with the anode as the working electrode, the cathode (2.5 cm^2, 0.35 mg/cm^2; 10% Pt; E-Tek, NJ) as the counter electrode, and an Ag/AgCl reference electrode. Two different set ups were used according to the configuration of the MFC reactor i.e. c-MFC or h-MFC. Both set ups are described individually in Chapter 4 and Chapter 6. The working electrode was programmed to linearly ramp with through time in an oxidizing and reducing cycle. The cycle was conducted in triplicates using a voltage range of -0.7 to +0.7 V; scan rate was differed between 10-100 mVs^{-1}. The current at the working electrode was then plotted against the applied voltage to produce the cyclic voltammogram. From each voltammogram the oxidation peak current was further plotted against the concentration of VFA and is presented in Chapter 4, 5 and 6 in detail.

3.11 Molecular Analysis

3.11.1 Sampling, DNA Extraction and Measurement

1cm^2 sections of anode electrode was sampled in an anaerobic chamber (Coy Laboratory Products, Michigan, USA); by aseptically excising with scissors on a pre-sterilized glass plate and to check the planktonic community 1 ml of liquid samples from the anode compartment were also collected for DNA isolation during methane inhibition study (Chapter 7). All test samples were immediately transferred for storage at -80°C prior to processing. The genomic double stranded DNA (dsDNA) was isolated from 1 cm^2 samples of carbon paper electrode and 1 ml from suspension. Samples were mechanically disrupted by bead beating and a phenol/chloroform/iso-amyl-alcohol extraction was carried out as the method described by (Oude Elferink et al., 1997). DNA concentrations were measured using a NanoDrop™ 1000 Spectrophotometer (Thermo Scientific) and these values were used as a measure of biomass/biofilm growth. DNA concentration has been previously shown to be an indicator of bacterial cell counts present in potable waters (McCoy and Olson, 1985) and correlations have also been established between DNA concentrations and levels of organic dry matter in reactor biofilms (Flemming et al., 2000).

3.11.2 PCR Amplification and DGGE Analyses

Amplification of DNA (16S rDNA) and PCR analysis were performed as previously described (Roest et al., 2005) using Archaeal primers 0968F-GC and 1401R, i.e. forward primer (5'-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG G AAC GCG AAG AAC CTT AC -3') and reverse primer (5'-CGG TGT GTA CAA GAC CC -3'). and universal bacterial primers 0109F-T and 0515R-GC i.e. forward primer (5'-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG G ATC GTA TTA CCG CGG CTG CTG GCA C-3') and reverse primer (5'-ACT GCT CAG TAA CAC GT-3'). For DGGE analysis a D-Code system (Bio-Rad, Hercules, CA, USA) was used with a 20% to 80% denaturing gradient (100% denaturant consisting of 7 M urea and 40% (v/v) formamide). 8% (w/v) polyacrylamide gels (acrylamide:bisacrylamide, 37.5:1, gel stock solution; Sigma, USA) in 1 × TAE (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 8.3), containing 0.11% (wt/v) APS (ammonium persulfate; Sigma) and 0.1% (v/v) TEMED (N',N,N,N'-tetra-methylethylenediamine; Sigma). Gels were run at 60°C at 200 V for 5 minutes, then 85 V for 16 hours and were

silver stained prior to gel imaging with a Fluor-S MultiImager (Bio-Rad, Hercules, CA, USA).

3.11.3 Pyrosequencing

Sequencing of selected anodic samples was performed (Macrogen Inc, Korea) utilising a GS FLX sequencer and titanium Series chemistry (454 Life Sciences, Branford, CT, USA). Archaeal PCR primers ARC346-F (5-GGGGYGCAGCAGGCG-3') and ARC915-R (5-GTGCTCCCCCGCCAATTCCT-3') were used and also adapters, keys and multiplex identifiers were included. Sequencing was performed using an emPCR Lib-A kit (Roche Applied Science). The BLAST algorithm was used to analyse the sequences and in-house software was used for taxonomic assignment.

4 Microbial Fuel Cell Responses to Volatile Fatty Acids

This chapter outlines the development of a sensing strategy, following the first objective of the study i.e. determine the correlation between MFC based sensor signals and the VFA concentrations supplied as substrate or measured. The response time is one of the important factors for any sensor and considering that two electrochemical methods were investigated to quantify the correlation and are presented as follows:

1. By considering the Coulombic Efficiency (CE)
2. By considering the Cyclic Volatammetry (CV)

Each of these approaches is detailed below.

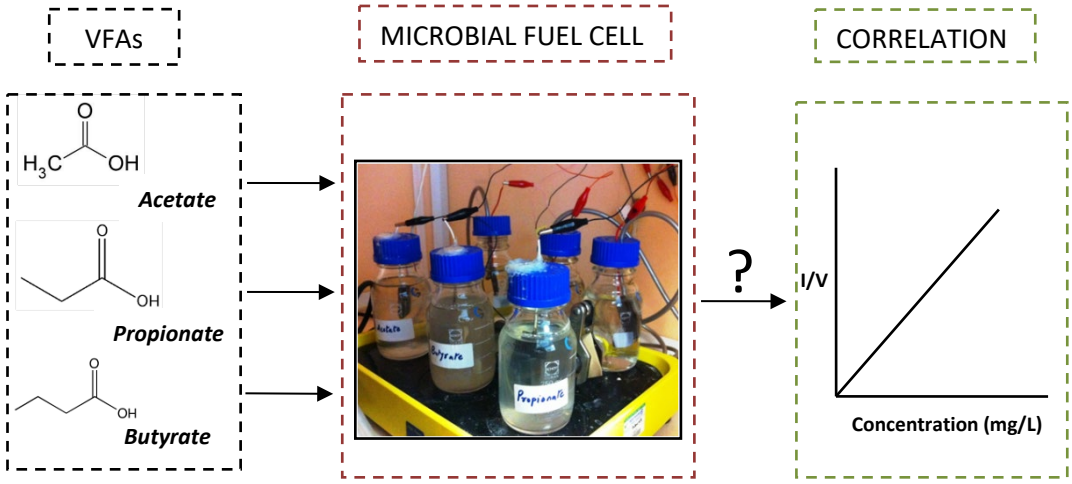

Figure 4.1: Schematic representation of the motivation behind the study

4.1 Results

The following results are presented to show the correlation between the H-Type MFC responses and VFA concentrations.

4.1.1 Enrichment of h-MFC with Acetate, Propionate and Butyrate

Figure 4.2 shows the typical voltage generation from three MFCs during the enrichment process using 40 mM acetate, propionate and butyrate as the fuel. MFCs were inoculated with activated sludge and fed continuously with artificial wastewater with 1000 Ω load.

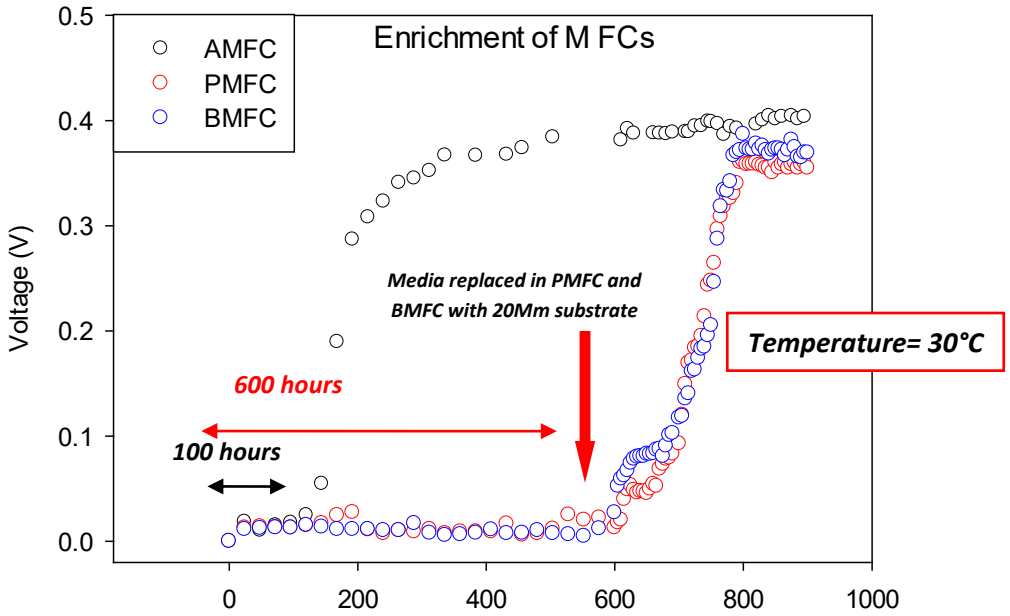

Figure 4.2: Voltage development from three MFCs during the enrichment process

After inoculation the start up took different amounts of time for individually supplied VFA species fed to the MFC reactors. The acetate enriched reactor (AMFC) showed a lag phase of approximately 100 hours followed by a rapid increase in cell voltage to 320 mV over the next 50 hours, which has also been observed by various other authors in different studies (Wang et al., 2009b, Kim et al., 2005b). In contrast, the MFCs fed with propionate (PMFC) and butyrate (BMFC) did not result in an appreciable voltage increase until 500 hours had elapsed and even after the whole media was replaced at 550 hours (<10 mV). At this point the pH of BMFC and PMFC were 6.5 and 6.7 as compared to AMFC (pH 6.9). The BMFC and PMFC showed rapid increases in voltage at 570 hours when the media was replaced with 20 mM of substrate instead of the previously supplied 40 mM. It can be seen in Figure 4.3 that while degradation of acetate is thermodynamically favourable as it is the end product of several metabolic pathways for higher order carbon sources. The degradation of propionate and butyrate in contrast is a multi-step process which requires the involvement of methanogens for complete electron transfer to the electrode. Propionate and butyrate degrade to lower molecular weight carbon compound and then transfer of the resulting electrons to the anode takes place. This process is thermodynamically a less advantageous process as compared to

acetate degradation to electrons. This shows that the syntropic bacteria need a longer period time in AMFC and PMFC to become stabilised and immobilised onto the electrode to generate voltage.

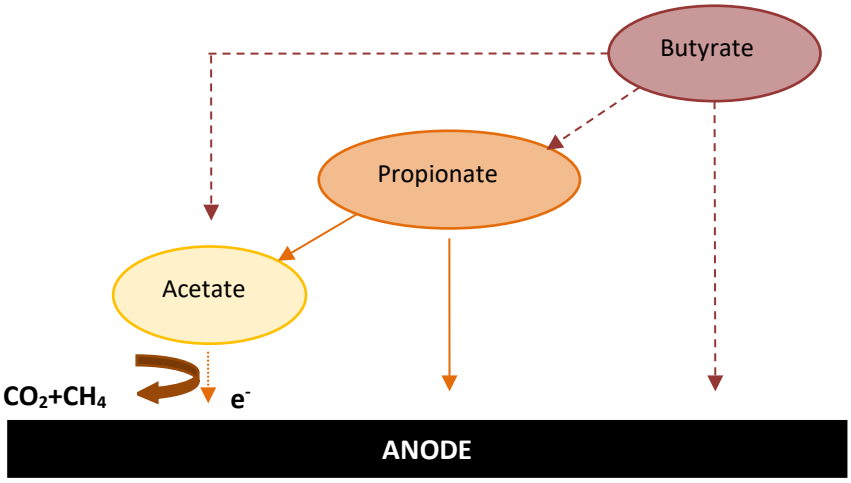

Figure 4.3: Biochemical Pathway of degradation of different VFAs in MFC

There are several factors such as temperature, pH, growth medium, oxygen diffusion to the anaerobic environment, and the availability of an electron sink which affect the start-up characteristics of an MFC fed on different substrates. Biofilm formation on the anode and its ability to liberate electrons from the oxidation of the organic matter influence the development of microbial community involved in the electrochemical reactions (Allen and Bennetto, 1993, Schroder, 2007b).

It is important to note that enrichment/initiation need only occur once, before the sensor is deployed. Once the reactors were fully enriched after two cycles of substrate replenishment and produced stable voltage generation, the maximum voltage was reached within 2-4 hours upon replacement of the media and VFA substrate. This indicates that the electrogenic biofilm for each VFA was established on the anode electrode and was resilient in the face of media replacement. When all the reactors were fully enriched after approximately 2 months, maximum current and power generated from each reactor was calculated to be 0.13 mW, 0.1 mW and 0.06 mW respectively, as shown in Figure 4.4 (A&B).

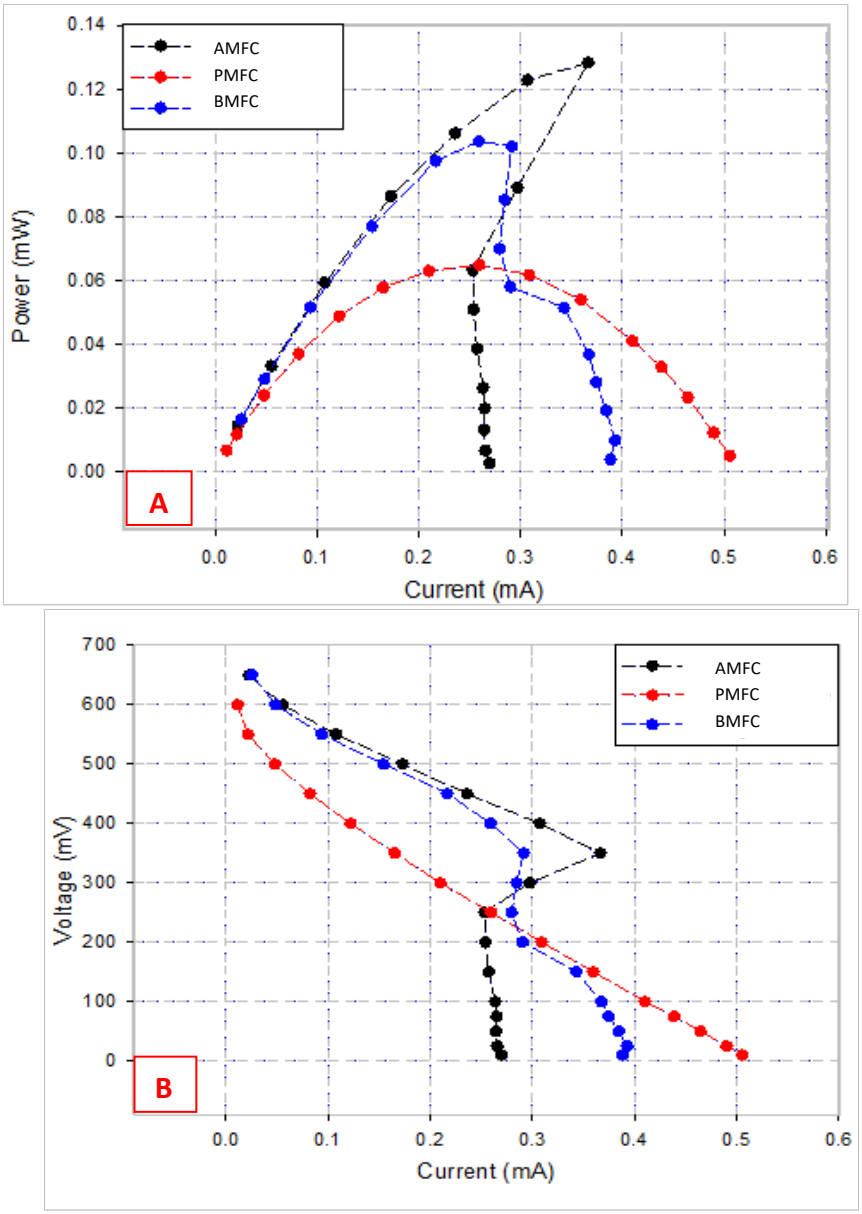

Figure 4.4: Power generated by AMFC, PMFC and BMFC after enrichment, with 1000Ω external resistance and 20 mM substrate concentration.

Figure 4.4 (A) shows the reflex behaviour of AMFC and BMFC which is considered as power overshoot. The power overshoot phenomenon is frequently seen, regardless of MFC configuration or operating conditions and is widely attributed to the biofilm on the anode (Watson and Logan, 2011, Hong et al., 2011). Immature biofilm can be one of the reasons for power overshoot as suggested by (Winfield et al., 2011) as the immature biofilm could not sustain high current demand in its early development Figure 4.4 (B). On the other hand the biofilm in case of PMFC was fully enriched and no power overshoot was observed in PMFC. Once the MFCs were acclimated on the specific VFAs

individually, an investigation of two methods was conducted into determining the most appropriate to discriminate between different species of VFA. The two methods are described as follows:

4.1.2 Coulombic Efficiency

The coulombic efficiency or the percentage of electrons recovered from the organic matter versus the theoretical maximum available, whereby all electrons would go to current generation, is an important measure of an MFC's performance in converting chemical energy in the substrate to electrical energy. CEs calculated for MFCs vary, but in general they increase with power density because there duration is shorter and there is less time for substrate to be lost to competing physical and biological processes (Logan and Regan, 2006). In a MFC based biosensor, CE could be one of the measures used to find the correlation between substrate concentrations and the total accumulated charge in coulombs produced at particular substrate concentrations. Many reports on MFC based BOD sensors have followed the same method and presented by various researchers (Chang et al., 2004, Di Lorenzo et al., 2009b). In this study the CE was also considered as one of the methods to quantify the VFA concentrations in respective VFA–MFCs.

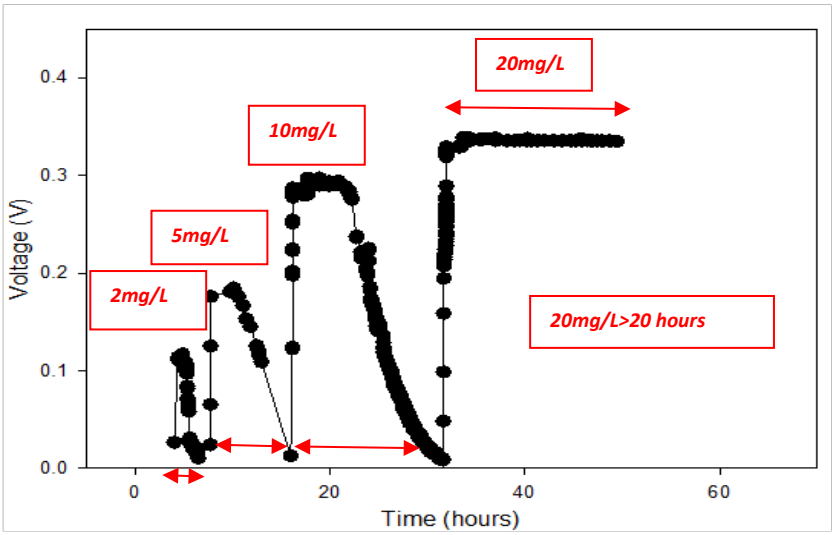

Figure 4.5: The time taken by BMFC to utilize different concentrations of butyrate.

In order to calculate the recovered electrons (charge) through the previously enriched electrogenic biofilm, and to determine the CEs as described in Section 3.9.1, the

substrate concentration was varied over the range of 2 to 20 mg/l and compared. The microbial community in each MFC responded in 1 to 2 hours on addition of VFA whereas coulombic yield of the MFCs varied with the initial dose of VFA. When VFA concentrations were higher than 20 mg/l, the maximum current values were similar, indicating typical saturation kinetics. The highest coulombic yield obtained from 20 mg/l of acetate an d butyrate were 92% and 7.2%, respectively when measured as a first batch feed, supplied just after enrichment. This value then decreased to 65% for acetate and showed no significant changes in the BMFC when measured after 4 batch cycles. CEs calculated for butyrate (4-8%) were significantly lower than those of acetate (50-90%), indicating that substantial electron losses to processes other than electricity generation.

The correlation between VFA concentration and charge (C) generation showed good linearity up to 10 mg/l concentration of acetate; and butyrate up to 20 mg/ l. However, the PMFC did not respond to concentrations below 20 mg/l but achieved maximum voltage when supplied with 50 mg/l of substrate with a maximum CE of 34%. In the case of AMFC, the maximum CEs varied within the range of 70-85% measured 4 times after enrichment. The highest CE was observed just after enrichment in the first batch fed operation, which then decreased and became consistent at <80%. However, PMFC and BMFC with propionate and butyrate feed, showed significant variations ±20% and ±30-35%, respectively. Figure 4.5 shows the time taken by BMFC to utilize different concentrations of butyrate. It is clearly evident that the higher the concentration, the more time the bacteria take to consume the substrate, which results in a slower sensor response. CE as above, is unlikely to be useful for rapid or frequent on-line measurements of concentrations and species of VFA as the sampling time (>24 hours for 20 mg/l initial substrate concentration), would be excessive.

4.1.3 Cyclic Voltammetry

Cyclic voltammetry (CV) tests were conducted to investigate current generation and its coupling to VFA oxidation; and to examine if the concentration of the substrate can be determined from the current produced. The experimental set up for CV measurement during this study is shown in Figure 4.6 and explained in Section 3.9.2. The oxidation peaks on CVs from each VFA enriched MFC were monitored at different VFA

concentrations. The amplitude of oxidation peak current obtained from CV showed a linear correlation to the supplied VFA concentration, when CVs were conducted at a consistent scan rate.

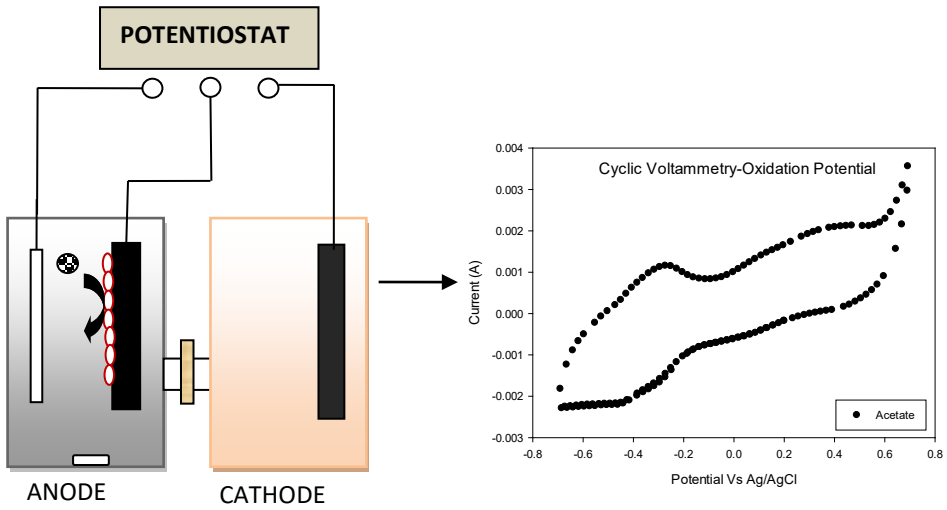

Figure 4.6: Experimental set up for measuring cyclic voltammetry for h-MFC based VFA sensor configuration

The oxidation peaks obtained from each VFA were also found to be very distinct in shape. Different VFA concentrations were tested in the individual VFA enriched MFC reactors. Furthermore, each enriched MFC was subjected to the VFAs that were not used in its enrichment to determine the enriched electrode's CV and current response to different VFA. Hence AMFC was subjected to propionate and butyrate, BMFC was subjected to acetate and propionate and so on. The range of potentials used in CV was between -0.65 V and +0.65 V. Along with the careful choice of the potential window, the scan rate should also be justified. To check the effect of scan rates on oxidation peak currents, the enriched electrodes were scanned within the potential range at varied scan rates. Figure 4.7 shows data where electrode potentials were swept at the rates of 10, 20, 50 and 100 mVs^{-1}. The location of the peak shifts with scan rate with increase in its amplitude (current).

It was determined that sweep rates up to 50 mVs^{-1} did not alter the shape or features of the waveform. A further increase in the scan rate to 100 mVs^{-1} alters the shape of the voltammogram and the oxidation peaks in the forward scan started losing definition. So,

a scan rate of 50 mVs^{-1} was selected as a good compromise between measurement response time and information content in the CV for all MFC reactors.

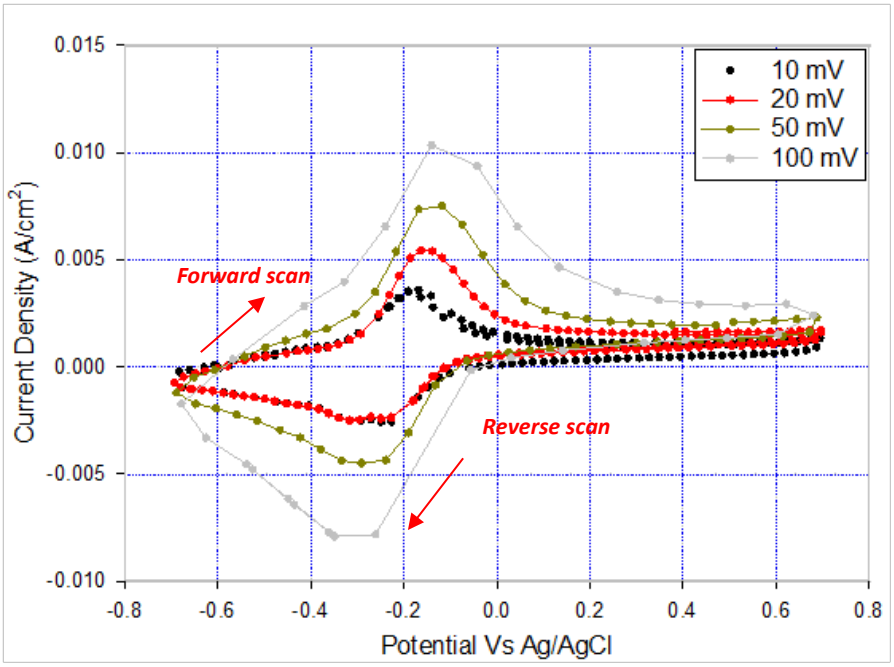

Figure 4.7: The dependency of peak current response on scan rates in enriched MFC showing typical changes in the voltammogram shape at 100 mVs^{-1} (vs Ag/AgCl reference electrode, saturated in KCl) The red arrows indicate voltage sweep direction.

Before measuring the CV on individual VFA enriched MFCs the anode chamber was washed with distilled water and then refilled with fresh phosphate buffer containing vitamins and minerals without any substrate (electron donor). Then the current peak was stabilized by repeated cycles of CV and once a stable peak current was obtained the concentration of respective electron donor was increased in steps. In AMFC when 5 mg/l acetate was added, an oxidation peak was obtained at -0.205 V vs. Ag/AgCl, with peak current of 1.01 mA. A slight shifting of potential with increasing substrate concentration was observed though. The concentration of acetate was then increased in the solution and the peak current was again observed. The current peak increased as acetate concentration was increased, indicating that these peaks resulted from acetate metabolised through the enriched electrochemically active bacteria at the anode. Typical CV traces showing the current variation resulting from biofilm electrochemical activity at the electrode, when subject to different concentrations of VFAs, are shown in Figure 4.8.

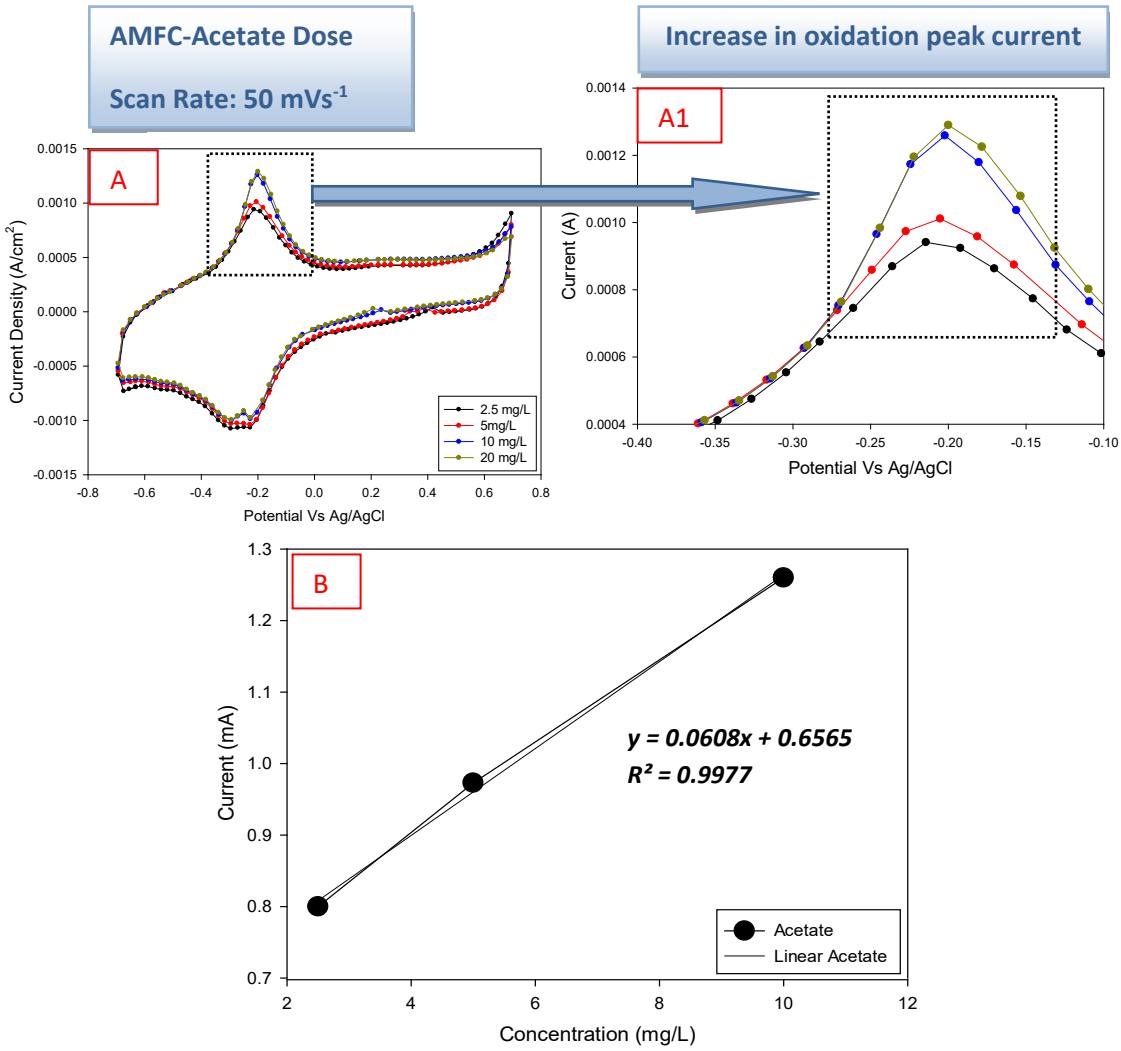

Figure 4.8: Cyclic Voltammetry based oxidation peak current response to the addition of different concentrations of acetate in AMFC, at an oxidation potential of -0.205 V

It can be clearly seen in Figure 4.8 (A and A1) that the peak current increased as a result of higher metabolic activity in the biofilm and saturates after 10 mg/l of acetate concentration. Saturation might be due to the thickness of the biofilm which may not have allowed higher acetate to pass through the biofilm within the applied scan rate. It is known than at flat planar electrodes, the diffusion quickly becomes rate limiting. As can be seen in Figure 4.8 (B), the linearity of AMFC to changes in acetate concentrations can be observed between 2.5-10 mg/l, with R^2=0.9977. The typical definition of detection limit for any sensor is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) within a stated confidence limit (generally 1%). The detection limit is estimated from the mean of the

blank, the standard deviation of the blank and some confidence factor (MacDougall and Crummett, 1980). Whereas in this study the detection limit was taken as the visible response obtained from the lowest VFA concentration which was found to be 2.5 mg/l in AMFC after measuring blank sample without any VFA.

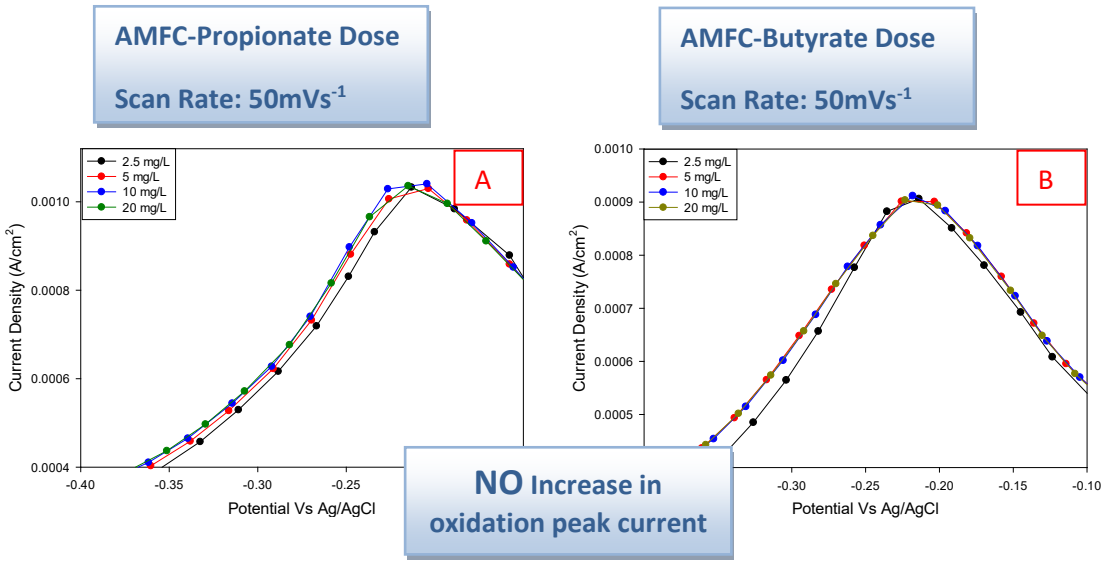

Figure 4.9: Cyclic Voltammetry based oxidation peak current response to the addition of different concentrations of (A) propionate and (B) butyrate in AMFC; at a potential of -0.205 V

Further CV scans were performed to determine the cross response of the enriched biofilm in AMFC to propionate and butyrate, as shown in Figure 4.9 (A and B). The AMFC enriched biofilm did not show an increase in peak current with concentration of propionate or butyrate, indicating that AMFC might have additional metabolic capability for degrading butyrate and propionate over acetate. This result suggests that specificity of the AMFC enriched bacterial community towards acetate exists. Therefore, the acetate acclimated bacterial community uses only acetate as a carbon source, and can be used to measure acetate concentrations in the MFC and possibly, if incorporated into any wastewater treatment system up to certain range of concentration.

Similarly, CVs were measured on the PMFC to measure responses to propionate acetate and butyrate to determine the responses from the other individual VFAs dosed. The CV scan of the anode colonized in PMFC exhibited two oxidation peaks (−0.378 V, 0.24 mA and -0.231 V, 0.37 mA) as shown in Figure 4.10 (A), out of which only one oxidation peak

at -0.231 V showed an increase in peak current on the addition of propionate to higher concentrations.

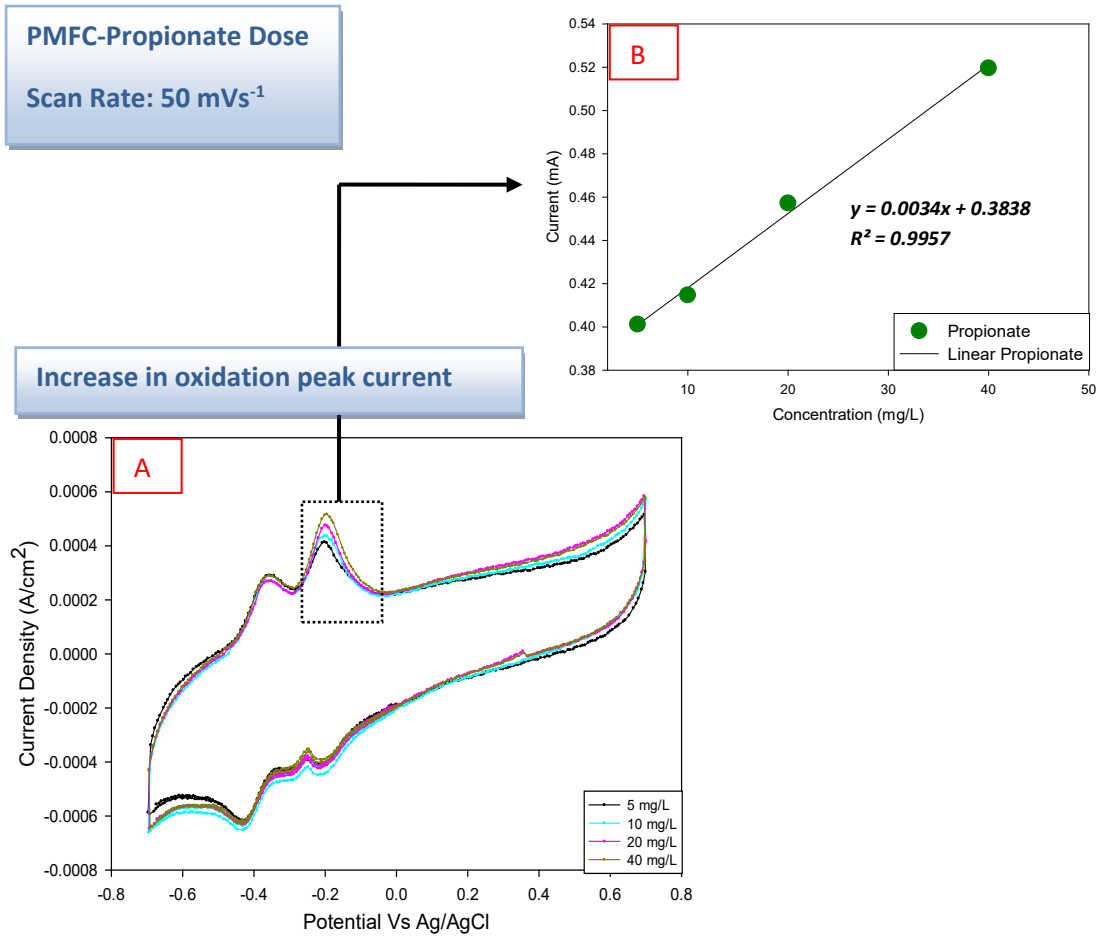

Figure 4.9: Cyclic Voltammetry based oxidation peak current response to the addition of different concentrations of propionate in PMFC, at a potential of -0.231 V

The peak current increased up to a concentration of 40mg/l showing linearly with R^2 = 0.99 as shown in Figure 4.10 (B). Where in AMFC the saturation reaches at 10 mg/l of substrate concentration, PMFC showed linearity up to 40 mg/l. The thickness of the biofilm which normally grows 12-27µm could be the reason for the rate of diffusion of the substrate in individual VFA enriched MFC (Kato Marcus et al., 2007). It has been shown by (Michie et al., 2011) that acetate fed biofilm grows faster and thicker compare to a butyric acid acclimated reactor. Although the study also concluded that the electrogenic activity was found to be higher in the case of acetate, but it was the case when reactors were in fed batch mode. The exposure of the biofilm to a specific substrate during CV is much lower compare to a fed batch system and rapidly reaches

saturation in the short time span made available by the scan rate, due to the biofilm thickness, which might resulted in reduced diffusion. A linear relationship was found within the range of 5-40 mg/l, after this point there was a decrease in the current response.

The response from the PMFC was also investigated for propionate responses and the same range of acetate and butyrate concentrations. When acetate concentrations to the PMFC were increased, a decrease in current was observed Figure 4.11 (A). On the other hand the increase of butyrate concentrations showed no effect on the current output Figure 4.11 (A).

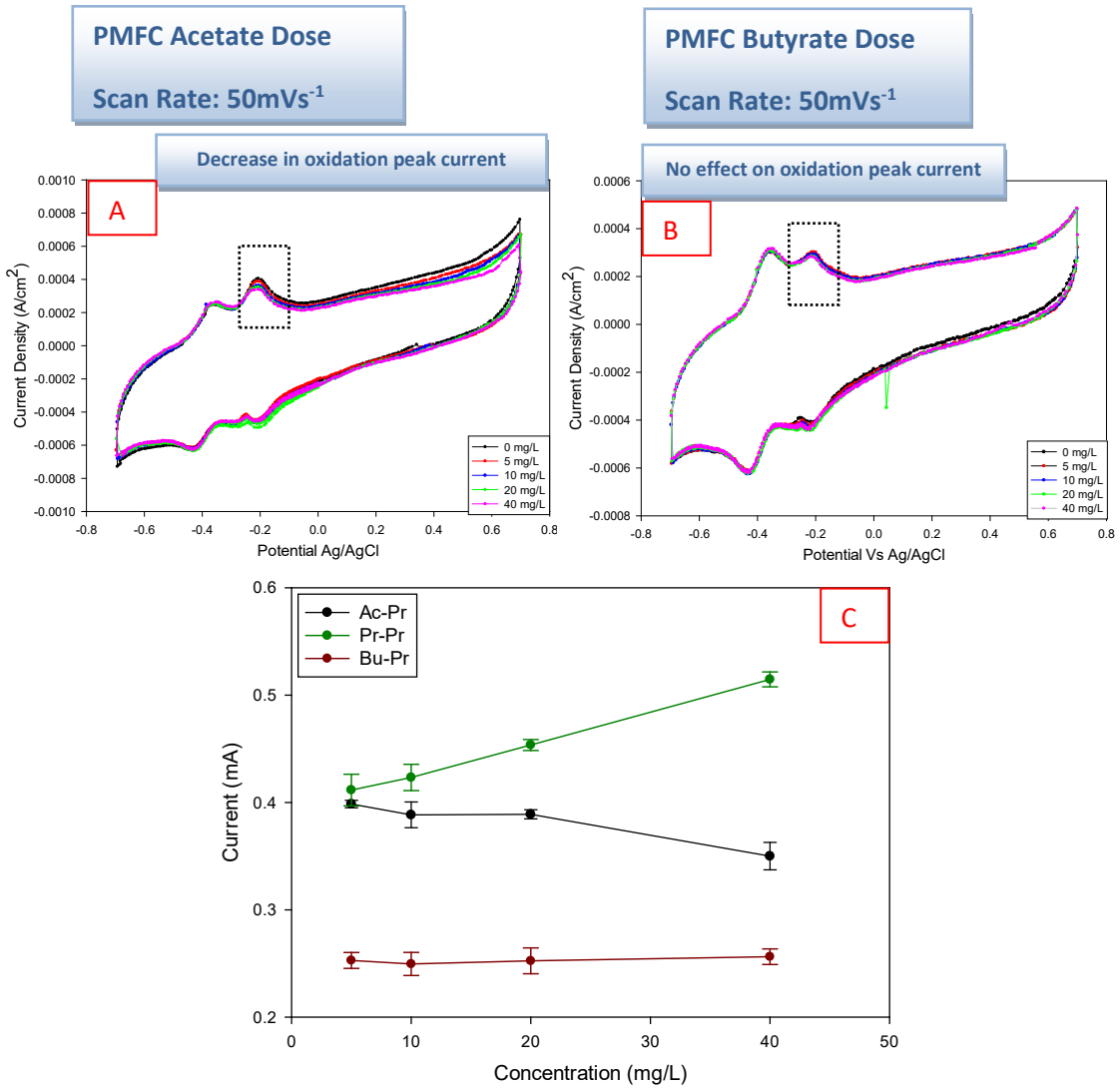

Figure 4.11: Cyclic Voltammetry based oxidation peak current response to the addition of different concentrations of acetate and butyrate in PMFC, at potential -0.231 V

This suggests that the specificity of the PMFC enriched anode bacterial community towards propionate is high. The metabolic pathway switch from the degradation of propionate to the degradation butyrate was evidently not quick enough to respond during the <2 minutes of CV measurement. It has been observed by (Teng et al., 2010) that butyrate contributes considerably less towards electricity generation compare to acetate and propionate. The response to acetate concentration variations was also found to be negative in PMFC during CV measurement and the reason could be the same as the metabolic pathway switch to different substrates may not be possible in short time periods. Figure 4.11 (A, B and C) show CV responses obtained from acetate and butyrate samples supplied to PMFC. The flat line (Bu-Pr and Ac-Pr) i.e. PMFC responses to butyrate and acetate in Figure 4.11 (C) indicated the no positive response from VFA species other than propionate.

The third reactor (BMFC) was tested for its response to the three VFA species. The shape of the CVs from BMFC showed one oxidation peak at (0.0373 V, 2.20 mA) which is very distinctive and different from peaks generated by AMFC and PMFC, in terms of its peak current potential as shown in Figure 4.12 (A). Initially, butyrate concentrations from 5-100 mg^{-1} were measures and a linear relationship was found exist up to 40 mg/l with R^2 =0.99 (Figure 4.12 A1 and B). Similarly to the PMFC, BMFC also saturated at 40 mg/l and the current output decreased with increasing butyrate concentrations above 40 mg/l. To investigate the cross responses from other VFAs, increasing acetate concentrations were then supplied to the BMFC. In contrast to AMFC and PMFC, the BMFC responded to all of the VFA species, but the degree of response was substantively different. A very sharp increase was observed when 5 mg/l concentration of acetate was added to BMFC. There was an increasing current up to *circa* 20 mg/l acetate, after that the current response was observed to saturate in BMFC, as shown in Figure 4.13 (A).

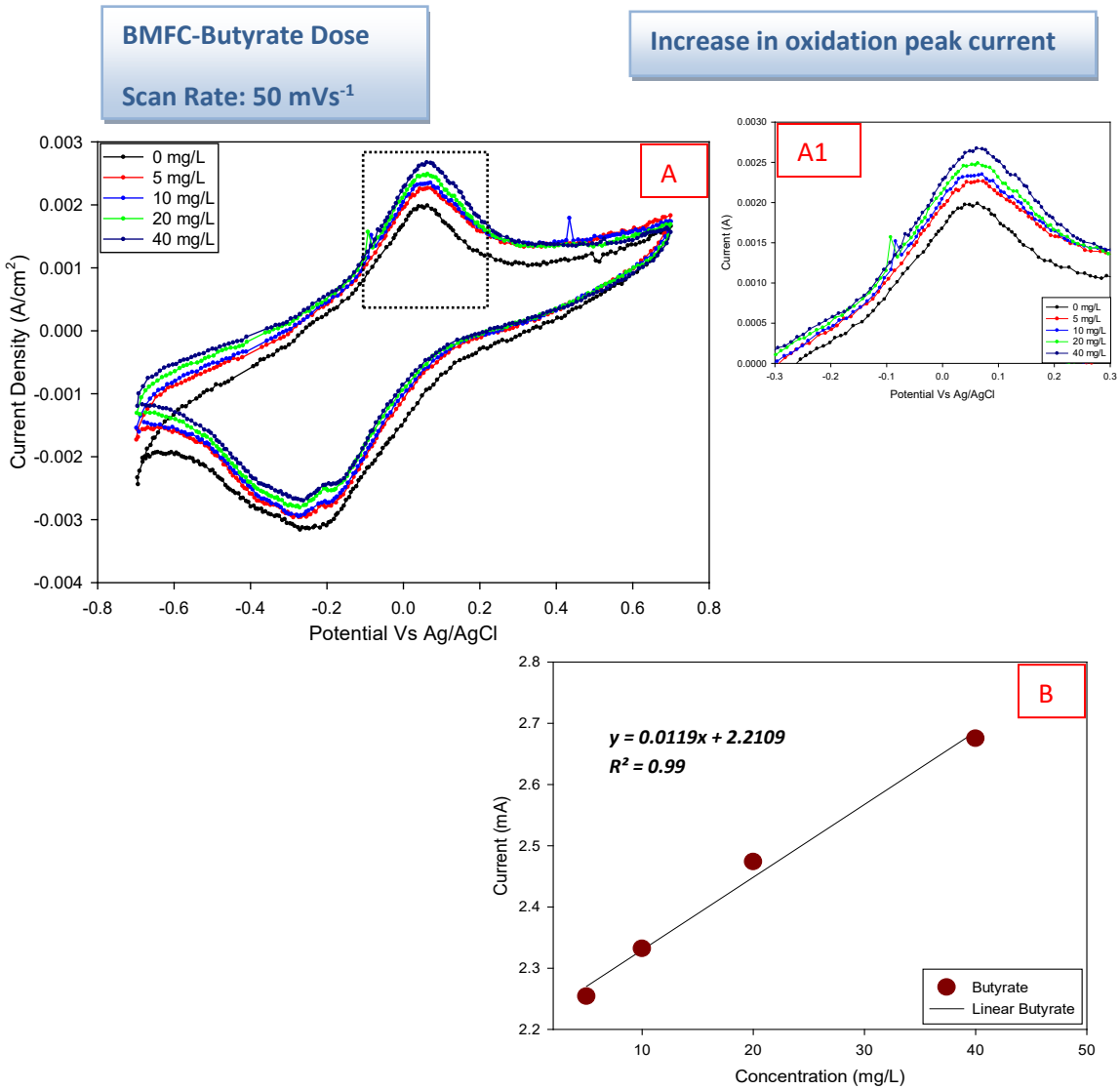

Figure 4.12: Cyclic Voltammetry based oxidation peak current response to the addition of different concentrations of butyrate in BMFC, at a potential of 0.03 V

The same range of concentrations for propionate was investigated in order to check its corresponding current responses Figure 4.13 (B). Butyrate showed a linear response up to 40 mg/l although the difference in current response was very limited, but above this no change in response was observed. On the basis of the above CV results, the responses to the three VFA species supplied to AMFC, BMFC and PMFC separately, where difference as described above were evident, it may therefore be possible to discriminate between different concentrations and species of VFAs.

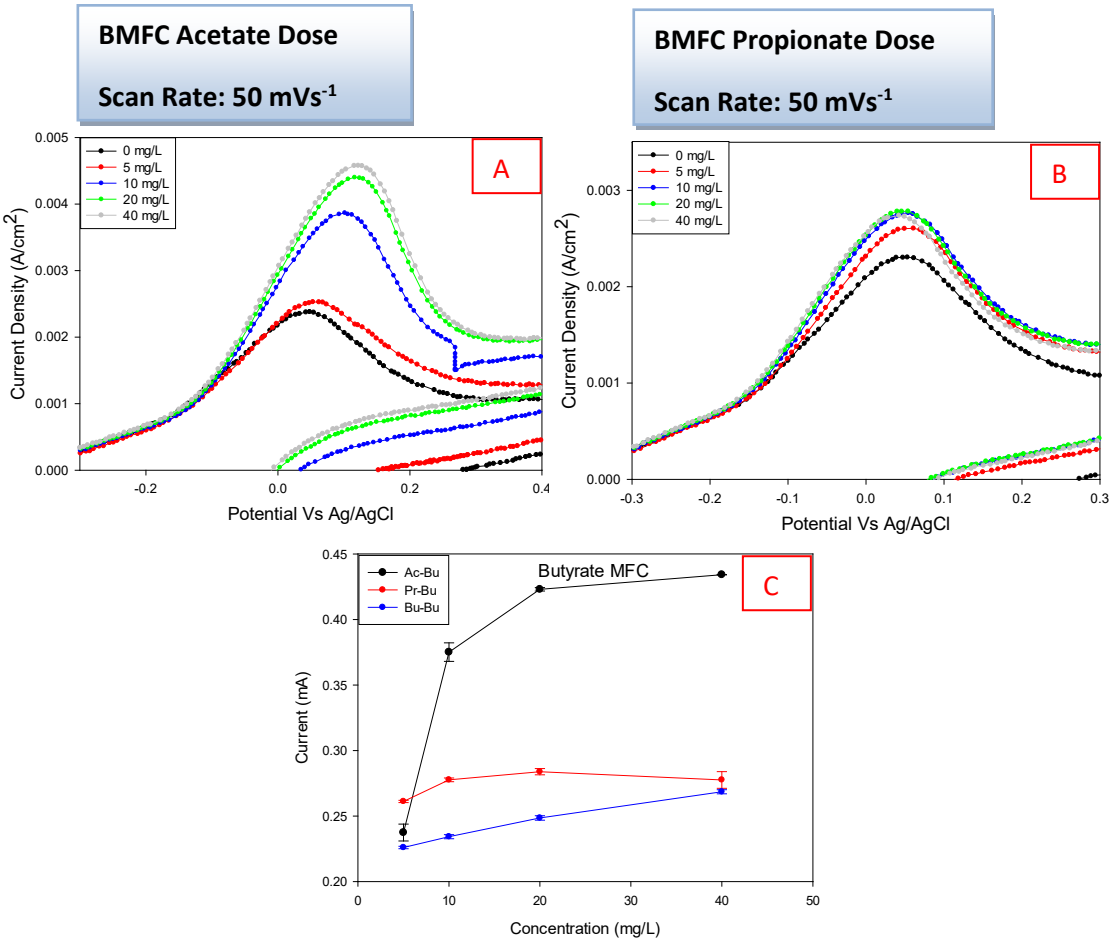

Figure 4.13: Cyclic Voltammetry based oxidation peak current response to the addition of different concentrations of acetate and propionate in BMFC, at potential -0.031 V

Although the range within which discrimination of their concentrations was limited to *circa* 20-40 mg/l, samples at higher concentrations could be diluted to extend the range and so measure the VFA concentrations. Table 1 summarizes the cross-sensitivity responses of individual VFA enriched MFCs to the respective VFA species.

Table 4.1: Cross-sensitivity of MFC reactors enriched on acetate, propionate and butyrate, to each species of VFAs

MFC Reactor	*Sensitivity of reactors to VFA species at 5mg/l concentration*		
	Acetate	*Propionate*	*Butyrate*
AMFC	+	-	-
PMFC	-	+	-
BMFC	+	+	+

Where "+" and "-" represents response and non response from individual VFA species

A variety of substrates, such as acetate, butyrate, sucrose, cellulose lactate, and wastewater have been considered by various researchers as electron donors in MFCs in order to investigate their potentials for electricity generation (Deepak Pant et al., 2010). The energy recovery efficiency varied considerably when different electron donors were supplied and a similar strategy was applied to each pure VFA enriched MFC.

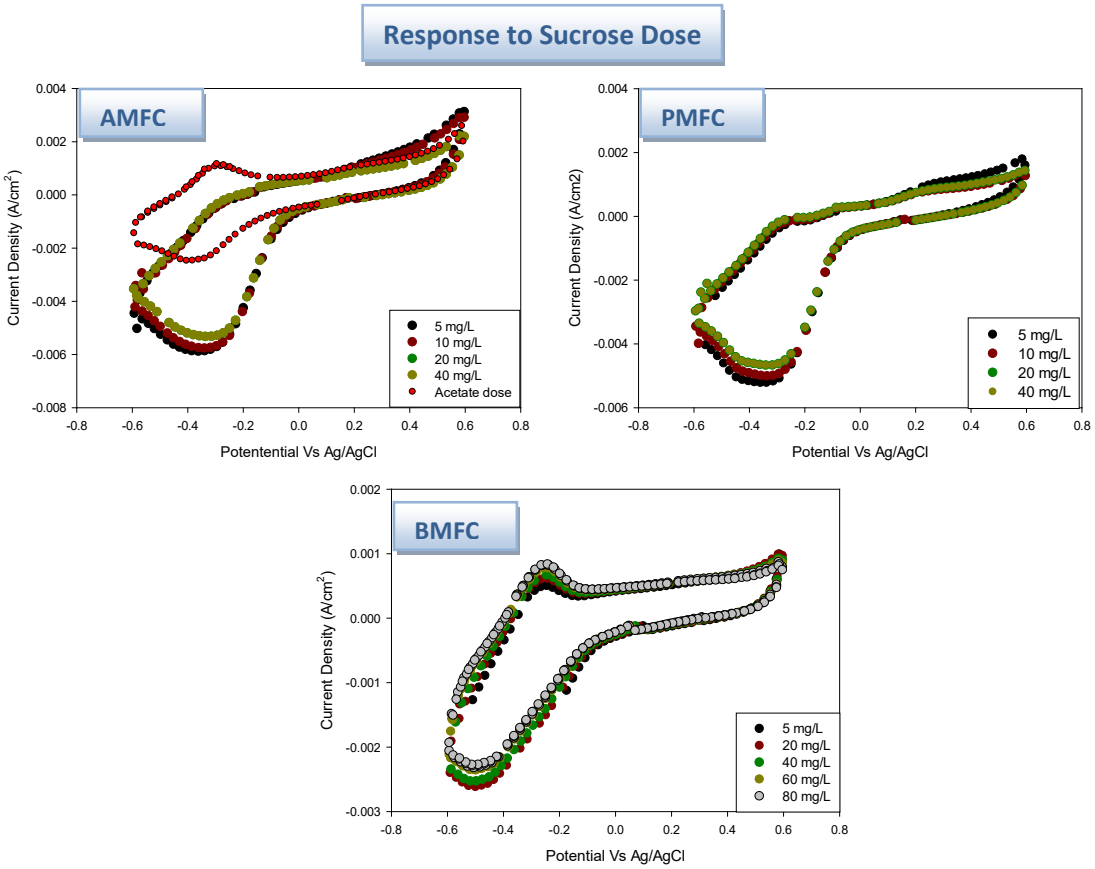

Figure 4.14: Cyclic voltammetry based current response from AMFC, PMFC and BMFC on addition of selected sucrose concentrations. No oxidation peak was observed in AMFC and PMFC but a clear peak is observed in BMFC, with small increase in current with sucrose concentration

The samples from the sucrose fed tubular MFC reactors are considered in Chapter 6 in the context of offline measurement. To check the response from non-degraded sucrose in all reactors, a set of concentrations were tested in individual fed reactor. Figure 4.14 (A, B and C) shows typical response obtained from each VFA enriched MFC on addition of increasing concentrations of the sucrose. Concentrations of sucrose from 5-60 mg/l were tested. In the case of AMFC and PMFC, no oxidation peak was observed within this range. It has previously been observed (Kim et al., 2013) that sucrose degradation mainly

gives rise to high concentrations of acetate and butyrate, from a mixed acid fermentation process, which resulted in no oxidation peak or current increase in AMFC and PMFC. But when acetate concentration was added in AMFC it immediately showed oxidation peak which was not observed on sucrose addition. On the other hand BMFC showed a small increase in the current peak at -0.260 V on addition of sucrose concentration but saturated at 60 mg/l. The response from sucrose might result from the high concentration of acetate and butyrate due to the rapid degradation of sucrose by the enriched biocatalyst. The saturation is achieved rapidly with very slight changes in the current output

4.2 Molecular Analysis

After 1 year of fed batch operation and development of the biofilm in the individual VFA enriched MFCs, DGGE analyses were performed to determine the bacterial community present in each VFA reactor (AMFC, BMFC and PMFC for both biofilm and planktonic phases). Sequencing of selected anodic samples was performed as described in Section 3.11.3. The BLAST algorithm was used to analyse the sequences and in-house software was used for taxonomic assignment. The types of dominant microorganism present at the anode result from the given substrate in each of the specific MFC system. Community analysis from sludge inoculated MFCs, subsequently incubated for extended periods on acetate, propionate and butyrate individually have resulted in identified bacteria belonging to the Taxa *Firmicutes, γ-Proteobacteria, β-Proteobacteria*, *α-Proteobacteria and Actinobacterium.*

Table 4.2: Summary of bacterial community composition in individual VFA enriched reactors

	Phylum %				Class %			
Reactor	*Proteo-bacteria*	*Actino-bacteria*	*Firmicutes*	*Others*	*α-proteo-bacteria*	*β-proteo bacteria*	*γ-proteo bacteria*	*δ-proteo bacteria*
AMFC	***69.9***	***10.8***	***7.5***	***11.7***	***30.5***	***32.1***	***2.0***	***19.3***
PMFC	***81.0***	***6.0***	***5.0***	***8.0***	***5.4***	***62.8***	***0.67***	***18.4***
BMFC	***40.9***	***47.9***	***5.34***	***5.9***	***8.2***	***56.1***	***1.2***	***13.2***

Table 4.2 shows the percentage microbial population present in each VFA enriched MFC. Where the percentage of phylum *Proteobacteria* was approx 70-81% in acetate and propionate reactor; butyrate reactor was found to be equally enriched with *Proteobacteria* (40.9%) and *Actinobacteria (47.9%).* Very low percentages of other taxa were also present which includes *Firmicutes, Bacteroidetes, Spirochaetes, Synergistetes* and *Chloroflexi.* The main electrogenic bacteria found in all reactors was *Geobacter* spp.

Archaea community analysis was also done and most dominant methanogenic genera in all three individually enriched reactors were *Methanosaeta, Methanobrevibacterium*, *Methanothermococcus* and *Methanobacterium.* The presence of high number of methanogens in all reactors especially in the propionate and butyrate fed reactors, resulted in very low coulombic efficiencies and loss of energy in methanogenesis instead of electrogenesis.

4.3 Conclusions

On the basis of the results presented in this chapter it is suggested that an acclimated mixed community can be operated stably in an MFC and could be used as the basis for off-line or on-line VFA monitoring. MFCs can be enriched with different VFAs and the acclimatized fuel cells can be used to monitor the VFA concentrations over a limited range. CE and CV methods were found to represent good measures for detecting VFA concentrations between certain limit and able to qualitatively discriminating between the VFA species, although CE would require excessive sampling times. These results are encouraging and demonstrate the possibility of practical application of MFC technology in VFA sensing. In so far as applications are concerned, it is foreseen that anaerobic processes in e.g. still waters, sewers and primary wastewaters, industrial wastewaters and other diluted environments may usefully be monitored for VFAs at low concentrations. Also, the application of such sensors at very low-concentrations e.g.in BES, such as microbial electrolysis or desalination, or MFCs themselves, which are able to reduce concentrations to lower level of VFAs, are considered to be important. The control of these devices or indeed aerobic or membrane effluent polishing systems, could all benefit from the measurement of VFAs in the ranges presented, or close to these ranges. Such a sensor is based on self replicating whole cell (bacteria), which will

be constantly fed with a specific VFA substrate, while periodically (at a predefined sampling interval) receiving samples to measure. The stability of such a sensor will therefore be expected to be substantial as compare to any enzyme based sensor. MFCs have been stably operated over periods of years and there is no cause to doubt that a suitable instrument could be contrived to achieve such longevity.

5 Stability, Sensitivity and Recovery of Sensor Response

The sensing strategy as presented in Chapter 4 is capable of measuring total VFA concentration in a controlled synthetic solution and can also be used to quantify individual VFA under similar circumstances. The microbial consortia used in this study were of mixed unidentified species and not a single or a number of pre-selected known species, and these mixed inocula would be constantly exposed to a non-sterile sample matrix. The enriched biofilm community on the anode of a MFC sensor could therefore be altered by sustained contact with the sample matrix; therefore it could be very important to protect the characteristic distribution of metabolic functionalities present in the enriched biofilms of AMFC, BMFC and PMFC, from such community changes to ensure repeatability and stability of the sensor (Shen et al., 2013). The choice of a suitable immobilization matrix to retard any biofilm changes is an important consideration for any biosensor, and the system proposed is no exception. Various natural polymers have been studied for their capabilities towards immobilization of whole cells for example by Yujian et al.,(2006) Nassif et al.,(2002) Karube et al.,(1976) Adinarayana et al.,(2005). Conducting polymers have also emerged as strong contenders as immobilization matrices, which can be used to immobilize microorganisms. It has also been reported that conducting polymers deposited electrochemically onto an electrode can play an important role in electron transfer (Umana and Waller, 1986, Kuwahara et al., 2008).

The aim of this study was to enhance the temporal stability, repeatability and recovery of an MFC based VFA sensing approach, by using various natural and conducting polymers as immobilization matrices. Figure 5.1 explains the principle behind the study. The fabrication of comparable bio-anodes with positively functionalised pyrrole polymers (Ppy^{+}) as shown in Figure 5.1(a) to attract negatively charged bacteria and electron; and which facilitates the electron transfer; and presents a more stable and durable sensor response was seen as the hypothetical ambition. However, to establish its primacy, it was necessary to compare against other polymeric methods of immobilisation. Natural polymer with redox mediator materials included as shown in Figure 5.1(b) could enhance the current output, which may increase the range and sensitivity of the sensor.

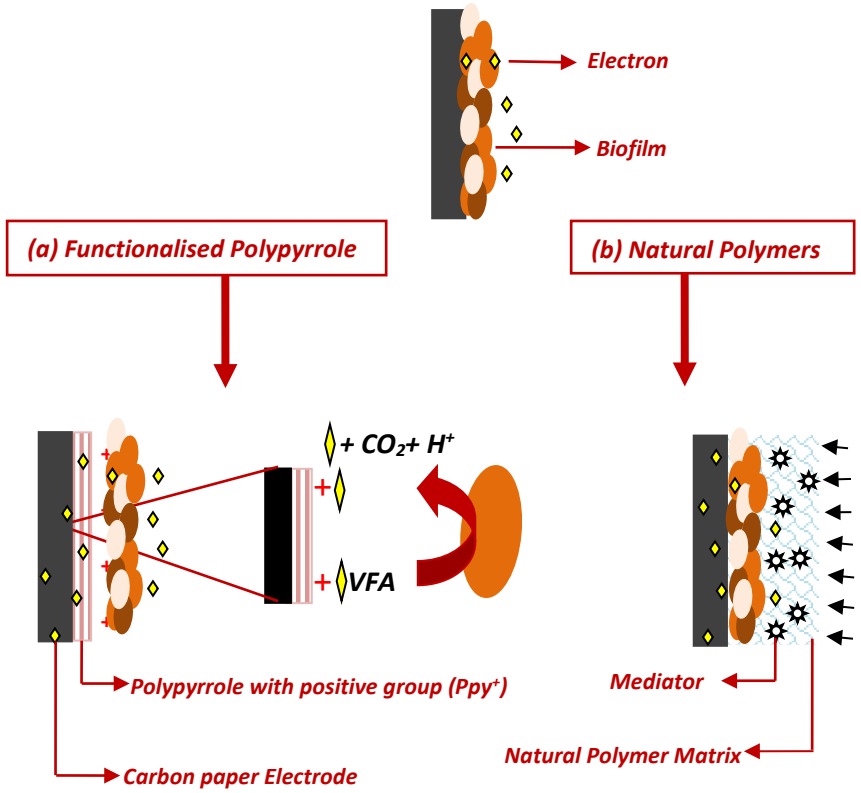

Figure 5.1: Principle behind the study and modification of electrodes with natural polymer and positive functionalised pyrrole polymer (Ppy^+)

In this study, consideration is given to both natural and conductive polymers to immobilize the biofim and to see their effect on the stability and recovery of the proposed sensor response. Two studies were conducted, with two different approaches used to modify carbon paper anode electrode surfaces; one employed natural polymers and the other employed conductive polymers. In the latter case, functionalised pyrrole was used as the conducting polymer for anode fabrication, with pyrrole (Ppy) and pyrrole modified with negative (Ppy^-) i.e. pyrrole propanoic acid and positive (Ppy^+) i.e. pyrrole alkylammonium functional groups used separately. Agarose, polyacrylamide and polyvinyl alcohol with calcium alginate were considered as the natural polymer for immobilisation. The fabrication methods used for modifying carbon paper electrode with natural and conductive polymers is described in detail in Section 3.2.2. The use of carbon/conductive polymer composite electrodes to provide a mechanism to maintain prolonged anodic syntrophic microbial community biocatalysis in an MFC based VFA sensor, to the author's knowledge has not previously been reported in the context of biosensor stability and repeatability. The effect of both sets of polymers on the static

response of the biosensor was also investigated and the results obtained from this study are presented in this chapter and advantages along with limitations are discussed.

5.1 Results and Discussion

The effects of electrode surface modification on the start-up time, sensor stability, current response and signal recovery at different VFA concentrations, were investigated. The major results from this work are discussed as follows:

5.1.1 Accelerated Start-up of MFCs with Ppy^{+} Electrodes

During implementation of the MFC based sensor, rapid start-up is desirable if they are to be cost effectively used in the monitoring of bioprocessing systems. There are various factors which have been considered to decrease the start-up time of MFCs and ultimately to increase the performance, which includes nutrient supply, flow rate, pH and temperature (Catal et al., 2011, Rabaey et al., 2004, Liu et al., 2004b). One of the most important and most investigated factors is the anode potential at which the MFC is operated, as it controls the theoretical energy gain for microorganisms. Reported start-up times for MFCs vary depending on the substrates examined and the reactor architecture, ranging from hours to several months (Feng et al., 2008, Liu et al., 2008). It has already been discussed in Chapter 4 that PMFC and BMFCs with unmodified electrodes previously took more than 700-800 h to acclimate to their maximum stable voltage and acetate fed MFCs take approximately 100 h. It is important to reduce this lag phase to increase the possibility of implementation of the sensor. The results obtained from Ppy^{+} modified electrode showed significant decrease in the lag phase in all substrates.

All reactors with modified electrodes showed a small voltage signal immediately after inoculation, when connected to 1000 Ω external loads. This voltage may have been facilitated by natural redox mediators released by the bacteria in the inoculum sludge (Kim et al., 2004).

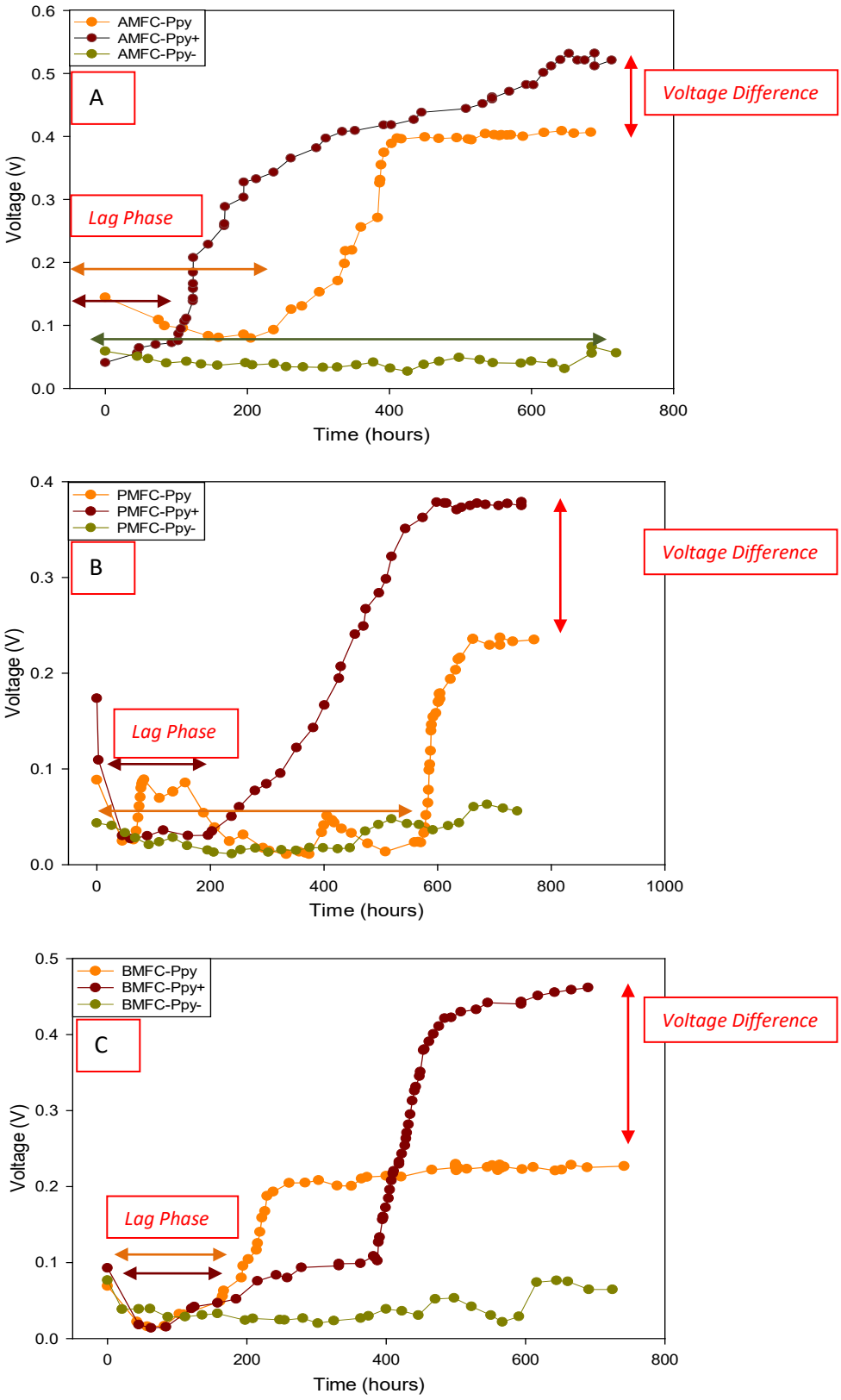

Figure 5.2: Typical voltage generation from AMFC, PMFC and BMFC with Ppy, Ppy+ and Ppy- fabricated anodes during the enrichment process.

Figure 5.2 (A, B&C) shows that in individual VFA-MFC with Ppy^+ modified electrodes designated as AMFC-Ppy^+, PMFC-Ppy^+ and BMFC-Ppy^+, the start-up time has been reduced by approximately 50 hours for AMFC-$Ppy^+$and <200 hours for PMFC-Ppy^+ and BMFC-Ppy^+ as compare to AMFC, PMFC and BMFC previously described in Chapter 4, Figure 4.2. In contrast to Ppy^+, the MFC reactors with Ppy modified anodes designated as AMF-Ppy, PMFC-Ppy and BMFC-Ppy, took almost double the time to start-up as highlighted in Figure 5.2. On the other hand, in BMFC-Ppy^+ the start-up time was slower than the equivalent acetate and propionate device, but soon attained an almost 70% higher voltage (0.45V) compared to control in BMFC calculated in relation to the voltage generated previously by the control BMFC i.e. 0.32V (Figure 4.2) and the voltage generated by the modified electrode colonised and acclimated by a biofilm 0.45V (Figure 5.2) during enrichment period for first few weeks.

From the typical enrichment behaviour obtained from different modified electrodes, it can be suggested that improved electron transfer between bacterial cells and the anode surface by virtue of the conductive polymers resulted in higher voltage output. This is attributed to the greater number of electron transfers with respect to time. Furthermore, it is postulated that the positive charge functionality increases the density of negatively charged electroactive bacterial cells and plays an important role in accelerating the initial bacterial colonisation and adhesion to the anode surface. This decrease in enrichment time provides evidence of an improved local environment for electrogenic metabolism and increases the practical applicability of the sensor device. The potential generated by the different VFAs and their metabolic pathways are likely to differ because of thermodynamic constraints in the electron transport chain and metabolic pathways within the cell. The efficiency of the electron transfer mechanisms between the bacteria and the electrode, the transfer of cationic species from the biofilm and the coupling of these processes through the electrochemical cell are also implicated. The negative functional group electrode modification failed to enrich and generated <0.04V in individual VFA-MFCs i.e. AMFC-Ppy^-, PMFC-Ppy^- and BMFC-Ppy^- as shown in Figure 5.2. This suggests that bacterial cells are attracted to the electrode at a faster rate by a positive functional group and this may improve the response and stability of the sensors.

5.1.2 Performance of the MFC Sensor with Fabricated Anodes

A clear increase in the maximum stable voltages with Ppy^+ modified electrode in all reactors, as compared to the control and other modified anodes, was observed. Figure 5.2 and 5.7 shows the difference in the voltage outputs where the H-type AMFC, PMFC and BMFC, on 40mM of acetate, propionate and butyrate of substrate respectively produced maxima of 0.45V, 0.32V, 0.35 V. with the same inoculum. However, AMFC-Ppy^+, PMFC-Ppy^+ and BMFC-Ppy^+ showed a significant increase in the maximum voltages i.e. 0.55V, 0.39V and 0.49V respectively; which also increases the current and power from the MFCs.

CV was used to study the performance characteristics of each modified electrode after a stable notionally maximum voltages was achieved after enrichment. The current response was determined at oxidation peaks potential of -0.201, -0.230 and 0.034 V for AMFC, PMFC and BMFC respectively with ±0.50V of potential shift during measurements. The linearity range remained similar to that observed before electrode modification and after the fabricated/modified electrodes using different polymers were deployed.

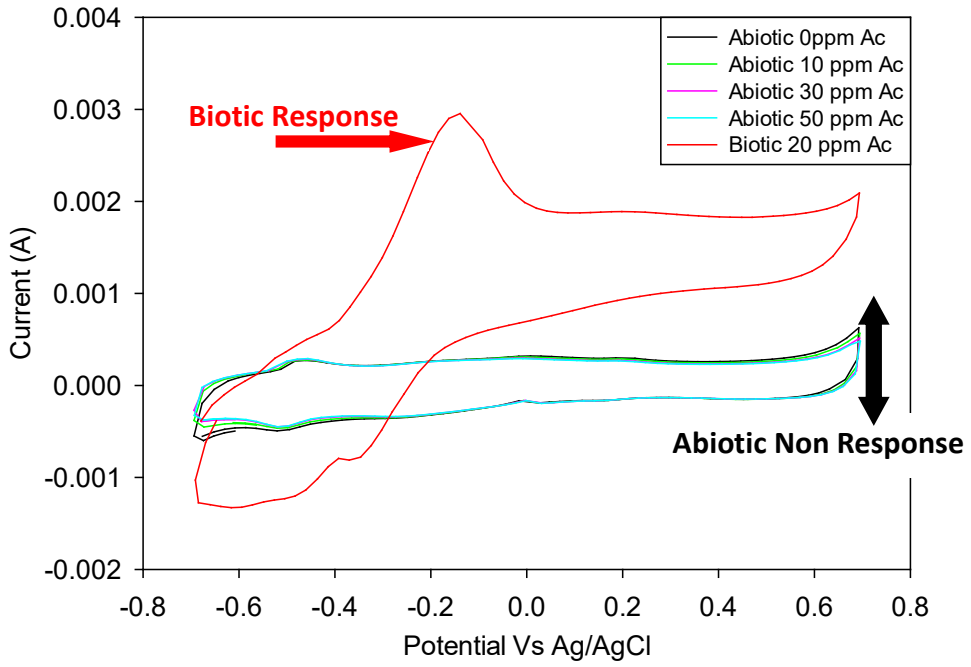

Figure 5.3: A comparison of abiotic and biotic current production in AMFC-Ppy^+ on addition of acetate concentrations

All modified electrodes were checked for any electrochemical activity prior to inoculation. Figure 5.3 shows the CV response from AMFC-Ppy^{+} under abiotic and biotic conditions and it can be clearly seen that a negligible current was produced even on increasing the acetate concentrations during abiotic condition. Whereas, the response of anodes once acclimated deliver discernable and useful signals as shown in Figure 5.3. However the natural polymers were coated over the biofilm and hence the abiotic activity is uncertain.

For investigations considering electrode modification with natural polymers, three sets of H-type MFCs with carbon paper electrodes were acclimatised with three VFA species as described in Figure 3.5 previously. Once enriched, three different natural polymers were applied to the anode surfaces over the biofilm by following the methods described in Section 3.3.2, in order to study their effect on voltages, current, stability and repeatability. There was a slight increase in voltage with MFC-AG+NR (MFC with agarose and neural red modified electrode) and PA+NR (MFC with polyacrylamide and neural red modified electrode) gel coatings, but decreased by 20% in the case of MFC-PVA/CA/C (MFC with polyvinylalcohol, calcium alginate and carbon powder modified electrode), which may be due to mass transport limitation. The voltage increase was stable for 3 days and later decreased to levels slightly higher (4-5%) than the voltages recorded prior to the application of the immobilization coating. The voltage increases may be due to the presence of mediator (neutral red) in the anolyte. The system was stable for only 3 days due to the loss of mechanical and chemical stability between the electrode and gel matrix. The layer started coming off the electrode and this resulted in the sensor response deteriorating after 3-4 days, which is an observation also reported by (Murano, 1998). Although it is possible to use natural polymers and the sensor could be readily disposed after 2-3 days of operation depending upon the stability of the response. But as compared to natural polymer modification, anode fabrication with Ppy^{+} modified electrodes presented a more prolonged stability by maintaining bacterial cell contact to the surface over longer periods, with higher voltages and currents outputs. So the Ppy^{+} modified electrodes were considered for further investigation in this study.

5.1.3 Stability and Reproducibility of Sensor Response

It is essential for a sensor to generate repeatable responses and to maintain reproducibility over a reasonable operating period. Considering it as an important factor the MFC sensor stability was evaluated by applying standard VFA concentrations over a period of 2 weeks. Once the polypyrrole fabricated electrodes were checked for their electrochemical activity in abiotic conditions, the electrodes were then enriched and were used for the analyses with different VFA concentrations on each fabricated electrode. The MFC with the ppy^{+} fabricated electrodes had good reproducibility and stability. The response time of the MFC sensor was 2-4 minutes depending upon the stabilization of the peak current. All measurements were carried out in duplicates by injecting standard concentrations from 100 mg/ml stock solution, into the 250 ml of sensor volume. The pronounced differences in the sensing behaviour were observed in all the different fabricated electrodes and results are presented in Figures 5.4 and 5.5.

The response obtained from natural and conductive polymer modified electrodes were compared with the control (without any modification i.e. carbon paper electrode), for their stability of responses, on the basis of standard deviations determined during the operational period. Figure 5.4 shows respective responses from AMFC, PMFC and BMFC sensors with natural polymer modified electrodes to each VFA concentration, revealing that the responses were relatively stable for 3 days, with low SD when using natural polymers. However, anodes fabricated with Ppy^{+} showed relatively stable responses over a 2 weeks period, with very low standard deviation (SD=±0.03-0.08%) compared to the control which showed high standard deviations (SD=±0.9-1.5% over the 2 weeks period) as shown in Figure 5.5. The stability of the sensor response for 2 weeks as compared to the other electrode modifications would increase the possibility of online implementation of the sensor and shows a major improvement as compared to the non fabricated electrode. The regions of the graphs which showed linearity between current and ln(concentration in mg/l) in Figure 5.4 and 5.5 were selected, enabling straight line curves were fitted to all the data sets (albeit on a natural logarithmic scale). From the equations of the straight lines, the static sensitivity of the response for each MFC sensor was determined with respect to each of the three VFA species, as shown in the Figure 5.4 and 5.5 (A1, B1, C1). Where Figure 5.4 shows stability of the response obtained from

different natural polymer modified electrodes, Figures 5.5 illustrates stability and static sensitivity (K) obtained over the linear range of each MFC sensor. If the linear region of the natural logarithm/concentration data is selected as illustrated in Figure 5.5 (A1) then Table 5.1, summarizes the static sensitivity of the MFCs to respective VFAs. The low K values show the lower sensitivity of responses to some VFA species. Negative static sensitivities show an inhibitory response of the individual reactor to other VFAs and suggests a degree of specificity of the sensor.

Table 5.1: Summary of the static sensitivities from linearised straight line fitting obtained for each VFA sensor response to cross VFA species

MFC	VFA	R^2	Range *[mg/l]*	Equation of straight line fit *I=Kln(conc)+i*	Static sensitivity *K [mA/ln(mg/l)]*
AMFC	Acetate-control	0.9299	2.5-60	I=0.2254ln(x)+1.1106	**0.2254**
	Acetate-Ppy^+	0.9928	2.5-60	I=0.4082ln(x)+1.6073	**0.4082**
	Propionate	0.2609	2.5-60	I=0.0041ln(x)+1.0177	**0.0041**
	Butyrate	0.0126	2.5-60	I=-0.0008ln(x)+0.8235	**-0.0008**
PMFC	Acetate	0.942	2.5-60	I=-0.002ln(x)+0.0422	**-0.002**
	Propionate-control	0.9891	2.5-60	I=0.0069ln(x)+0.0414	**0.0069**
	Propionate –Ppy^+	0.9978	2.5-60	I=0.0068ln(x)+0.0549	**0.0068**
	Butyrate	0.0115	2.5-60	I=2E-05ln(x)+0.0259	**2E-05**
BMFC	Acetate	0.7431	5-60	I=0.2534ln(x)+3.4735	**0.2534**
	Propionate	0.5903	2.5-60	I=-0.1521ln(x)+2.9673	**-0.1521**
	Butyrate-control	0.7142	2.5-60	I=0.1385ln(x)+3.155	**0.1385**
	Butyrate-Ppy^+	0.9886	2.5-60	I=0.2621ln(x)+4.0272	**0.2621**

R^2=coefficient of determination; I=current [mA]; K=static sensitivity; ln(x)=natural log of VFA concentration

The current production increased in the cases of MFC-AG/NR, MFC-PA/NR and all VFA-MFC-Ppy^+ anode electrode for each substrate, which suggests improved interactions between bacterial communities and the electrode. Higher current was generated through improved bacterial cells adherence to the positive functional group of Ppy^+ and more stable interactions were apparent in the case of natural polymers. Reproducibility of the MFC based VFA sensor was established using twice weekly CVs, with the same standard concentrations of VFAs in each individual VFA enriched sensor. Figure 5.4 and 5.5 shows responses of the current under VFA concentration ranging from 0 to 100 mg/l. The MFC responded with reasonably constant current when the VFA concentration was the same.

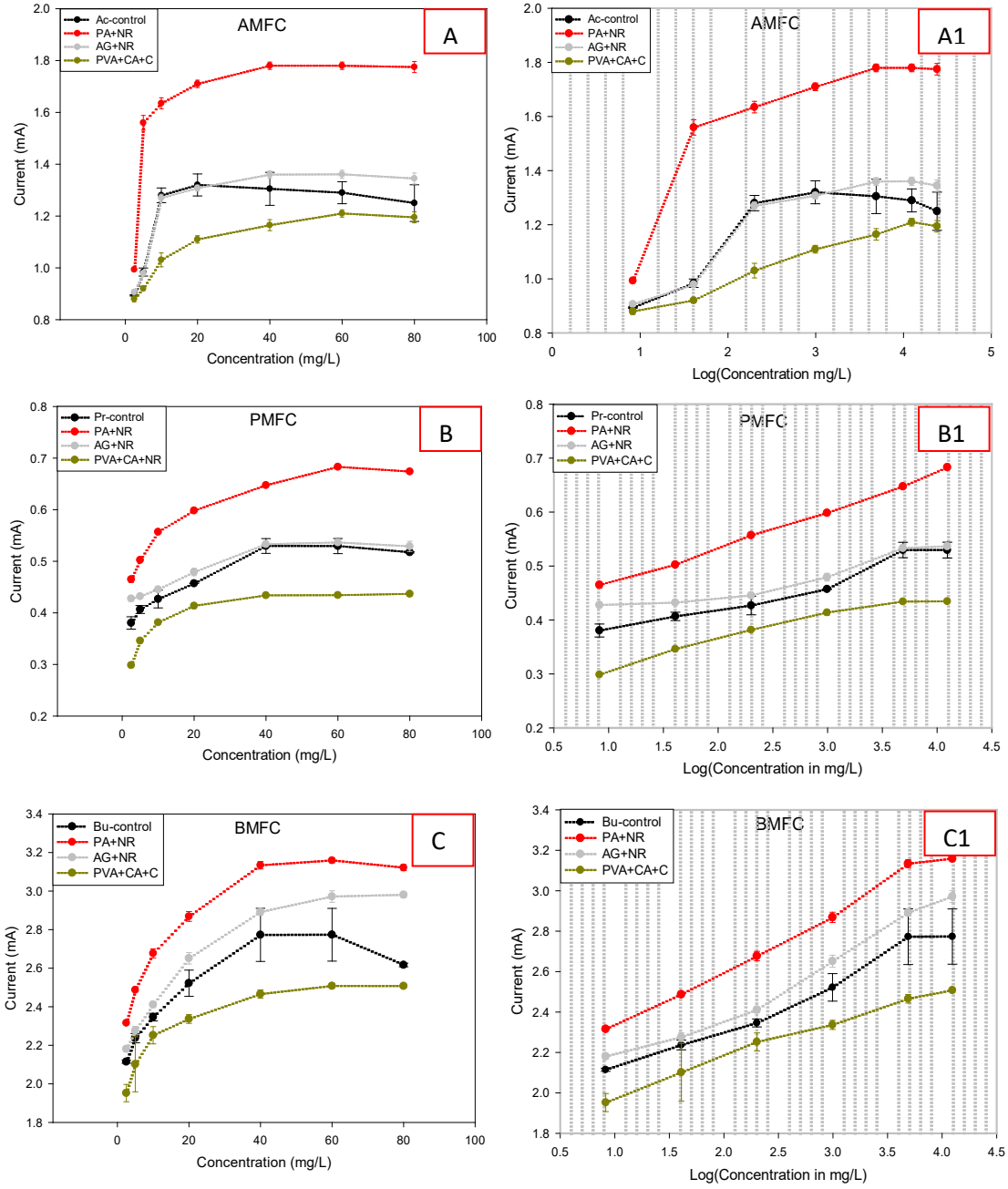

Figure 5.4: Cyclic Voltammetric response from (A, A1) AMFC, (B, B1) PMFC and (C, C1) BMFC from different natural polymer modified anodes with respective VFA dosage for the period of three days. Error bars showing standard deviation of the response during the period of 3 days. Where (Ac-control, Pr-control and Bu-control) were MFC with electrodes without any natural polymer modifications

For example, when the reactor AMFC-Ppy^{+} was supplied a concentration of 10 mg/l of acetate, the current output was 2.49 mA with SD=0.05%, over a 2 weeks duration; whereas in control (i.e. ac-control), for the same concentration, SD was 0.9%. In the Ppy^{+} modified electrodes, it was observed that the sensor gave a consistent response over

the testing period, whereas the response from the control showed an increased SD=1.5%, indicating that the bacterial electrode community's response to VFAs varied continually, probably in response to population dynamics and preponderance.

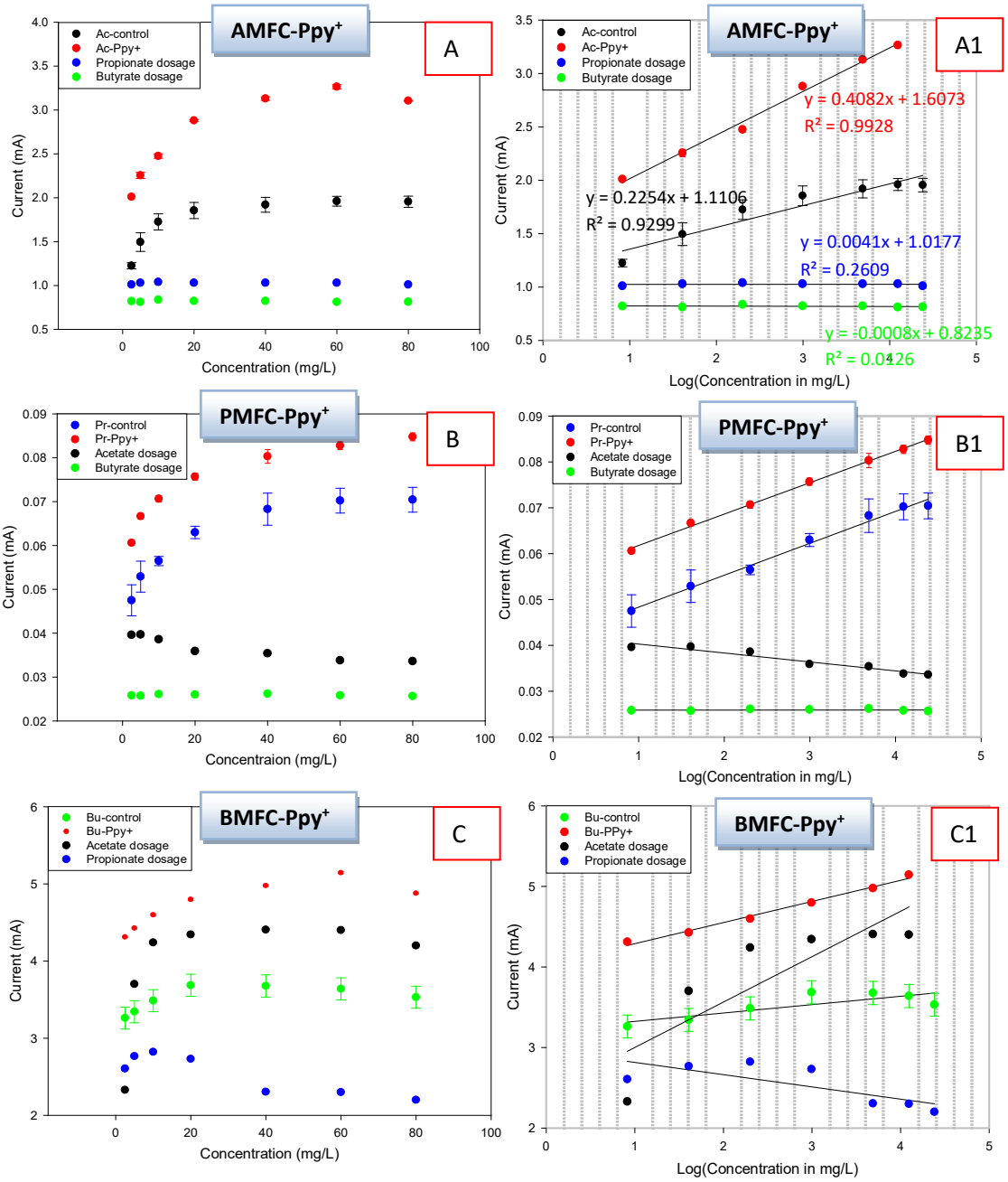

Figure 5.5: Cyclic Voltammetric response measured in individual VFA-MFC sensor (A, A1) AMFC-Ppy+, (B, B1) PMFC-Ppy+, (C, C1) BMFC-Ppy+ and from control (Ac-control, Pr-control and Bu-control) with different VFAs (acetate (ac), propionate (pr) and butyrate (bu)) during the testing period of 2 weeks. The error bars indicated the standard deviation of the sensor response measured during the testing period.

With natural polymers however, the sensor response showed stable results over 3 days, although the detection limit increased to 60 mg/l as compared to 20-40 mg/l presented in Chapter 4.

The major limitation of anode fabrication was the deterioration of mechanical strength and the bonding between planar electrodes and matrices used with time.

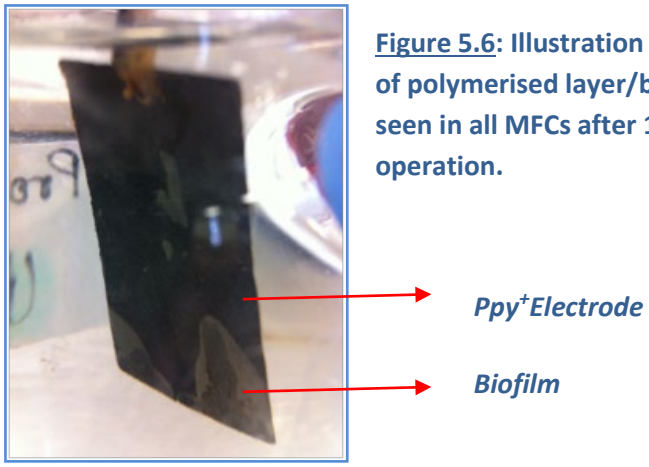

Figure 5.6: Illustration of the loss of polymerised layer/biofilm seen in all MFCs after 1 month of operation.

Where in natural polymers the layer came off the electrode in <3 days, the voltage in the case polypyrrole modified electrodes suddenly dropped to <10 mV and lost activity when media was replaced after one month; and a clear detachment of layer was observed as shown in Figure 5.6. The reason behind this sudden drop of voltage is still not clear and was not analysed further.

5.1.4 Recovery of the Sensor

Recovery time of a sensor is another major factor which affects overall performance. It is important for a sensor to recover rapidly so that measurements can be done without excessive delay. The shorter recovery times were achieved by using Ppy^+ modified electrodes as compared to the control/unmodified anodes. To check the recovery of the sensor steady state voltages, the 'before and after' measurement of standard concentrations were determined. The measurement time was not considered within the recovery time of the sensor.

Figure 5.7 shows the recovery times of the AMFC, PMFC and BMFC (control) and AMFC-Ppy^+, PMFC-Ppy^+ and BMFC-Ppy^+ which reflects that different modifications to

electrodes took different periods of time to recover after the measurement period. Recovery time was affected by the surface modification of the anodes but also depended upon the introduction of cross VFAs to each MFC sensor. After CV measurement with the 10 mg/l standard acetate concentration, the electrode was washed with buffer solution and reinserted into the previous concentration to establish a new steady state voltage.

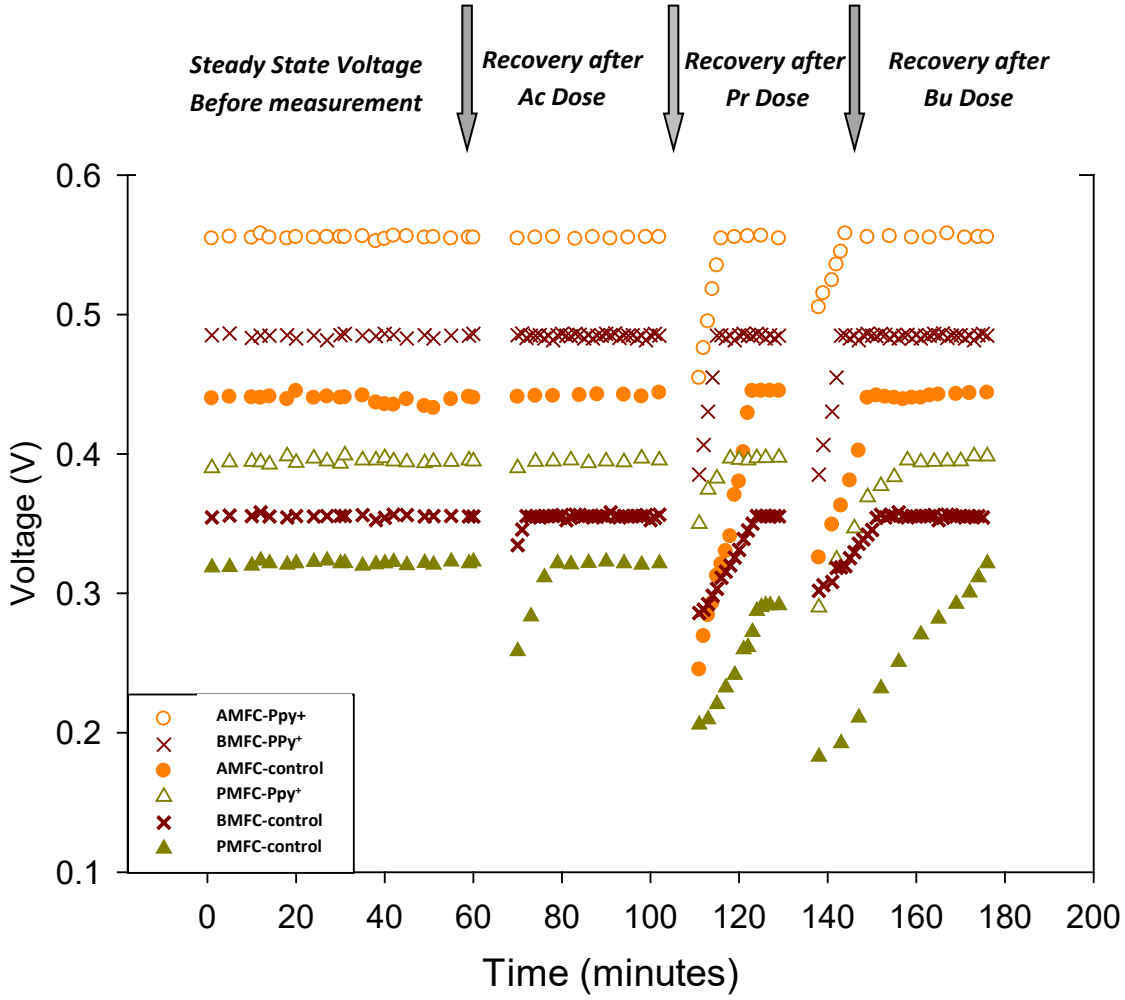

Figure 5.7: Sensor recovery after each dose of VFA in positive functional group modified electrodes in comparison to control (not modified)

It can be seen in Figure 5.7 that there was no difference in the voltage for both cases and the recovery time was measured in seconds; but when propionate and butyrate concentrations were measured in the same MFCs and subsequently washed out, the new steady state voltage achieved represented a drop in voltage and the recovery time for AMFC was longer than AMFC-Ppy^{+}electrode. Similar voltage drops and recovery

times were observed in PMFC, BMFC, PMFC-Ppy^+ and BMFC-Ppy^+ as shown in Figure 5.7. Recovery times for all VFAs were improved by the Ppy^+ modified electrodes and were much more rapid (<2-10 minutes) than the control (10-30 minutes) for 10 mg/l concentration of each VFA. The bacterial community in all Ppy^+ modified MFC sensors recovered faster and was more resilient. The results remained consistent throughout the experiment as a consequence of the positively charged Ppy^+ retaining bacteria on the electrode for a longer period compared to the unmodified control.

5.1.5 Conclusions

The current study presents another step towards the improvement of the sensor introduced in Chapter 4 by stabilizing the microbial community for longer period of time with anode surface modification. Anodic microbial biocatalysed electrodes, when connected in MFCs, are able to act as sensing elements; sensitive to acetic, butyric and propionic VFA species. Particularly the anode system fabricated with a) natural polymers polyacrylamide with neutral red (PA+NR) and b) conductive polymer poly pyrrole with positive functional groups Ppy^+ i.e. poly (pyrrole-alkyl ammonium) can significantly improve the performance of the sensor array in terms of voltage output, start up, current production, stability for 2 weeks and recovery rate of the sensor's response. The sensitivity of the electrode can improve the sensor applicability and reliability for wide range of systems containing the VFAs considered, and which need to be monitored, often to prevent failure.

6 Improved Range and Practical Measurement

For a sensor to meet the expected criteria of a robust, deployable VFA sensing system, the range and selectivity of the sensor system requires improvement on the results presented above. This chapter presents the results which indicate an improved range for the sensor, achieved by using specific poised potentials on the basis of oxidation peaks obtained from CV in relation to each VFA enriched MFC sensor. Offline testing of 'real' samples collected from a scaled-up MFC running on sucrose and an electrodialysis cell with sucrose and grass separately as a feed was also undertaken to investigate if the industrial implementation of the sensor could be plausible. The results obtained from this work are presented in this chapter.

In an MFC, the biocatalyst present in the anodic chamber influences several aspects of the overall performance. Anode potential is one of the important factors controlling the synergistic interactions within and between microbial population and current flow as it can influence the electron liberating capacity of the biocatalyst (Schroder, 2007b, Aelterman et al., 2008). The anode potential can vary over a wide range, which thermodynamically alters the energy available for bacterial-electrode electron exchange. Setting the anode potential to a fixed voltage using a potentiostat in BES, can exert effects on system performance, bacterial growth and/or composition of consortia (Aelterman et al., 2008, Kumar et al., 2013). The positive effects of setting anode potential has already been presented with pure cultures 0.095V to 0.595V (vs SHE) (*Shewanella putrifaciens*) (Carmona-Martinez et al., 2013), -0.16V to 0V (vs SHE) (*Geobacter sulfurreducens*) (Wei et al., 2010) and 0.27-0.32V (vs SHE) (*Pseudomonas putida* F1) (Friman et al., 2012). While the use of set potential for efficient performance of bioanodes has received significant attention in the context of improving start-up time (Aelterman et al., 2008, Chou et al., 2014, Huang et al., 2011), power generation and bioelectrochemical behaviour (Wagner et al., 2010, Friman et al., 2012), only a limited number of reports have been published regarding the application of set potentials to improve the degradation of recalcitrant organics and also the importance of set potentials in measuring different substrates have not been published so far for MFCs based sensors. The anode potential of MFCs regulates the possible energy gain used for

growth and maintenance of electrogenic bacteria. The potentiostatic control of the anode is expected to provide greater accuracy than the use of a traditional MFC arrangement (Soon Bee Quek et al., 2014).

6.1 Results and Discussion

6.1.1 Cyclic Voltammetry

CV based sensors are the most common of all biosensors, but different strategies may be found. Nonetheless in this type of sensor the measured parameter is oxidation potential of an electrochemical reaction. The working principle relies on the fact that when a voltage is applied to an electrode in solution, a current flow occurs because of an electrochemical reaction. The voltage at which these reactions occur indicates a particular reaction and/or particular species (D'Souza, 2001). CV has also been well studied to understand the behaviours of electrogenic bacteria in donating electrons. There is still little agreement however, on what the optimal anode potential should be for the exploitation of an electroactive consortium, but an appropriate anode potential could improve the MFC's operation (Chou et al., 2014). So, the following strategy was considered for the selection of poised potential to improve the range of the sensor and for the speciation of VFAs. In Chapter 4, CV tests were conducted to demonstrate that current generation was coupled to VFA oxidation and to examine if the concentration of the substrate can be detected from the current produced. It was concluded that CV can be used to measure and speciate VFAs in MFCs for a limited range of concentrations. The characteristic shapes of the cyclic voltammogram obtained in Chapter 4 were very distinctive for each VFA enriched MFC. The CV to select the potential was repeated in a new set of H-type (h-MFCs) and c-MFCs with different inoculums as used in Chapter 4. The peak potential for acetate and propionate were consistent for both configurations i.e. (-0.260 V and -0.220 V) with ±30mV of peak shift. In contrast to acetate and propionate enriched MFCs butyrate showed a shift of peak current potential ranging between ±0.100 V. The potential for BMFCs were then selected on the basis of peak potential measure just before calibration of the sensor, which was (-0.100 V in both configurations). This potential shift is believed to be because of the degradation of butyrate to short chain fatty acids as the BMFC sensor gives a current response to all three VFAs investigated. The selection of potentials was based on the experimental

results, as theoretically the redox potential for all the VFAs lies between -0.290mV to -0.280mV. Figure 6.2 shows the different oxidation peak potentials in cubic AMFC and PMFC designated as c-AMFC and c-PMFC (Figure 6.2 A&B) and H-type BMFC (h-BMFC) and cubic BMFC (c-BMFC) (Figure 6.2 C&D) respectively.

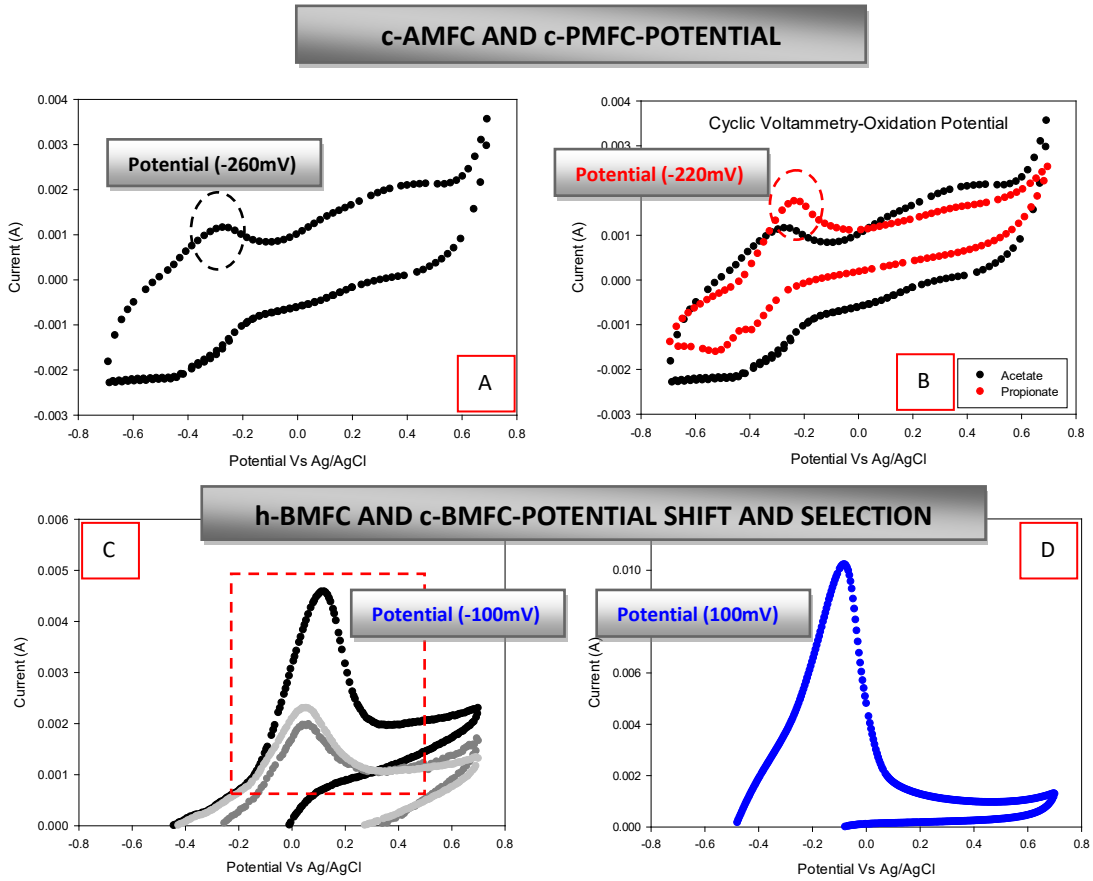

Figure 6.1: Cyclic Voltamogram showing the difference in oxidation potential with 10 mg/ l substrate concentration between (a) (AMFC: -0.260 V, (b) PMFC: –220 V, and (c) BMFC: -0.100V can be noticed

6.1.2 Anode Poised Potential Investigation

The anode potentials were selected and were then imposed as a poised potential on individual VFA enriched h-MFCs and c-MFCs to measure standard VFA concentrations and real samples as described below.

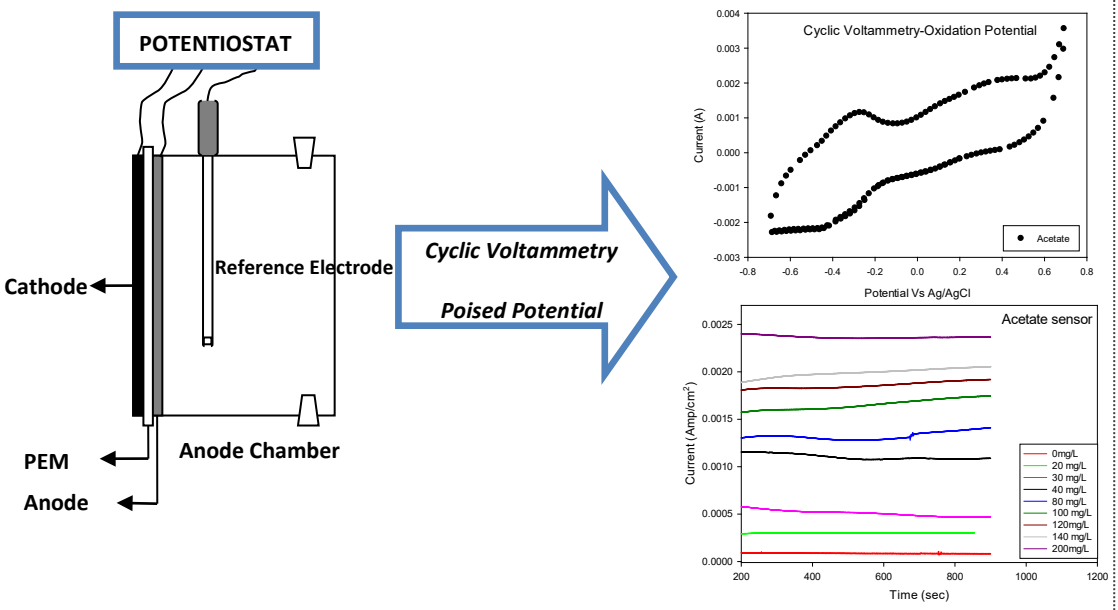

Figure 6.2: Experimental set up during cyclic voltammetry and anode poised potential with Ag/AgCl as reference electrode saturated in KCl.

6.1.2.1 Standard Calibration Curves (For Both h-MFC and c-MFC)

h-MFCs and c-MFCs were enriched with acetic, butyric and propionic VFAs individually, the constant voltage/current was maintained by operating the h-MFCs and c-MFCs on fed batch mode with 20 mM and 5 mM substrate concentration respectively. The calibration of individual VFA enriched sensor was done by using standard respective VFA concentrations ranging from 0 to 200 mg/l. Before calibration, reactors were shifted to the anaerobic chamber to keep the anode environment anaerobic and also to maintain the temperature at 30°C. The working electrode, counter and reference electrode were then connected to the potentiostat. Two possible methods were considered for calibration:

1) The anode chamber was rinsed with phosphate buffer and then refilled with media without any substrate in it. The current was stabilized for 1 hour at pre-selected and respective poised potential for the individual VFA. This stable current was taken as a baseline current. Then concentration of the respective substrate was increased stepwise with equal intervals in the same anolyte and measured for 10 minutes to get the stable current.

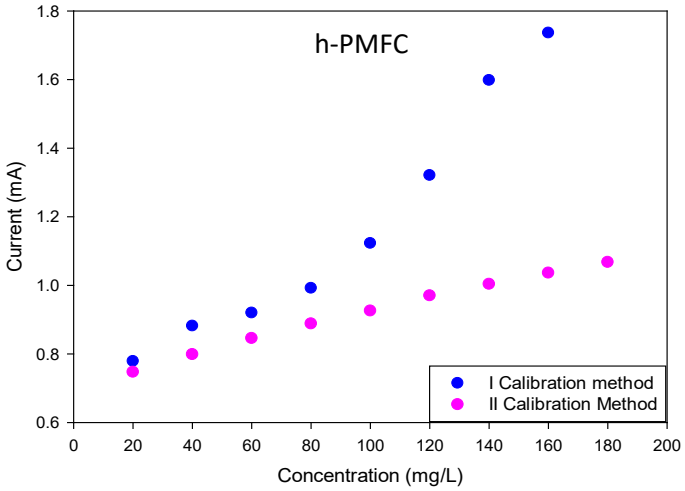

Figure 6.3: Difference in the current response measured from two different calibration methods in h-PMFC reactor. Similar results were observed in other VFA-MFC sensors.

2) The same procedure was again followed, but the anode chamber was rinsed and refilled with fresh media for measurement with increasing concentration as opposed to rinsing with buffer solution. Before measurement, the current was stabilized to the baseline current. This baseline current was then subtracted from each current output.

There was a considerable difference in current output measured from the two different methods. When the current outputs from the two methods were compared, it was found that the current production in the case of first method was higher than the current measured by the second method. Figure 6.3 shows the difference in the current outputs, with both methods measured in the h-PMFC. In first method, the high current might have been caused by the constant utilization of the available substrate by the bacterial community when concentration of the substrate was increased in the same solution without rinsing. To remove this factor, the second method was considered for calibration. In case of microbial reactions when the substrate concentration is increased and saturation behaviour sets is normally seen. In those higher concentrations, dilution of sample prior to quantitative measurement might be necessary. Calibration is described here as opposed to Chapter 4 to maintain the integrity of the arguments. The step wise procedure followed for calibration is presented in Figure 6.4.

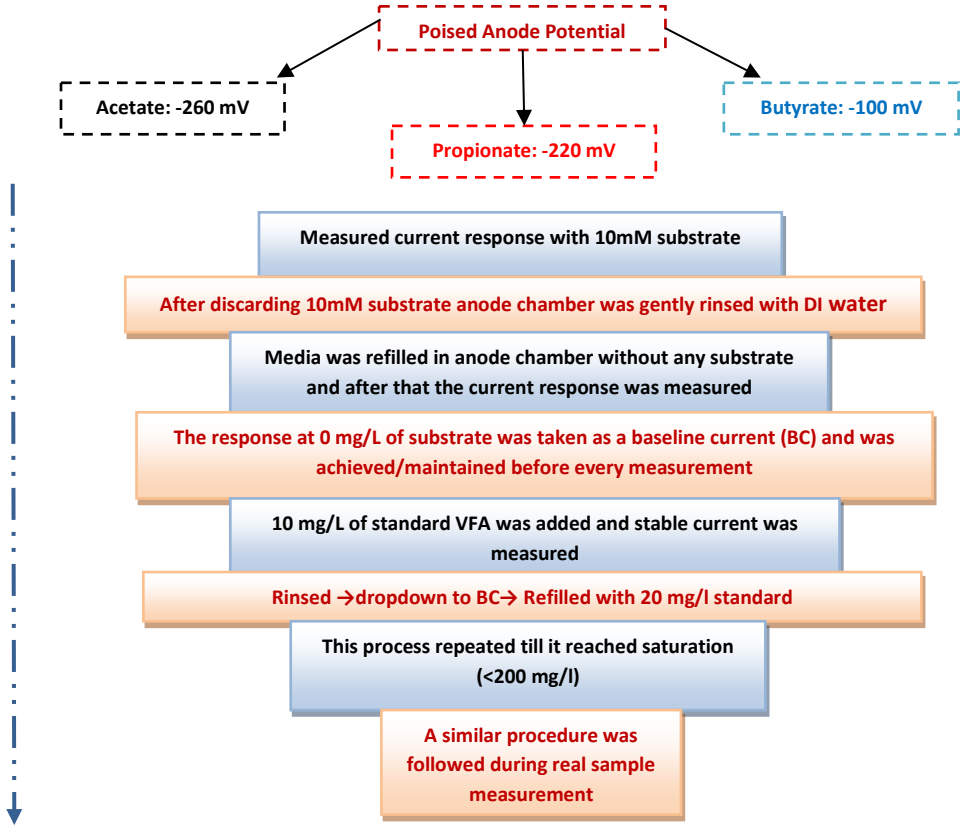

Figure 6.4: Stepwise calibration of the MFC sensor for measurement of real samples in the h-MFC and c-MFC.

The current responses at the fixed working electrode potentials were measured for each VFA sensor in response to a range of concentrations (0-200 mg/l) of the corresponding and other VFA species. The current outputs were recorded at an interval of 30 minutes, within which period the chamber was washed and refilled with the initial substrate concentration and the current was allowed to reach its initial state or baseline condition (BC). Before the anolyte was refreshed for each measurement, the anode chamber was washed using DI water to remove the residual substrate that may have remained on the chamber wall etc. The stable current was then plotted against the VFA concentrations, individually in AMFC, PMFC and BMFC, revealing linear relationships with relatively high R^2 values (0.97, 099 and 0.99 respectively). When corresponding VFAs were supplied to the sensors, the range of the sensor was increased from 40 mg/l to 200 mg/l with no response to other VFA species (in AMFC and PMFC). This implies that the poise potential did not alter the specificity observed during CV measurements discussed in Chapter 4 and maintained the selectivity and applicability of the sensor.

For the implementation of such sensors, it is important to reduce the working volume as much as possible. This will improve the dynamics of the sensor and possibly minimise the size of the sensor. The sensor volume was kept to a millilitre scale as micro-MFCs are still in their infancy and the small power output and high fabrication cost militate against their application in large numbers (Qian and Morse, 2011). Also owing to the flexibility in adopting favourable configurations for reducing internal resistances and improving mass transport, the best results have been achieved from millilitre scale MFCs. Considering all these factors, the working volume was reduced from 250 ml to 10 ml; with a commensurately dimensioned air cathode. Although the dynamic response may be improved further by reducing the sensor reactor volume to millilitre scale, the author found it difficult to enrich smaller devices, especially those to be acclimated with propionate and butyrate substrates. The 10ml c-MFCs were also calibrated using the same procedure that which was followed for h-MFCs and will be discussed later in this chapter.

6.1.2.2 Sample Sources and Preparation

Keeping in mind the application of the sensor presented in this study we considered the samples from tubular MFC and electrodialysis cell (ED Cell) running on complex substrates. One tubular MFC shown in Figure 6.5 (A[3]) enriched with acetate before it was fed on 10 mM of sucrose for 1 week and operated at 30°C. The voltage reached a maximum in 10-12 hours and the samples were then collected after one day from the start of fed batch cycle. A total of five samples were collected and were analysed for their VFA content through GC. The maximum and minimum concentrations of mixed VFA in all samples are summarized in Table 6.2. A total of five samples with varying range of mixed VFA concentration listed in Table 6.3 were collected from an ED Cell Figure 6.8 (B[4]).

[3] Tubular MFC was maintained on acetate by Hitesh Boghani as a part of his PhD studies. The same MFC was run on sucrose and samples were collected by the author.
[4] ED cell was run and samples were provided by Rhys Jones, PhD Student

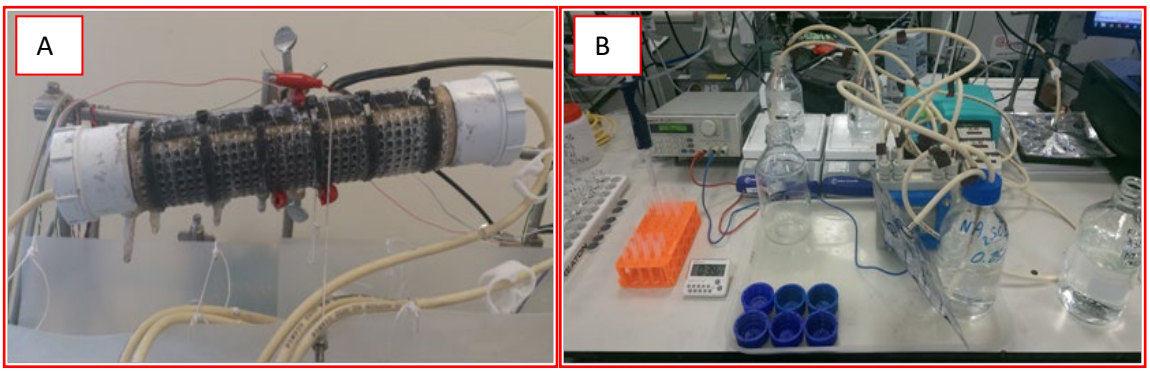

Figure 6.5: Sources of real samples (A) sucrose fed tubular MFC, (B) ED Cell

6.1.2.3 Measurement of 'Real' Samples in h-MFCs

The tubular MFC was continuously operated on 20 mM concentration of acetate as substrate. The samples were collected on alternate days for a week providing 3 samples i.e. *sample 1, sample 2 and sample 3*. The GC analysis was done before measuring these samples in the VFA sensor array, to ensure appropriate sample dilution. The GC measured concentration of acetate in each sample was 998, 839, and 818 mg/l with very low concentrations of propionate and no traces of butyric acid. The pH levels of the samples were checked and were found to be 6.4, 6.5 and 6.7. The samples were then filtered through a membrane filter of pore size 0.2 µm to remove all unwanted bacterial contaminants. After filtration samples were kept in a freezer to limit all biocatalytic reactions and were then measured simultaneously. After calibrations the anode chamber was rinsed and returned back to the baseline current for at least 15 minutes with blank (i.e. media without substrate). After a defined incubation period of approximately 5 minutes the samples were measured using the set up described in Figure 4.5. The current response was then compared with the calibration curve and the concentration measurement was determined. Figure 6.6 is showing the calibration of each VFA sensor alongside the responses obtained from the three samples derived from an unrelated but operational tubular MFC. The individual VFA sensor outputs of the concentration of acetate, propionate and total VFA (TVFA) in the tubular MFC are therefore presented for comparison with GC measures.

As the working volume of the h-MFC sensor was 250 ml, dilution and separate sample preparation was not required prior measurement; and the appropriate volume (5ml) of

sample was directly injected into the media used for calibration of the sensor arrays i.e. phosphate buffer with vitamins and mineral, keeping dilution within sensor range i.e. 20-160 mg/l for acetate. The current response obtained from *sample 1* reflected the concentration of acetate <20 mg/l as the sample was highly diluted due to the large sensor working volume. This concentration, corresponding to the stable current output, was then multiplied by the dilution factor to obtain the real VFA concentration (here acetate). For example the GC determined concentration of sample was 998 mg/l, which when diluted in measuring solution became 20.4 mg/l. When measured in h-AMFC the stable current gave a concentration value 19.9 mg/l. From these values real concentrations can be calculated.

The percentage error was then calculated as described in Section 3.9.3 from the MFC and GC responses and it was observed that the AMFC gives promising results to acetate concentrations with low percentage error <2.5% in all samples. Figure 6.7 shows the comparison of concentration responses obtained from MFC array with GC responses and Table 6.1 summarises the percentage errors calculated for MFC responses to *sample 1, 2, and 3* individually. The same sample i.e. *sample 1* was then measured in h-PMFC and in h-BMFC sensor to find out the concentration of propionate and TVFA and it was observed that the response in PMFC was even less than the zero substrate current value on the calibration curve. So it can be concluded that the propionate concentration in the sample was negligible or zero and also reflects the specificity of the propionate sensor to the respective VFA concentrations.

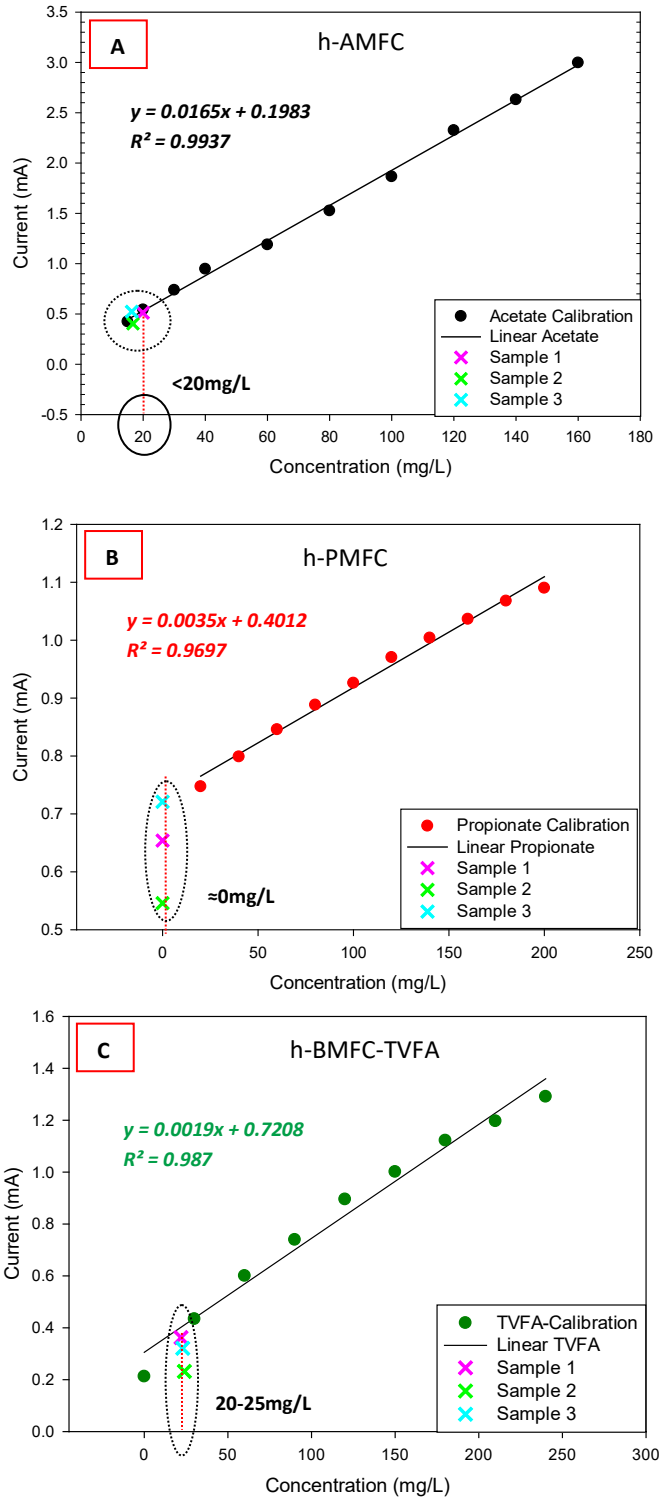

Figure 6.6: Shows the amperometric response of samples collected from scaled up MFC running on acetate as a sole source of carbohydrate, in array of sensors enriched and maintained on different VFAs at respective poised potential

Finally, *sample 1* was measured in the h-BMFC sensor to estimate TVFA concentration. The current output obtained from *sample 1* was 0.3621 mA, which corresponds to a

concentration of 22 mg/l. This when entered in equation 6.1 gives the TVFA concentration of 1078 mg/l with a 7.4% error when compared with GC values. The % error was higher with *sample 2 and sample 3*. It has also been observed and discussed in Chapter 4 that MFC gives higher current responses to acetate concentrations as compare to butyric acid when measured by CV. Similar results were obtained in this experiment and h-BMFC gives responses to all VFAs, and it was calibrated for various TVFA concentrations (Appendix 1). Although there was no butyric acid in all three samples, nevertheless the response obtained from the BMFC showed TVFA values with moderate percentage error comparable to GC. All together, from individual concentration measurements, the TVFA and butyrate concentrations were then calculated. In a similar manner another two samples were also tested in the array of individual VFA sensor and the real concentration was then calculated and compared with GC results. The error range for responses obtained from the MFC VFA sensors when compared to GC responses were found to be <2.5 in AMFC and <25.7 % in BMFC. As the error % in BMFC for TVFA concentration was considerably higher, it was difficult to estimate the correct value for butyric acid concentration in the samples.

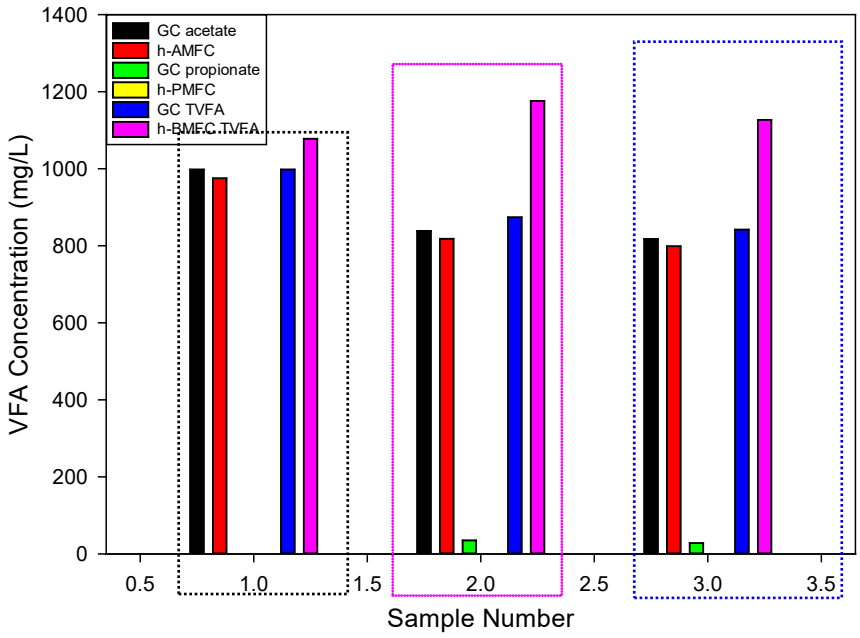

Figure 6.7: Comparison between VFA results from GC with those obtained from h-MFC sensor array

Table 6.1: Showing percentage error between the GC and MFC sensor responses obtained from three different samples collected from tubular MFCs.

Reactor/VFA response	Sample 1			Sample 2			Sample 3		
	GC	MFC	%Error	GC	MFC	%Error	GC	MFC	%Error
AMFC/acetate	998	975	2.3	839	818	2.5	818	798	2.4
PMFC/propionate	0	0	na	35	0	na	28	0	na
BMFC/TVFA	998	1078	7.4	874	1176	25.7	846	1127	24.9

It can be concluded from the response obtained from the h-MFC sensor array, to the samples from the tubular MFC, that the sensor can be deployed with decreasing working volume to enhance the applicability by reducing the dilution factor, giving less error of dilution in 10 ml reactors. So on the basis of the results obtained from h-MFC sensor array, further testing of samples from different sources were considered in the 10ml volume reactor/sensor.

6.1.2.3.1 *Comparison of Microbial Community Before and After Measurements (H-Type Reactor)*

An experiment to determine the changes in the biocatalyst community resulting from the measurement of 'real' samples was conducted. As it was not possible to collect anode samples from the 10 ml reactor due to the complexity of the set-up, the community analysis was done on anode samples from h-MFCs after testing number of samples obtained from tubular MFCs.

Figure 6.8 shows the DGGE profiles obtained from the h-AMFC, h-PMFC and h-BMFC before and after measurements were performed. Both archaeal and bacterial communities were considered in the investigation. In all three individual VFA enriched MFCs, it was observed that the community remained the same even after the exposure to samples. As the samples were filtered and pH was then neutralized, the chances of contamination which would alter microbial composition were very low. Although it would be interesting and important to study the community changed when the sensor is in continuous operation for online measurement of the sample, this has not been done to date.

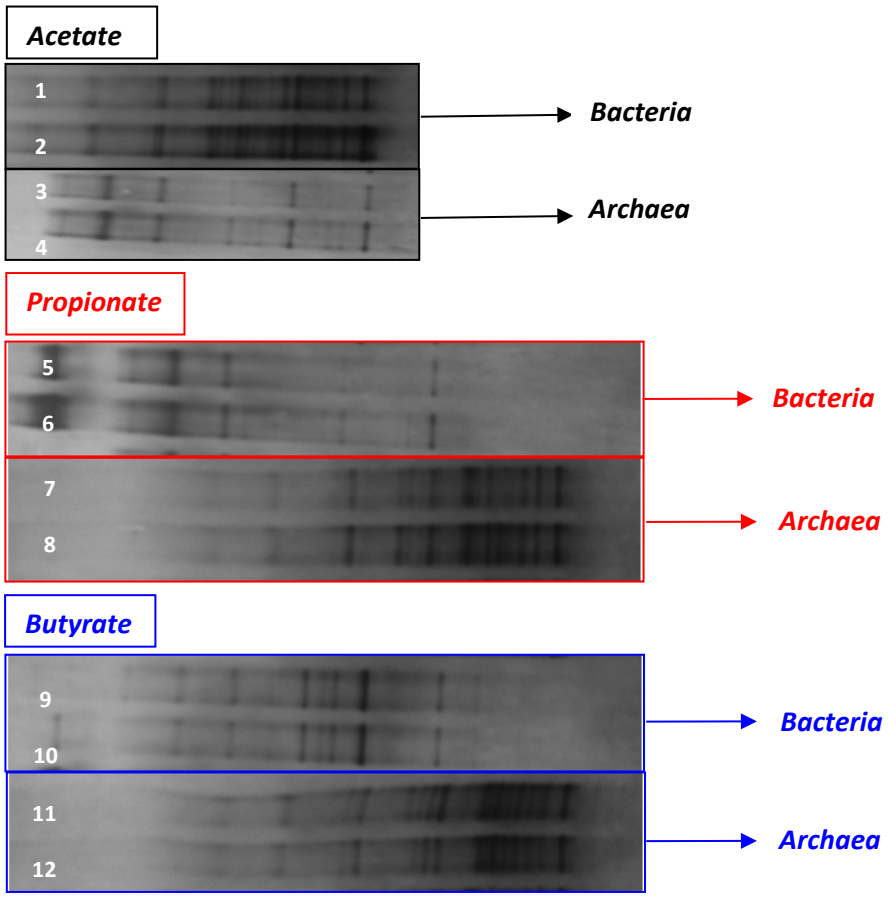

Figure 6.8: Bacterial and archaeal DGGE analysis of anode samples from individual VFA enriched h-MFC to check the effect of real sample measurement on microbial community.

It may be possible that the community would be affected by long term operation of the sensor which would affect the sensor's response. However it is also believed that with frequent calibration of the sensor system this would not be an insurmountable issue, though it may affect the applicability of the sensor. In summary, from results obtained from h-MFC sensor array we can say that the sensor could be used to discriminate VFA species when using anode poised potential; but as the concentrations were low in the measured samples, further analyses using a low volume reactor and more complex samples are needed for better understanding of the performance characteristics.

6.1.2.4 Offline Measurement of Samples in c-MFCs

A study of the application of biosensors to real-world samples is necessary before moving toward the ultimate ambition for any such device's commercialization.. As the volume of the reactor-MFC was 250ml, resulting in high dilution of the samples, the volume was reduced to 10ml in the c-MFC. The sensor responses were checked and

compared against the GC results. Also the concentration of the samples collected from tubular MFCs had very low or negligible amount of propionate and butyrate, so the samples from different feed stocks were considered and collected from different sources to check the reliability of sensor responses in the presence of other. The results obtained from 10ml c-MFCs are discussed as follows.

6.1.2.4.1 Acetate and Propionate Responses

A similar procedure to that described in Figure 6.2 was also followed to calibrate 10 ml c-MFCs. The range achieved and R^2 values are summarised in Table 6.2 with the equation of linear fit and respective static sensitivities also included.

Table 6.2: Shows the linearity obtained from calibration curves in each VFA-MFC with respective R^2 value and range

MFC	*VFA*	*R^2*	*Range [mg/l]*	*Equation of straight line fit*	*Static sensitivity*
c-AMFC	Acetate	0.9833	20-160	y= 0.0009x + 0.1731	0.1731
c-PMFC	Propionate	0.9833	20-140	y=0.0012x + 0.5098	0.5098
c-BMFC	Total VFA	0.9955	30-250	y = 0.0019x + 0.0164	0.0164

In order to test the ability of the 10 ml sensor array to mixed VFAs contained in samples, the specific potential was poised for the individual c-MFC using miniaturised Ag/AgCl saturated in KCl as reference electrode. Most of the samples obtained from the sucrose fed tubular MFC and ED cell were within the range of c-AMFC and c-PMFC and were not diluted further for measurement. All samples were measured twice and the mean value of current was plotted on the calibration curve (dotted lines), analyzed at the same time and under the same conditions, to derive a value of equivalent target compound concentrations.

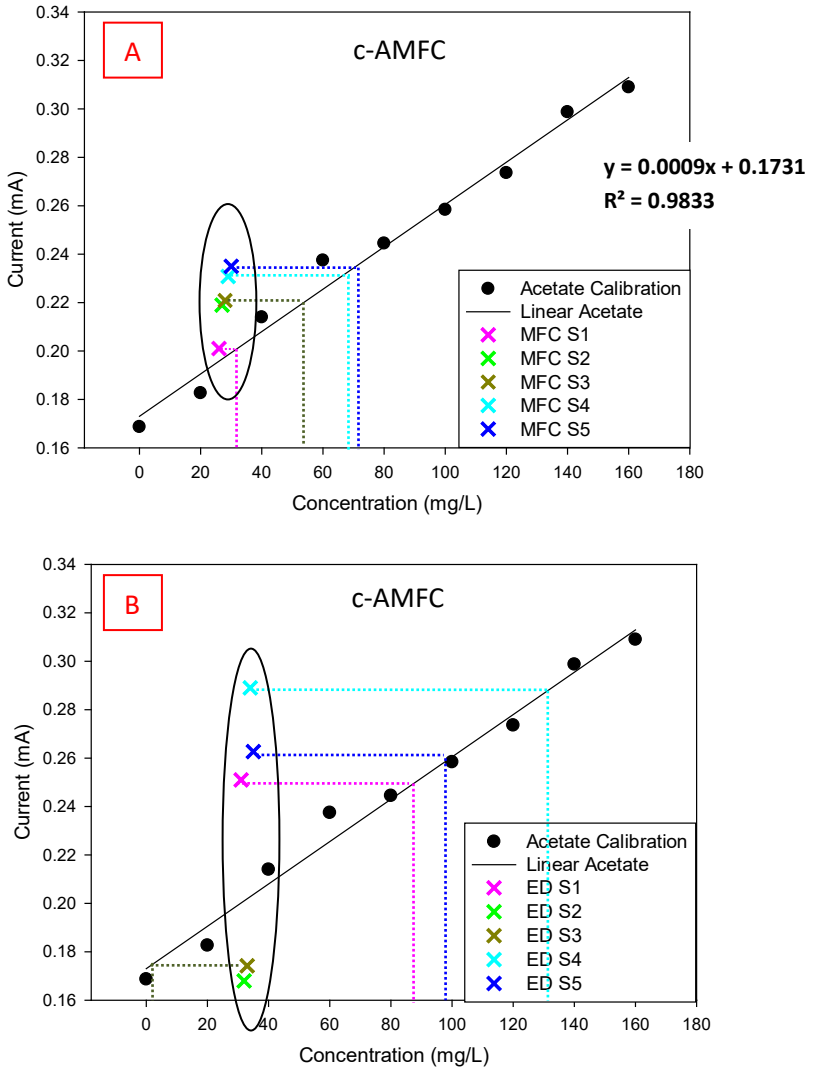

Figure 6.9: Shows the amperometric response of samples collected from (A) tubular MFC and from (ED Cell) in c-AMFC at -260mV anode poised potential Vs Ag/AgCl, saturated in KCl

Figure 6.9 (A &B) shows the typical responses obtained from c-AMFC with respect to each sample. The maximum and minimum concentrations of acetate in samples were 148 mg/l and 0 mg/l respectively. For zero concentration the response was <20 mg/l on the calibration curve, which indicates a low amount of acetate in the sample, possibly due to the degradation of the butyric acid in the samples. Both concentrations gave very close responses with <10% error when compared with GC response, even in the presence of other VFAs, which shows that c-AMFC could be a promising candidate for measuring acetate concentrations in low VFA content samples.

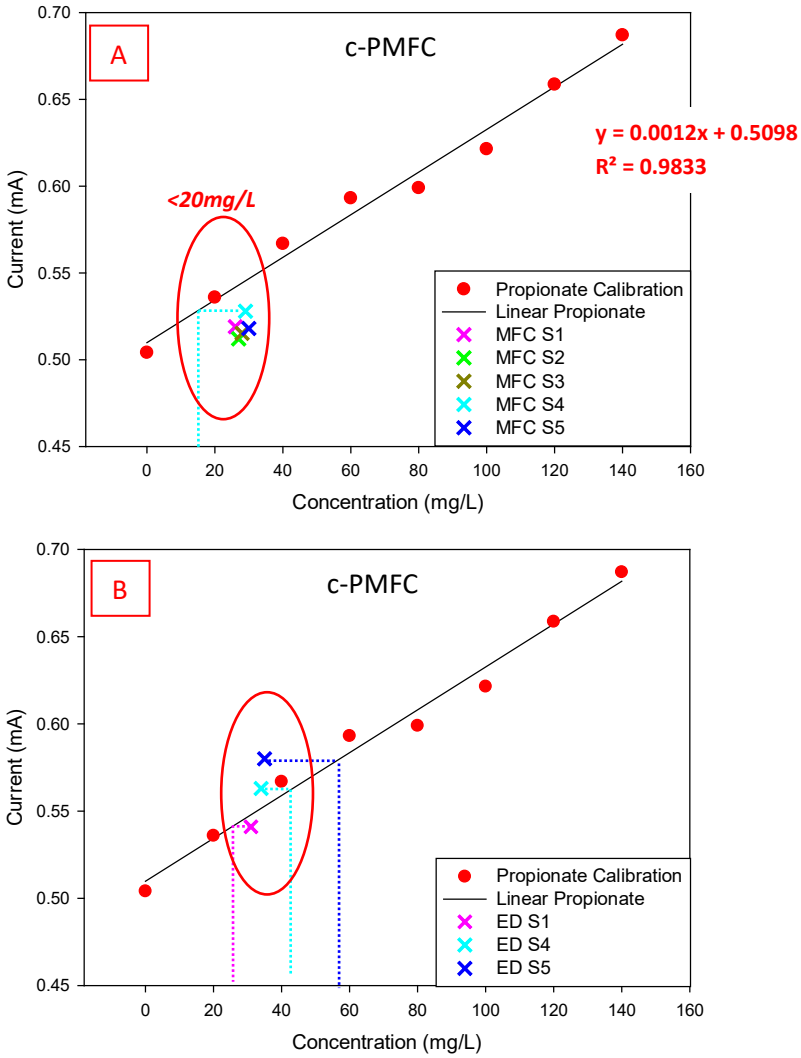

Figure 6.10: Shows the amperometric response of samples collected from (A) tubular MFC and from (ED Cell) in c-PMFC at -220mV anode poised potential Vs Ag/AgCl, saturated in KCl

The propionate sensor was then tested for measuring propionate concentrations within the samples and also the effect of other VFAs in the samples. In contrast to c-AMFC, the concentration for propionate in all samples was very low i.e. <70 mg/l with a maximum in the sample from ED S10; and the minimum was 0 mg/l. When measured in c-PMFC these samples showed responses <50 mg/l. Figure 6.10 shows the responses obtained from MFC and ED derived samples in c-PMFC. Although the concentrations were not measured exactly as by the GC, no higher current was generated even in the presence of high butyric acid concentrations. This shows that the propionate sensor was specific to its corresponding VFA and might be inhibited by other unknown factors, resulting in

lower response. It is important to analyse the system performance with a larger number of samples with higher concentration of propionate to assess how accurate quantification of the target compound is.

6.1.2.4.2 Total VFA Responses

The samples were diluted in the ratio of (1:2) wherever required, with the same buffer solution that was used during the calibration. As BMFC responded to all VFAs, it was calibrated for both butyrate and TVFA (Ac+Pr+Bu) concentrations with increasing and equal proportions of each VFA. Where c-AMFC and c-PMFC showed comparable results to GC measurements, it was found that in c-BMFC it was difficult to determine the accurate concentration of the VFAs. The current responses obtained in c-BMFC were higher than expected in almost all samples. The current responses obtained are shown in Figure 6.11 (A&B), which indicates a low accuracy and repeatability of the measurement outputs from c-BMFC. In Table 6.3 the highlighted red negative values of % error shows the greater responses and high % error with respect to GC responses. As the butyric acid enriched sensor always gives a higher response to acetate and propionate concentration as opposed to the butyric concentration, the higher current responses were expected. The synergistic effect of combined VFAs in samples affected more the c-BMFC, compare to c-AMFC and c-PMFC. The reliability of the sensor response decreases when butyric concentration in samples is ≥ 100 mg/l. In sample MFC S3 and ED S1, the concentration of the butyric acid was >100 mg/l, which resulted in the randomly decreased current output with high %error. This decrease might be due to the maximum detection limit of the c-BMFC for butyric acid, beyond which the concentration inhibits the microbial respiration.

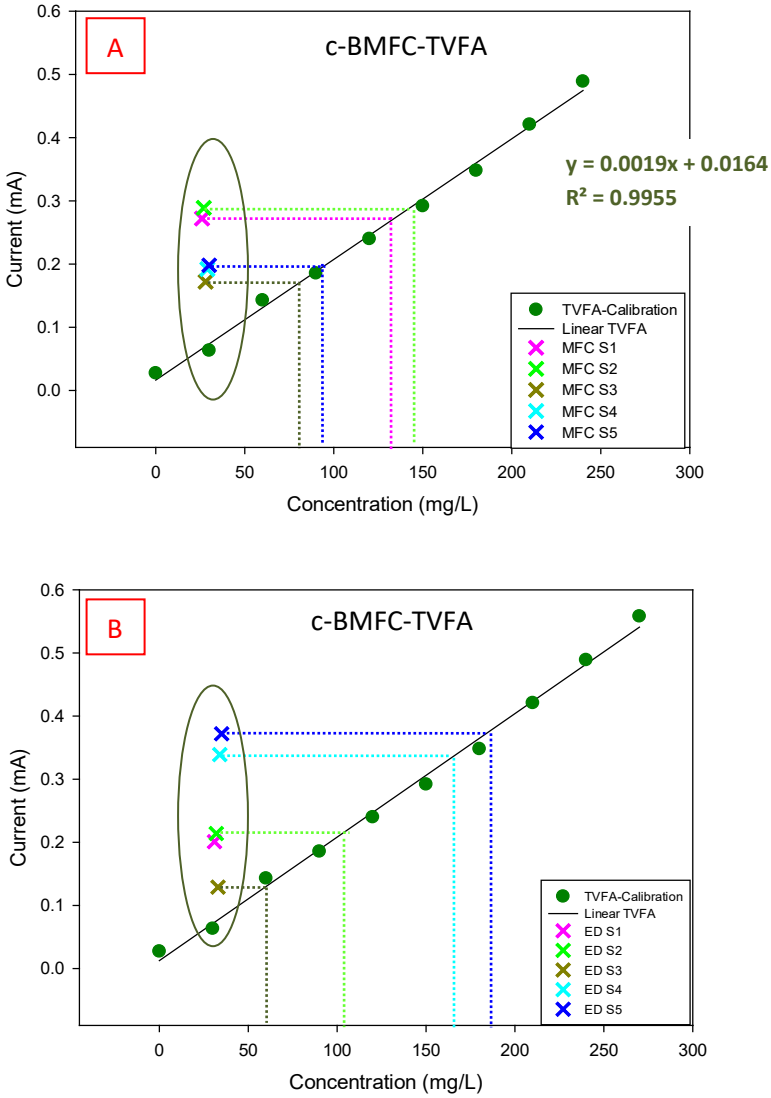

Figure 6.11: Shows the amperometric response of samples collected from (A) tubular MFC and from (ED Cell) in c-PMFC at -100mV anode poised potential Vs Ag/AgCl, saturated in KCl

Table 6.3: Summary of responses obtained from real samples in VFA-MFC array of sensor with respect to GC and their corresponding percentage errors calculates as in Section 3.9.3

		Gas Chromatography Response (mg/l)				MFC Responses (mg/l)					
Sample Number	*Source*	*Acetate*	*Propionate*	*i+n Butyrate*	*Total VFA (1:2) dilution*	*AMFC*	*%Error*	*PMFC*	*%Error*	*BMFC*	*%Error*
1	MFC S1	40	0	180	220 (110)	32	20%	<10	-	140	-21.4%
2	MFC S2	56	24	200	270 (135)	44	21.4%	<20	-	150	-10%
3	MFC S3	62	20	240	332 (166)	54	12.9%	<20	-	80	**51.8%**
4	MFC S4	84	20	158	262 (131)	68	19.04%	<20	-	100	23.6%
5	MFC S5	100	20	152	272 (136)	75	25%	<20	-	100	26.5%
6	ED S1(Sucrose)	62	32	242	336 (168)	88	-29%	24	25%	98	**41.6%**
7	ED S2(Sucrose)	0	0	72	72	<20	-	-	-	104	-30.7%
8	ED S3(Sucrose)	0	0	36	36	<20	-	-	-	62	**-48.4%**
9	ED S4(Grass)	148	68	76	282 (141)	134	9.45%	40	41.7%	172	-18%
10	ED S5(Grass)	72	72	144	288 (144)	98	-26.5%	58	19.4%	189	-28.8%

6.2 Conclusions

The results obtained showed a behaviour which might support interpretation from the developed array of VFA biosensors, when applied to environmental applications. They were able to generate significant responses, although high complexity in analyzing the samples may present interferences which defy simple analysis. In future, to obtain a more precise quantification, larger numbers of samples are required to establish the calibration for mixed VFA concentrations. Due to the mixed consortium of microorganisms the mechanisms behind the responses still needs to be understood for a clearer picture. Using membrane filtration processes for extracting specific VFAs could be another possibility to achieve clear response from MFCs. Where the acetate and propionate poised 10ml MFCs sensors gave comparable results, the butyrate sensor needs further calibration and deeper study is required to understand the complex chain of mechanisms. The degradation of butyrate by mixed consortia is not first order as it follows different metabolic pathways by different micro organisms and hence it was difficult to determine the concentration of the butyrate within the mixed total VFA solution. More analyses of the VFA biosensor are recommended, submitting it to different environmental samples. Convincing data have been produced which demonstrate a degree of robustness, selectively good measurement accuracy (in AMFC and PMFC) when compared with GC analysis. It is proposed with some confidence that the presented study on VFA sensing will contribute to understanding the place of MFC technology in the sensing of VFAs.

7 Methanogenesis Suppression in Microbial Fuel Cell based VFA Sensor

In addition to electricity producing bacteria in the MFC a proportion of the active community can be sustained by alternative metabolisms such as fermentation and methanogenesis and numerous catabolic processes, which utilize substrate but do not directly contribute in electricity production. Improving MFC performance by suppressing methanogenesis is an ongoing issue in the area of BES; including (MFCs). There are various factors which affects the performance of MFCs and various studies have already been published in this respect (Liu et al., 2005, Oh et al., 2004, Zhao et al., 2009). The energy diverted to the methanogenic archeae from substrates is of considerable importance, beyond the above mentioned factors (Chae et al., 2010a, Ishii et al., 2008b). External energy is required for methanogenesis to occur as it is thermodynamically and relatively unfavourable (Gerardi, 2003) and utilizes energy which affects the overall performance of any BES. Methane is also known as a green house gas and its emission is of great environmental concern (Wahlen, 1993, Minamikawa et al., 2006).

It would be advantageous to reduce substrate oxidation by metabolic processes other than those leading to electricity generation and thus increase the preponderance of electrogens to increase the overall performance of MFCs. This can be assisted by the elimination of methanogens from the anode. This is almost regardless of MFCs application, which may include scaled-up systems for treating industrial or municipal wastewaters; or small devices used as sensing elements. However, in the context of this thesis, the effect of methanogenesis and other notionally parasitic reactions are of considerable importance to the measurement of biodegradable components in a waste or effluent stream. It has already been investigated by Feng et al., (2013b) that the inhibition of methanogens in a MFC based sensor can improve sensor response by delivering a lower detection limit which can be generated by a higher peak area, a higher CE or a wider dynamic response range. However for potentially large scale MFCs on complex waste treatment, which target effluent polishing for example, a very high CE may not be as important. The focus of this study was to inhibit methanogenesis by using different operating strategies. A novel approach based on different open circuit /closed

circuit (OC/CC) operating regimes and starving[5] the MFCs is considered, which may further enhance the sensor response and its performance. The relevance of the operating strategies was studied by completely removing the methanogens from the reactor, depending on the field of application.

An investigation into the suppression of methanogenesis was conducted using h-MFCs and results obtained are discussed in this chapter. Also, the metabolic pathway used in the oxidation of recalcitrant fermentation end products such as propionate and butyrate, known to be difficult to degrade in bioprocesses, were investigated in these BES. A preliminary set of experiment was set up to study the degradation patterns of individual VFA enriched MFC over 5 days in OC, followed by 5 days of CC operation. The results obtained were analysed to see the effect of this operating regime on methanogenesis, leading to a second set of experiments with different OC/CC regimes discussed later in this chapter.

7.1 Results and Discussion:

7.1.1 Open and Closed Circuit Operation (5days each)-Substrate Utilization

h-MFC reactors were fed on 20 mM of respective VFA concentration and were then left for 5 days of OC operation. During the latter, liquid and gas sample were taken each day and were analysed to see the rate of utilisation of the substrate. After 5 days of OC operation the anode chamber was refilled with fresh media and 20 mM of respective VFA. The reactors were then operated for 5 days in CC and liquid and gas samples were taken and analysed. Figure 7.1 shows that the metabolic pathways for the degradation of propionate and butyrate were affected by the electrode reaction, which could overcome the effects of VFA accumulation in e.g. an anaerobic digester. VFA degradation was more rapid in the reactor in CC operation than in OC operation. The starting concentration of each VFA in both OC and CC was 20 mM and at the end of 5 days operation in OC the substrate consumed was significantly less than in CC i.e. 1350

[5] Starvation experiment was conducted by Hitesh Boghani, PhD student and published together with this work as Kaur et al 2014, Bioresource Technology (Accepted)

mg/l and 1620 mg/l as compared to 392 mg/l and 253 mg/l in PMFC and BMFC respectively.

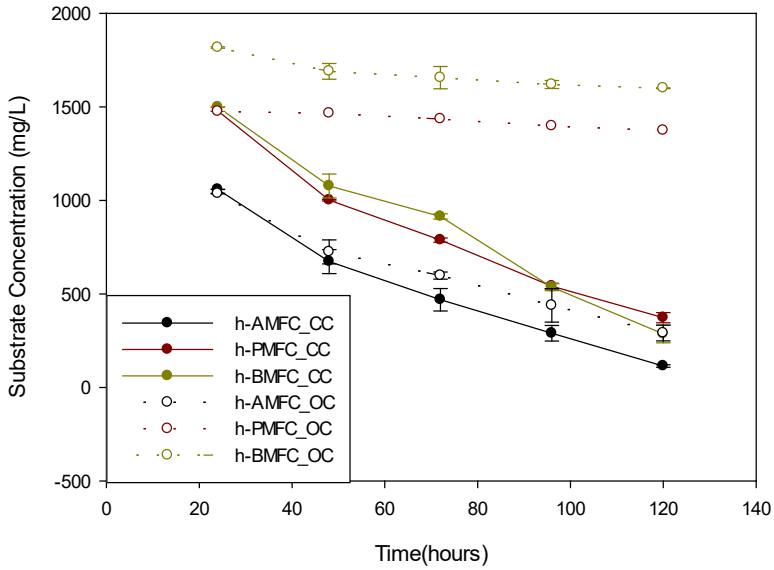

Figure 7.1: Headspace gas analyses from individual VFA reactors during OC and CC operation. Error bar represents range between duplicate experiments.

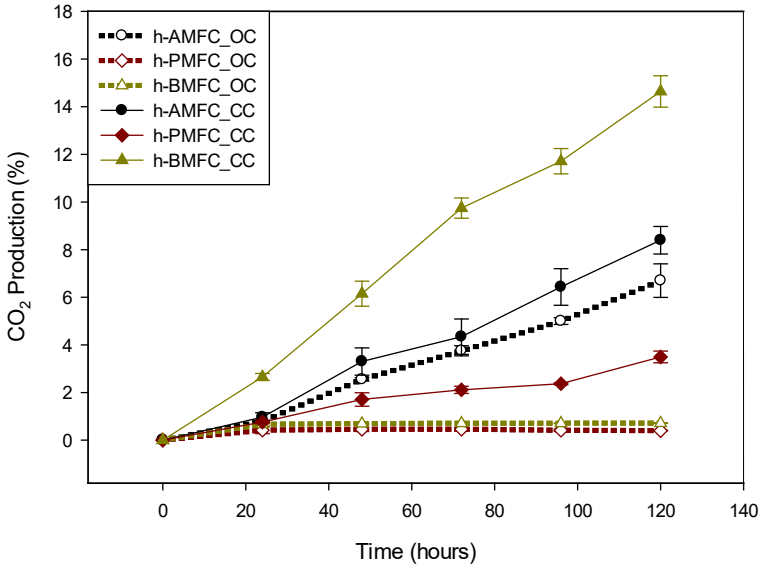

Figure 7.2: Headspace gas analyses from individual VFA reactors during OC and CC operation. Error bar represents range between duplicate experiments.

Acetate eventually degraded in both systems, in contrast to propionate and butyrate and the consumption rate of acetate was much faster in the CC operations. In the h-BMFC, concentration of acetate (<250 mg/l) was also measured and showed that butyrate first degrades to acetate, which then contributes to the electricity production. On the other hand no acetate in h-PMFC was detected in OC operation but very low fractions of acetate (<100mg/l) were produced during CC operation. Figure 7.2 shows that propionate and butyrate degradation resulting in the accumulation of 3.4% and 14% of CO_2 in the headspace respectively, after 140 hours for the CC operation. Carbon dioxide production also occurred in OC, but not until 24hours had lapsed and at a lower final concentration <0.7% in the headspace. However, methane was not detected in the h-BMFC and in very low concentrations in h-PMFC in either OC or CC operation.

The preceding results presented in Figure 7.1 and 7.2 on enhanced degradation of recalcitrant organic compounds shows that the electrode reaction (acting as terminal electron acceptor or a simple bacterial attachment medium), plays a very important role in the degradation of propionate and butyrate. The slower but effective removal of propionate and butyrate has been studied by Liu et al.,(2004a), Rabaey et al.,(2005). In this study, OC and CC operation reveals that MFC technology can be used to overcome thermodynamic hurdles which normally require syntrophic methanogenesis to proceed. It is suggested that propionate and butyrate can be more efficiently degraded in MFCs, compared to the syntrophic degradation in an anaerobic digester (Liu et al., 2004a, Deepak Pant et al., 2010). However, the metabolic pathway is not clearly understood in either OC or CC operation, but the dependence of VFA degradations on the electrode reaction is clear from results presented in Chapter 4 and 7. The results obtained from DNA analysis indicate a predisposition of the microbial community to be in solution when there is no current flowing and no terminal electron acceptance by the electrode (discussed in detail in Section 7.2.2). However, this phenomenon varies for different species of VFAs. The effect of current flow on VFA degradation and the consequent microbial products in the anode liquid phase and headspace were analysed for each MFC for different VFAs and OC/CC modes. An enhancement of the degradation was sought, for the recalcitrant fermentation end products such as propionate and butyrate using these BES, hypothesising that the syntrophic relationship between acetogens and

methanogens can be replaced by an electrode reaction. This manner of syntropism bioelectrochemically could be useful in enhancing the sensor's discriminatory responses to these recalcitrant products in developing whole cell based VFA sensors.

7.1.2 Methane Inhibition during different OC/CC Operating Regimes

The suppression of methanogens affects the CE of MFCs, although the importance of methane suppression is application dependent; i.e., avoiding methanogenesis may be essential for MFC based sensors in order to increase accuracy but methanogenic activity may be less critical in scaled-up MFCs designed to remove COD from waste streams. From the perspective of MFC VFA sensing, methane suppression is desirable as a high CE is necessary to discriminate the concentrations of VFAs in complex matrices that would be found in environmental or wastewater samples. The illustration of the experimental set is described in Figure 7.3 as shown below.

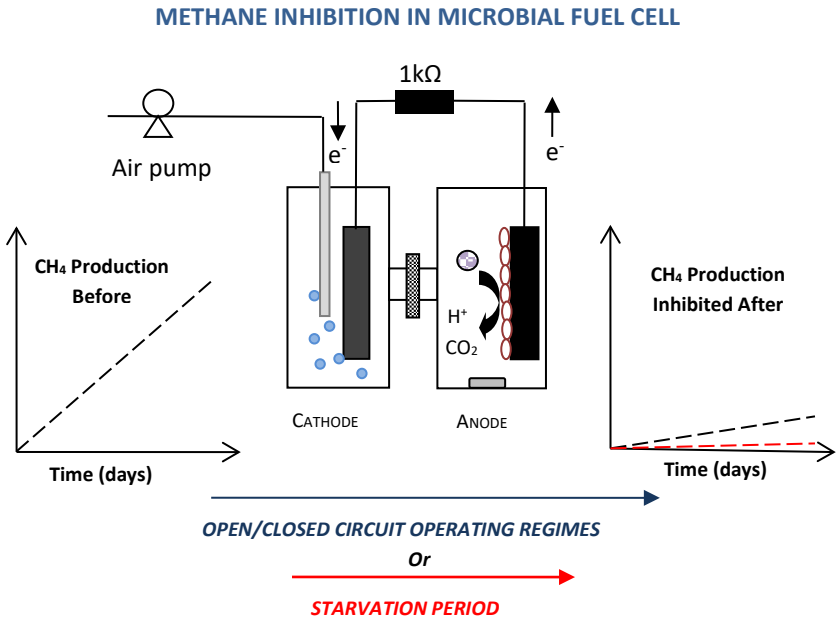

Figure 7.3: Illustration of the experimental set up for open/closed operation in MFC reactors fed on acetate, propionate and butyrate individually and starvation of the MFCs fed on acetate.

On the basis of results obtained from 5 days OC/CC operation, further investigations of MFCs fed on VFAs was conducted to understand its effect on methnogenesis. The subsequent study presented here, investigates for the first time the effects of different durations of open and closed circuit operations on the inhibition of methane production

and on methanogens in MFCs. Prolonged lack of substrate supply to the anode and its effect on methanogens and electrogens were also studied. Two different sets of h-MFCs, as described in Section 3.3.4, were utilised for the study and results were analysed. The methanogenic community on the electrode and in the liquid phase from an MFC operated under OC (no current generation) and CC conditions over different periods of time were compared using DGGE analysis. The results obtained from this study are presented and discussed as follow:

7.1.2.1 Gas Analyses during OC/CC and Starvation Period:

Gas composition in the headspace of the anode was analysed in each MFC while they were operating on different OC/CC regimes. As observed in 5 days OC operation followed by 5 days CC experiment the VFA degradation was more rapid in the reactor under closed circuit than in open circuit operation, it was the case in all OC/CC regimes. Depending upon the time period for which the MFCs were operated in different operational states, different concentrations of the respective VFAs were introduced into each reactor. The starting concentration of each VFA in both OC and CC operation was 10 mM for 10 days and 40 mM for 1 month operation. It was observed that in all reactors, the percentage of methane in the head space from the open circuit state (Figure 7.4 A, B, and C) was higher than in closed circuit state i.e. 8.8%, 3.2% and 4.7% for acetate, propionate and butyrate respectively. Also, methane production was relatively low or negligible (<0.1%) after 1 month of CC operation. This indicated that a difference in redox potential might have caused the different behaviours of the bacterial community during operation in both circuit inter relationships (Ishii et al., 2008b). There was no methane detected in the MFC reactor running for more than 1 year on closed circuit, after having been subjected to various OC/CC operating conditions, which further supports the notion that methanogens are electrode-independent microbes (Chae et al., 2010a) and are deleteriously affected by current flowing through an external circuit when in an electrogenic community in the anode chamber, either by direct effects on their metabolisms or by an inability to compete with the affinity demonstrated by electrogens to the VFA substrates (Kim et al., 2011).

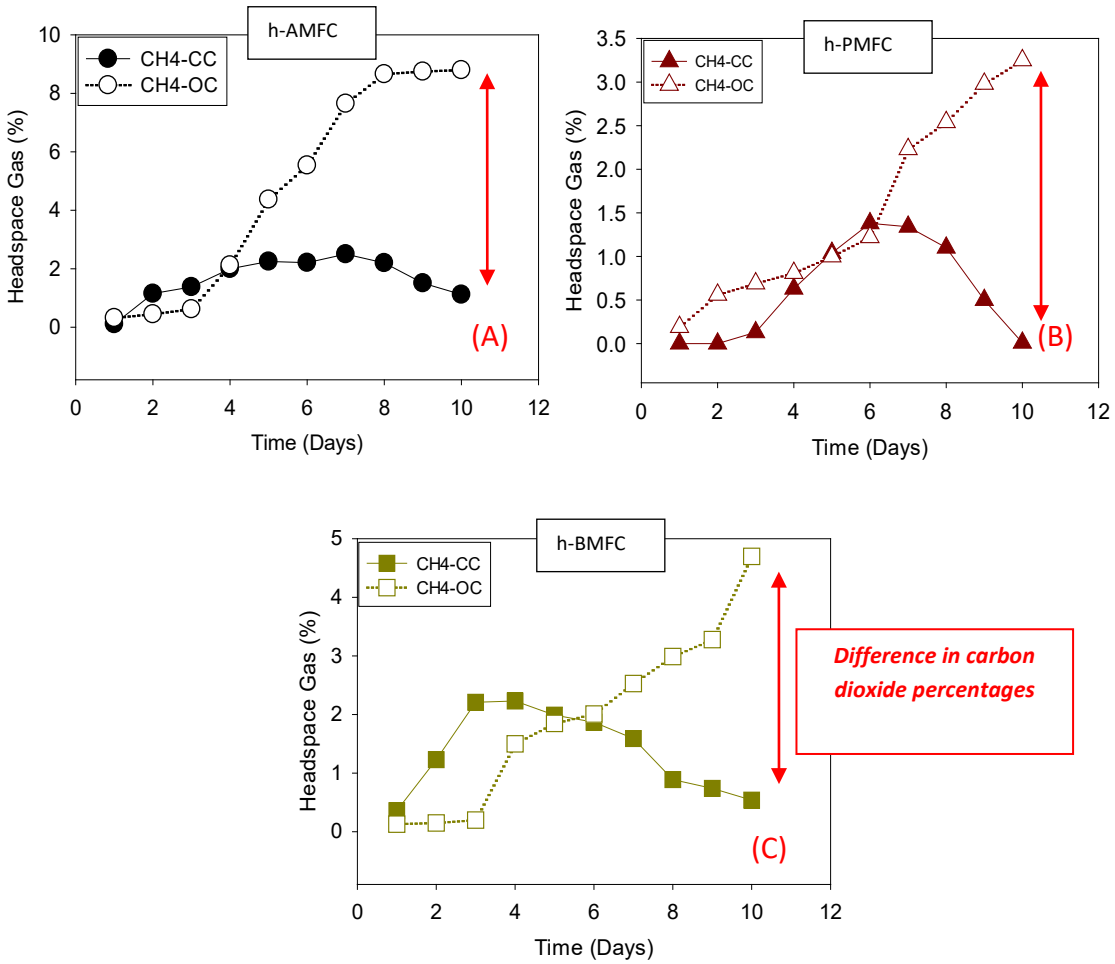

Figure 7.4: Head space percentages of methane from individual VFA reactor during 10 days open/closed circuit operation (A) Acetate (B) Propionate and (C) Butyrate.

It can also be seen in Figure 7.4 (A, B and C) that the small percentage of methane produced, evidenced by the concentration of methane in the headspace, during CC operation then decreased, which might be due to the high production of CO_2 affecting partial pressures in the headspace volume or less likely, due to the presence of methanotrophs.

For the same concentration of acetate, propionate and butyrate the headspace carbon dioxide concentration was relatively high i.e. >14%, >4% and >9% in contrast to the associated methane percentage in AMFC, PMFC and BMFC respectively during closed circuit operation (Figure 7.5 A, B and C). This indicates that the availability of the electron acceptor (anode electrode) enhanced the degradation of VFAs in CC as compare to OC in which the electrode would polarise without current flow. Due to wash out (during

media replacement) of the methonogenic community, in addition to planktonic bacterial community at the end of 10 days of closed circuit operation, methane was only present in the headspace at very low percentages (<0.1%); totally replaced by very high percentages of CO_2. The growth of methanogens as compare to other bacterial species in the MFC reactor takes a longer time to establish. This might have affected the recovery of methanogens after OC/CC operation and may be partially responsible for low methane percentages in the headspace.

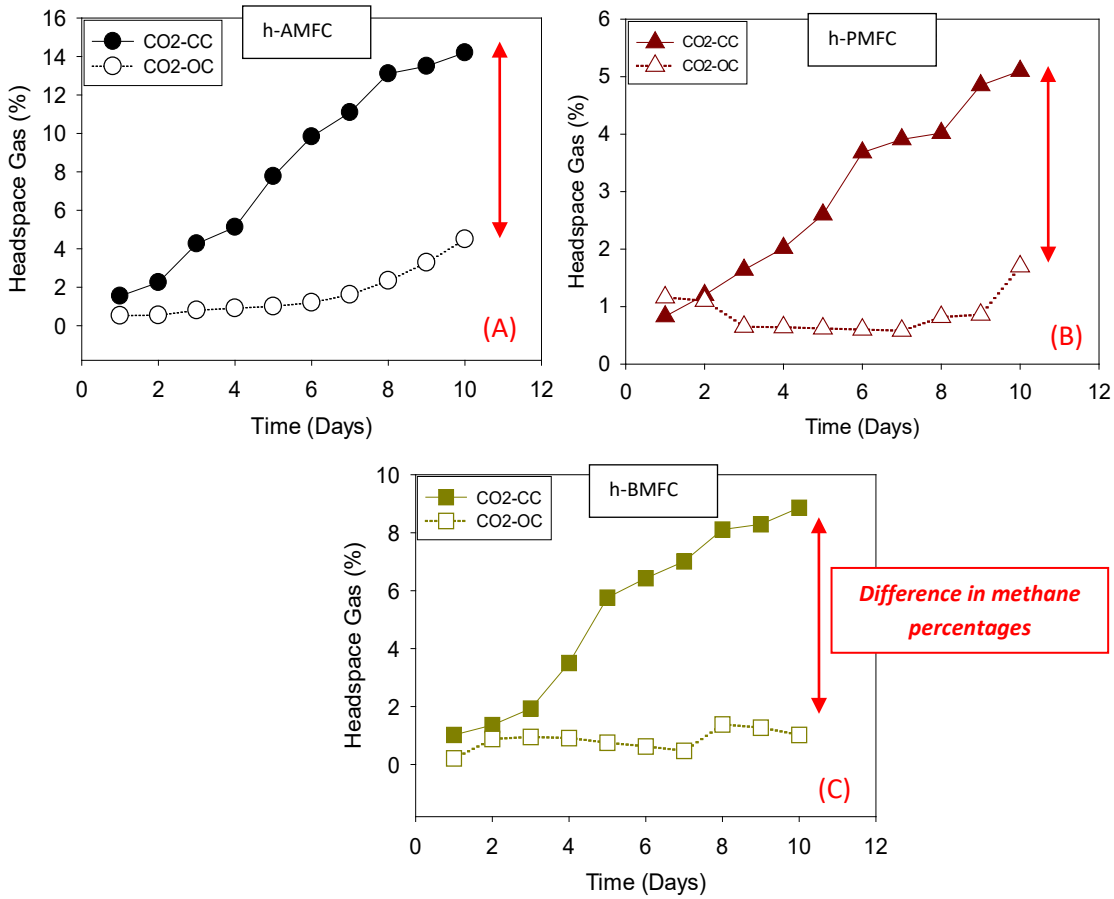

Figure 7.5: Head space percentages of carbon dioxide from individual VFA reactor during 10 days open/closed circuit operation (a) h-AMFC (b) h-PMFC (c) h-BMFC.

Table 7.1 shows the CE values from a MFC operated with maximum peak power point tracking (MPPT) which aims to maximum the instantaneous power production from the cell. The control strategy was published by Boghani et al., (2014). Furthermore, the results of gas analyses before and after starvation are included in Table 7.1. The MFCs were operated on acetate as sole source of chemical energy substrate and carbon. The methane was evident in the head space of anode chamber and % concentration was

measured as an indication of its production in all acetate fed MFC reactors. The percentage of methane increased when MFCs were fed with substrate concentration over a range of 0.5-5 mM acetate. The CE was determined to be between 61% and 87% prior to the imposition of a period of starvation. Methane was no longer detected in the anode chamber after a starvation period of 12 days. CE was affected and significantly lower than previously determined for 1 mM concentration of acetate (59.1% as compared to 86.77%). However, the drop in CE recovered to 86.98% during a batch cycle with 5 mM of acetate. There was no methane detected after feeding recommenced, providing sufficient substrate to the anode chamber (1 mM and 5 mM in batch cycles). This indicates that starvation may have affected bacterial growth negatively in the short term and archaea more dramatically, with the electrogens capable of utilised the energy supplied to recover without any further diminution of CE. As there was no methane detected even after providing substrate it is possible that deficiency of substrate supply might have allowed oxygen to diffuse through the membrane, this affecting methanogens specifically. Lack of CH_4 in the headspace gas samples taken after the starvation period suggests that the microorganisms responsible for producing methane (methanogens/hydrogenotrophs) were eliminated simply by depriving them of their food/substrate

Table7.1: Gas percentages in the headspace before and after substrate consumption and coulombic efficiencies in MPPT operated MFC

Substrate Concentration (mM)	Before /After Starvation	Start (All gases in %)				End (All gases in %)				CE (%)
		CH_4	CO_2	H_2	N_2	CH_4	CO_2	H_2	N_2	
0.5	Before	0	0.56	1.96	91.74	0.65	0.86	0.01	92.42	61.11
1	Before	0	0.65	1.23	92.49	0.98	0.44	0	93.35	86.77
2	Before	0	0.57	1.17	94.46	1.68	3.62	0	87.98	71.55
1	After	0	0.85	1.15	92.38	0	2.14	0	92.78	59.1
5	After	0	0.53	1.79	93.41	0	16.63	0	86.22	86.98

Comparing CO_2 (%) before and after starvation for the 1 mM concentration, it was observed that the CO_2 (%) was substantially lower before starvation (0.44%) compared to after starvation (2.14%). This might indicate that hydrogenotrophs could have been present (Conrad and Klose, 1999) to produce methane from CO_2 and H_2. A very high percentage of nitrogen was also detected in all gas samples. The media in the anode

chamber was always replaced by placing the MFCs in an anaerobic glove box which resulted the gas composition in the headspace being replaced with nitrogen (> 90%) and hydrogen (< 2%).

7.1.2.2 Molecular Analysis

As the methane percentages obtained during each OC/CC operations were found to be substantially different, the effect of OC/CC operation on the methanogenic community in individual MFC before and after the OC/CC regimes became an area of interest. The samples were collected from the electrode and solution during and after OC/CC operation. The DNA was extracted as described in Section 3.11.1. After extraction of the DNA amount present in both electrode and liquids samples was measured in ng/µl as discussed in Section 13.11.1. The different DNA concentrations measured from each sample are summarized in Figure 7.5 (A, B and C) showing a predisposition of the active biomass from electrode to migrate to the solution during OC operation. This is not entirely unexpected as living cells are typically charged. Hence, the results indicated the movement of biomass from electrode to the solution. The amount of DNA (ng/µl) in liquid was measure before starting OC/CC and was almost negligible and hence not shown in the Figure 7.5. However, after operating the MFCs on different OC/CC regimes the amount of DNA was found to have increased in suspension.

In Figure 7.6 it can be clearly seen that the amount of DNA on electrode before OC/CC operation was very high in all three individual enriched MFC reactors i.e. 326 ng/µl, 373 ng/µl and 319 ng/µl. After 10 days of OC operation, again samples were collected from the liquid phase and the electrode, which showed a decrease in DNA content on the electrode and increase in suspension. For example in the h-AMFC electrode and liquid phase DNA concentration after OC changed to 172 ng/µl and 102 ng/µl as shown in Figure 7.6 (A). Also, when the same reactor was operated in CC for 10 days, the amount of DNA was found to be far less when compared to OC operation i.e. 42 ng/µl in liquid and 136.5ng/µl on electrode.

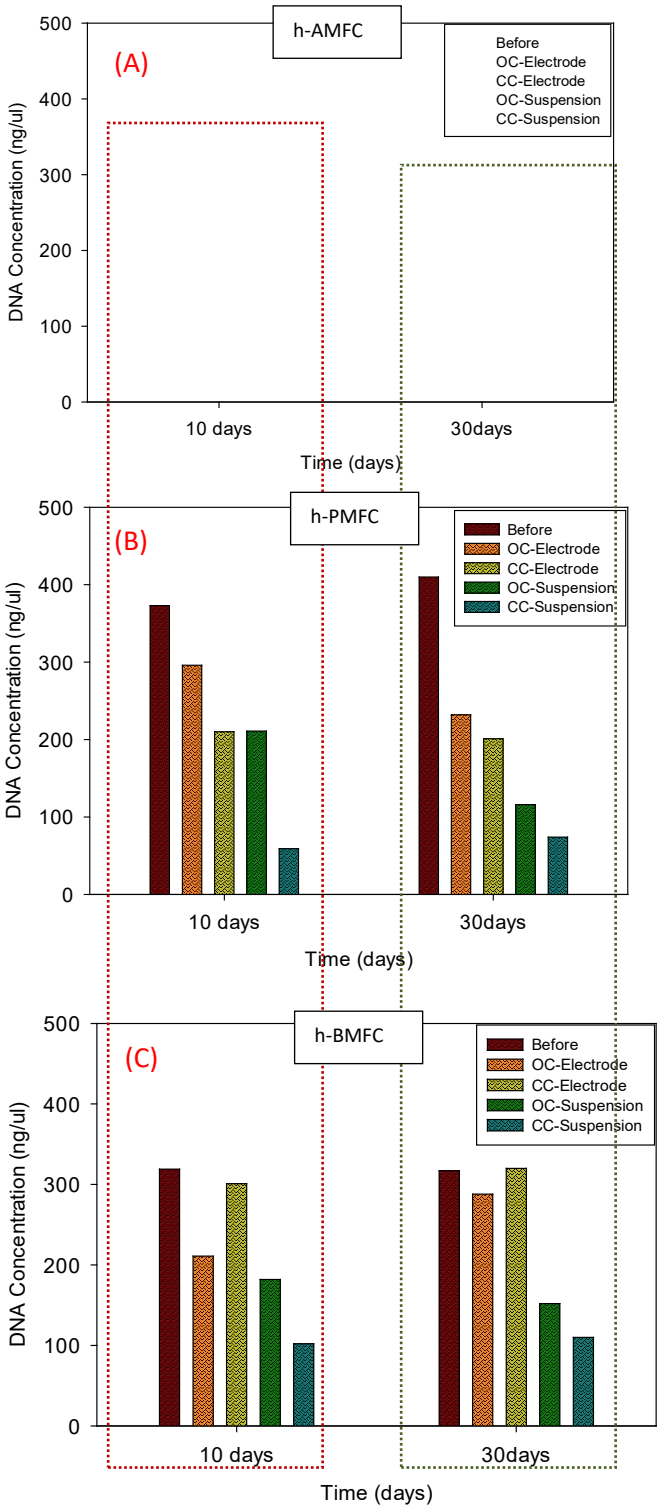

Figure 7.6: DNA concentrations in ng/µl measured on the electrode and in the liquid phase, before and after operating MFCs with individual VFA as substrate (A) acetate (B) propionate and (C) butyrate on different open and closed circuit regimes.

In the case of h-PMFC, similar results were obtained. In Figure 7.6 (B) the amount of DNA was high before starting in both 10 days and 30 days of operation i.e. 373 and 410 ng/µl, which then dropped down to lower concentration as can be seen in Figure 7.6.

Although in the case of the h-BMFC the concentration on the electrode after CC operation was found to be higher than OC operation, which might indicate that the microbial community recovered at a faster rate in h-BMFC as compared to h-AMFC and h-PMFC. Also it has been observed that the level of biomass on both the electrode and in the liquid phase in CC were lower than OC suggesting more active electrogens in CC and lower diversion of electrons into biomass, an observation which has also been made by Michie et al., (2011). This shows a tendency for active biomass to leave the electrode during open circuit, and this is specifically true of methanogens, which was confirmed by DGGE profiles obtained from open and closed circuit operation. This indicates that most organisms preferred to adhered to the anodes in the CC reactor, while planktonic cells were abundant in the OC reactor, an observation also made by (Ishii et al., 2008b).

The archaeal community analysis was done by using the DGGE technique to determine if any differences existed between methanogenic communities associated with individual VFA enriched MFCs during OCand CC operation. The archaeal community in both suspension and electrode were only identified before OC/CC operation and the most dominant methanogenic genera in all three individually enriched reactors were *Methanosaeta, Methanobrevibacterium*, *Methanothermococcus* and *Methanobacterium*. A very small fraction of some *Petrimonas, Methanosarcina, Methanosphaera* etc. were also present. Samples were taken after completion of every OC and CC operation. Figure 7.6 shows the difference between archaeal DGGE profiles before open/closed circuit operation and after 10 days, 1 month and 1 year operation in individual VFA enriched reactor. It was observed in the DGGE profiles that the density of archaeal bands reduced on the electrode, depending upon the OC operation period. Also the DGGE profile from the liquid samples showed some archaeal bands, which indicate the removal of methanogens from the electrode and shifting to the solution during OC operation.

In Figure 7.7 (A, B, and C) the red highlighted bands/methanogenic community were removed from the electrode, which was supported by the inhibited methane production in the respective reactors. Similar results were obtained in h-AMFC and h-BMFC as well (Figure 7.7 B and C).

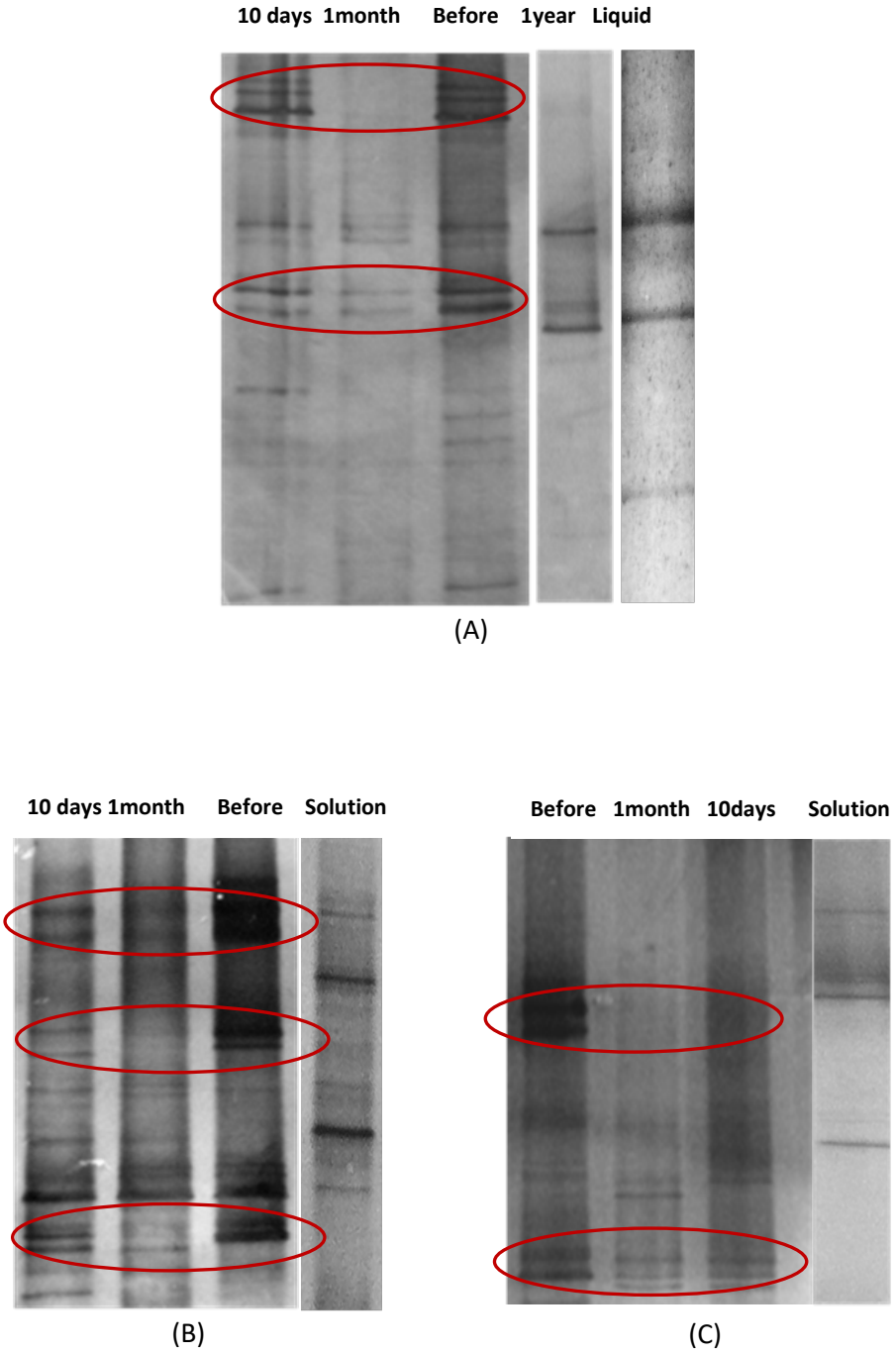

Figure 7.7: PCR-DGGE profiles of anode archaea communities obtained from the (A) h-AMFC, (B) h-PMFC and (C) h-BMFC after different OC/CC operation

After one year of closed circuit operation, only few common dominant species were found on the electrode in all the reactors, which shows the progressive elimination of methanogens from the electrode with time. DGGE profiles from solution during OC operation also indicates the movement of some biomass, both electrogens as well as methanogens, to the solution as shown in Figure 7.7. During open circuit, most of the methanogenic bacteria left the electrode and shifted to the liquid phase with small percentages of electrogens following suit. Bacterial DGGE profiles were also studied and it has been found that not much difference existed in the profiles from both open and closed circuit. This indicates that open/closed circuit operation affects methanogens more than electrogens.

On the basis of the results obtained from this study we can deduced that this work points out that the relevance of suppressing methanogenesis strongly depends on the application and areas of use. Mechanisms capable of inhibiting methanogenesis are of considerable interest, particularly in the study of methane production from ruminants and putative emissions from anaerobic processes. However, *in vivo* implementation of the mechanisms considered in this study may not be directly applicable, although plausible devices to displace methanogens might be inferred. Nevertheless, in BES or MFC applications, which may include scaled-up systems for treating industrial or municipal wastewaters; or small devices used as power sources for e.g. sensing elements, methane inhibition may have environmental and/or performance benefits.

In a multi-modular reactor, the CE varies between modules according to the substrate availability to electrogenic bacteria and their corresponding affinity in contrast to methanogens (Fradler, 2014). Furthermore, with other alternative electron acceptors such as nitrate-, sulphate- or with metal reducing bacteria (He et al., 2005, Logan et al., 2006, Schroder, 2007a), where lower CE may result when redox components in the media are directly and chemically oxidized and therefore artificially enhance the CE. The present study reveals for the first time suppression of methane production in a MFC by operating MFCs on different time scale of open and closed circuit regimes and by not providing enough substrate to the enriched biofilm. The methanogens can be outcompeted as electrogenic bacteria exhibit higher substrate affinity than methanogens when systems are operated on different OC/CC regimes. A cross inhibiting

method such as leaving the reactor for starvation period after open circuit operation, may lead to improved performance and also may have less effect on the bacterial community involved in electricity generation. This finding may also be used to find out whether the electrode reaction (acting as terminal electron acceptor or as a simple bacterial attachment media), plays an important role in the degradation of propionate and butyrate and whether this principle can be implemented in an anaerobic digestion process to increase its overall performance.

7.2 Conclusions

The findings within this chapter show that there are ways to prevent methanogenesis in MFCs, but depending on their application it has to be decided discretely, whether it is essential, as in sensors or of less important, as in scaled-up MFC reactors for COD removal. It may be particularly important to inhibit methanogens in MFC based VFA sensors, where methanogens might utilize the substrate and therefore affect the current response which will not then represent the concentration of a substrate component. However, *in vivo* implementation of the mechanisms considered in this study may or may not be plausible. Also the significance of such inhibition is likely to be application specific and more important in circumstances where non-electrogenic and parasitic reactions are deleterious to system performance. Whereas OC/CC operation reduced methanogenesis, starvation virtually eliminated methanogenesis in the MFC. Also, as methane emission is of great environmental concern, these results on suppression of methanogenesis in MFC system have important implications.

8 Discussion

The work of this thesis has indicated that it was possible to develop a proof of principle for a MFC based biosensor for the determination of the VFA (acetate, propionate and butyrate specifically) concentrations and to an extent their particular chemical species. The biosensor was prepared by acclimatizing different MFCs with the three individual VFA mentioned, in order to establish differing susceptibilities to a mixed sample of VFA and so be able to distinguish between them. A degree of complexity was expected in that there was unlikely to be highly specific and exclusive responses to the separate VFA species, when they were presented to a sensor array acclimated as described. Nevertheless, the signals produced had sufficient information content to deduce that the cross-coupling of the reactions to the VFAs could be understood. The acclimation period for individual VFA inoculated MFC took different periods of time. After enrichment the first objective was to find a way for discrimination between different species of VFAs and this has been presented in Chapter 4. Electrochemical analysis was considered to find the correlation between the current response and VFA concentration; two methods were used and these were, CE and CV. CE gave a slow response taking more than 20 hours, particularly at VFA concentrations of 20 mg/l in the case of the AMFC and BMFC, whereas in the PMFC, current responses were only generated with >20 mg/l substrate. Using CV to measure the oxidation peaks at a consistent scan rate showed that a linear correlation existed between the peak current measured and the VFA concentration; this was observed up to <40 mg/l and was achievable in a relatively rapid response time of 1-2 minutes. The results showed good correlations between the individual VFA species concentrations and charge, and also current generated. A MFC based biosensor array was produced capable of measuring individual acetate, propionate and butyrate concentrations with sensitivities down to 5 mg/l and up to 40 mg/l. The cross sensitivities of each MFC were also checked by adding other VFA species into the anodic measuring chamber. AMFC and PMFC sensors were selective in their response to the respective VFA; whereas the BMFC sensor responded to all VFAs and was considered to be promising for the detection of TVFA. The array of sensors was also tested for response to sucrose as the ‘real’ samples were mainly collected from the

sucrose fed scaled-up MFCs. Again the response to sucrose from AMFC and PMFC were negligible but response from the BMFC was significant.

Stabilizing the mixed community on the anode was another important part and a second objective of the study, as it is important for a sensor to maintain a temporally stable and repeatable response over a sufficiently long time period. Investigations were conducted to determine the effect of immobilization of bacteria by; modifying the electrode carbon surface with functionalised poly (pyrrole) coatings, increasing and holding the total number of negatively charged bacteria on the electrode; or by using natural polymers with mediators covering the pre-acclimated microbial community to improve catalytic action and to protect them from the sample matrix. Different natural and conductive polymer/carbon composite electrodes were used to increase the stability of the sensitivity, and recovery of the sensor. The natural polymers (Agarose, polyacrylamide, polyvinyl alcohol and calcium alginate) were modified by adding mediator (neutral red) to them to increasing the conductivity/electron transfer between the bacteria and electrode. These mediators have been used previously in solution with planktonic microorganisms to demonstrate electrogenesis and have been used in an immobilised fashion e.g. physic-sorption onto carbon electrodes, but had not been studied as a mechanism to stabilize MFC based biosensors. Likewise, the use of functionalised polypyrrole (in collaboration with UEA) had not previously been used for this purpose. CV was used to determine the oxidation peak potentials for three VFA species (acetic, butyric and propionic) after fabrication of the carbon paper electrodes and populating with biofilm. The dynamic behaviours and static sensitivities were recorded while the anode was supplied with each of the VFAs. The start-up and recovery of individual VFA sensors was significantly decreased by (pyrrole-alkyl ammonium) modified electrode. The positive charge on electrode attracted more bacteria and held them for a longer period of time. The sensors were then checked for their sensitivity and stability of responses and it was found that the range and amplitude of current responses was improved by polyacrylamide with neutral red as natural polymer and by (pyrrole-alkyl ammonium) as conductive polymer. The stability determined by a consistent response to the measurand was seen to be >10 days for the conductive polymers but was <3 days

for natural polymers due to the poor mechanical strength and separation of the matrix from the electrode.

The range of the sensor was another issue which was dealt by using different anode poised potentials selected from CV of each VFA enriched sensor. The anode was then poised at a potential corresponding to the oxidation peak from CVs with the specific VFA species. The sensors were calibrated for a range of standard concentrations correspondingly and cross VFAs using different calibration methods. The current responses at the fixed working electrode potentials were analysed for (0-250 mg/l). The specificity of the AMFC and PMFC sensors remained even in the face of corresponding VFAs as was also observed during CV analysis. When corresponding VFAs were supplied to the sensors, the range of the sensor was increased from 40 mg/l to 220 mg/l. The BMFC sensor responded to all VFAs, so it was likely to be suitable to measure TVFA and was calibrated for different combination of mixed VFAs. The sensor volume and configuration at this stage was decreased to 10 ml to increase the hydraulic kinetics, portability and implementation. The 'real' samples from scaled-up MFCs and ED cell were considered for off-line measurement in the array of the VFA sensors. Where AMFC and PMFC sensors gave promising and significant responses, the TVFA concentrations obtained from BMFC sensor were higher than the GC values when compared. The error % was higher in BMFC as compare to AMFC and PMFC. Appropriate data processing is yet required in order to disaggregate coupled information streams in order to deliver VFA species specific measurements. It is suggested and planned that computationally based numerical methods of data analysis, processing, classification and interpretation should be used for the correct interpretation of the sensor signal.

In addition to the two objectives mentioned so far, a third objective was considered to establish the effect of the suppression of methanogens which would consume the analyte species without contributing to the electrical signal to any great extent. Suppression of methanogenesis was therefore studied in the MFCs enriched individually with the three selected VFAs. The substrate degradation patterns during OC/CC operation reflected the role of electrode reactions to overcome thermodynamic barriers for the oxidation of recalcitrant fermentation end products. The syntropism between different bacterial communities can play a reduced roll when the electron acceptor

(here anode) is available. In addition to this, the further effects of OC/CC regimes were examined for the suppression of methanogens. Although the importance of suppression of methanogens is application dependent, it might be useful and possibly important to increase CE in VFA sensors and so accuracy. Different h-MFCs were enriched with acetate, propionate and butyrate individually and were operated under different open and closed circuit (OC/CC) regimes. The transfer of methanogens and some electrogens from anode electrode to the solution altered the product mix and lead to methane inhibition. The archaeal communities on the electrode and in solution were compared after different OC/CC regimes, and clearly indicated the shift of some dominant species from electrode to the solution during OC operation. The results indicate gradual inhibition of methanogenesis during open/closed operation and also complete inhibition after starving the MFC for a period of time. The findings showed that there are ways to prevent methanogenesis in MFCs, but depending on their application it has to be decided discretely, whether it is essential, as in sensors or of less importance as in scaled-up MFC reactors. It is particularly important to inhibit methanogens in MFC based VFA sensors, where methanogens might utilize the substrate and therefore the current response will not represent the actual concentration.

So, this work presents novel approaches to detecting short chain VFAs by using MFC technology and methods to improve MFC based sensing in this regard. The demonstration of the detection principle and methods used for identification showed promising results.

9 Conclusion and Future Research

9.1 Conclusions

This study suggested that an acclimated mixed community can be operated stably for a sufficiently long time period in an MFC and could be used as a commercial basis for off-line/on-line VFA monitoring. The following conclusions can be made from the results obtained from this study as follows:

- MFCs can be enriched with different VFAs and the acclimatized fuel cells have been shown to be capable of monitoring VFA concentrations over a limited range (i.e. 0—40 mg/l). The temperature at which the enrichment takes place plays an important role as enrichment failed below 30°C and the reactors were then operated at 30°C throughout the study. From Chapter 4 the CE and CV methods were found to represent good measures for detecting VFA concentrations up to 40mg/l and for qualitatively discriminating between the VFA species. The CE methods took >20 hours to analyse the concentration in the MFC. CV responses were quicker i.e.<2-3 minutes depending on the stabilization of the current. However, the saturation is reached very fast and further dilution of the samples is required to increase the range in both methods for the MFC arrangements studied. The AMFC and PMFC responses were specific for the corresponding VFAs on which they were acclimated, in contrast to BMFC which responded to all VFAs. The analysis of the CV response to sucrose showed sufficient specificity to VFAs and conversely BMFC showed an oxidation peak for sucrose as well as VFAs.

- Chapter 5 presented immobilization methods, by encapsulating enriched biofilm using natural polymer matrices with mediators included and functionalised polypyrrole fabricated anodes, improved performance was evident. These electrode when connected in MFCs, showed improved performance in context of start-up (<100 hours for PMFC-Ppy^{+} and BMFC-Ppy^{+}), stability (over 2 weeks), recovery (<10 minutes) and voltage/current output (double the amplitude compared to unmodified electrode). Of the six different modifications

considered, the anode system with a) natural polymers polyacrylamide with neutral red (PA+NR) and b) conductive polymer poly pyrrole with positive functional groups Ppy^{+} i.e. poly (pyrrole-alkyl ammonium) showed the most notable improved performance when compared with unmodified electrode (carbon paper). In particular, the concentrations of these organic acids in aqueous solutions can produce repeatable (SD=0.03-0.09% over 2 weeks) signals in MFC-Ppy^{+} to discern changes in concentration (ranging 0-60 mg/l). The sensitivity of the electrode can improve the sensor applicability for wide range of systems containing the VFAs considered, and which need to be monitored.

- The range of the sensor was improved from 40-60 mg/l to 20-200 mg/l by using specific poised potentials presented in Chapter 6. The individual VFA-MFC was poised at -260, -220 and -100mV for AMFC, PMFC and BMFC respectively based on their oxidation peak current from CV. The off-line testing of real samples in the poised potential sensor array, from different sample sources, revealed that the AMFC and PMFC gave promising signals when compared with the GC responses for the same samples. However, as BMFC when calibrated for butyrate saturated at 100 mg/l, and the 'real' samples gave random responses beyond butyrate concentrations of >100 mg/l. Also as BMFC responded to all VFAs with highest response from the acetate acclimated sensor, this made it difficult for accurate interpretation of the responses. So, accuracy and reliability of the sensor signals from MFC based sensors are still in question, especially from BMFC. As the biofilm grows with time and dead microbial mass remains on the electrode, this could eventually affect the signals from any type of MFC based sensors. To maintain the repeatability, MFC based sensor would requires frequent recalibration when used for real-time monitoring for extended period of time, which impacts the requirement for rapid monitoring of bioprocesses as also concluded by (Kim et al., 2003b). Although some studies seem able to remove such interference (Chang et al., 2005) and improves MFC operation in general, more accurate measurement requires that the applicability of these methods to sensor devices be investigated further.

- It is concluded from the methanogen inhibition study that methanogenesis can be inhibited in MFC, however its importance is application dependent. It may be less important in scaled up MFC systems but might be useful for sensor devices, particularly to assure accurate responses when substrate concentration measurements would affected by non-electrogenic metabolic processes. Methanogens might utilize the substrate and therefore the current response will not represent the real concentration. The study only presented two methods to inhibit the methanogens and did not proceed to show a causal improvement in accuracy. The effect on sensor signals should therefore be investigated. Also, as presented in Chapter 7 the enhanced degradation rate of propionate and butyrate in closed circuit operation would be advantageous for VFA sensing and indeed as a mechanism for organic contaminant removal. The rate of degradation of recalcitrant fermentation end products with 20 mM concentration was much faster and took less time in CC operation as compared to OC operation where at the end of 5 days there still remained high levels of substrate, which was also reflected in carbon dioxide percentages obtained during the 5 days OC/ 5 days CC operation.

It is envisaged that progress in VFA-MFC based sensor technology will further improve detectability and allow sensitive, fast, and cost-effective (bio)chemical analysis both in laboratories and in the field. The results indicate that the reported studies will further extend the potential of MFC technologies and allow them be used more widely. Given the existing advances in biological sciences, coupled with advances in various other scientific and engineering disciplines, it is imminent that the sensor presented in this study has potential to replace other expensive and time consuming methods. However, further advances are needed in the MFC sensor design and configuration in order to achieve a practical technology for application in bioprocess and effluent metrology.

9.2 Future Research

This study presents the basis for further implementation of the MFC based VFA sensor. There are many possibilities to further improve and to take MFC sensing closer to

commercialisation, with more detailed analysis of the data presented here and with further studies. Some of the possibilities are listed below:

- There are several parameters that still need to be optimized to ensure stable responses from the MFC based biosensors. These include external/internal resistance, electrode material, electrode size, electrode surface area to volume ratio, working volume of the sensor. All affect the sensitivity and robustness of the sensor response. The improvements in designs and advancement in the material used in the MFC based biosensors may increase the deployment of the sensor devices to real-world monitoring. Another major limitation MFC based biosensor express is that sensor signals reach saturation at certain and relatively low concentrations of measurand, so limiting microbial activity of the MFC anode. However, the linear range of MFC can certainly be enlarged by different approaches as have been discussed.

- Some of the obstacles common to biosensor technology include the diversity of compounds and the complexity of environmental samples. The sensor in this study was tested for off-line measurements of the 'non-synthetic' samples i.e. from waste waters but for further practical applications, the sensor should be tested for on-line monitoring of the sample from real wastewaters, by connecting them to bioprocessing systems such as scaled-up MFCs, and low VFA concentration containing wastewater streams.

- In the present state of the sensor it is recommended that the calibration should be done frequently to extract the more useful data. The calibration methods can be improved according to the implementation of the sensor system and the electrochemical method used for the analysis. In addition to the methods presented in this study more electrochemical analysis or methods should be taken into account for increasing the quantitative analysis of the responses.

- Further detailed analysis of the bacterial communities involved should be considered for better understanding of the sensor signals. The author have considered sludge as an inoculum for sensor acclimation but also believe that

more detailed knowledge of the known microbial communities could increase the applicability and repeatability of the sensor implementation. This would also help to understand the detailed mechanism behind the electron transfer between different bacterial communities and electrode on addition of individual VFA.

- Appropriate data processing in order to disaggregate coupled information should be considered in order to deliver VFA species specific measurements from mixed samples. It is suggested that computationally based numerical methods of data analysis, processing, classification and interpretation should be used for the correct interpretation of the sensor signal. Given existing advances in biological sciences, and coupled with advances in various other scientific and engineering disciplines, it is plausible that existing VFA measurement methods can be replaced by MFC based VFA sensor.

- Further to the work presented for inhibition of methanogens in the individual VFA enriched sensor by using OC/CC regimes and starvation methods, more detailed studies should be done to see it's the effects on the sensor response and overall performance of the MFC system derived from methane inhibition. The CE of the MFCs after methanogen inhibition should be considered specifically in OC/CC methods.

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11 Appendix

11.1 Appendix 1

In the beginning of this project the pure cultures were considered. But due to their restricted growth for over 6 months same strategy was applied to individual VFA enriched biofilms. The list of bacterial cultures tried in this study is as below:

1) *Syntrophomonas wolfei*
2) *Syntrophobacter wolinii*
3) *Desulfobacca acetoxidans*
4) *Desulfoluna butyratoxydans*
5) *Desulfobulbus japonicus*
6) *Methanospirillum hungatei*

11.2 Appendix 2

Table 11.1: Shows the mixed VFA concentrations considered for the calibration of h-BMFC and c-BMFC for TVFA

Number of Measurement	*Acetate (mg/l)*	*Propionate (mg/l)*	*Butyrate (mg/l)*	*TVFA (mg/l)*
1	10	10	10	30
2	20	20	20	60
3	30	30	30	90
4	40	40	40	120
5	50	50	50	150
6	60	60	60	180
7	70	70	70	210
8	80	80	80	240
9	90	90	90	270
10	100	100	100	300
11	110	110	110	330
12	120	120	120	360
13	130	130	130	390
14	140	140	140	420
15	150	150	150	450
16	160	160	160	480
17	170	170	170	510
18	180	180	180	540
19	190	190	190	570
20	200	200	200	600

11.3 Appendix 3

11.3.1 List of Publications

- **Amandeep. Kaur**, Richard.M. Dinsdale, Alan. J. Guwy, Giuliano .C .Premier (2014): "Improved Dynamic Response and Range in Microbial fuel Cell Based Volatile Fatty Acid Sensor by Using Poised Potential". WREC/WREN-III Conference Proceedings, 3rd -8th Aug, 2014, London.
- **Amandeep Kaur**, Hitesh. C. Boghani, Jung. R. Kim, Iain. Michie, Richard. M. Dinsdale, Alan. J. Guwy, Giuliano. C. Premier (2014): "Operating strategies to inhibit methane production by selecting electrogens over methanogens in microbial fuel cells". Bioresource Technology 173:75-81.
- **Amandeep Kaur**, Saad Ibrahim, Christopher Pickett, Iain Michie, Richard M Dinsdale, Alan J Guwy, Giuliano C Premier, (2014): "Anode Modifications to improve performance of Microbial Fuel Cell based Organic Acid Sensor". Sensors and Actuators B-Chemical 201:266-273.
- **Amandeep Kaur**, Jung Rae Kim, Iain Michie, Richard M Dinsdale, Alan J Guwy, Giuliano C Premier, (2013): Microbial fuel cell type biosensor for specific volatile fatty acids using acclimated bacterial communities. Biosensors and Bioelectronics 47: 50–55.

11.3.2 Platform Presentations

1. Biosensor 2012, Mexico – "A. Kaur, J.R. Kim, R.M. Dinsdale, A.J. Guwy, G.C. Premier 2013. Detection of volatile fatty acids using MFC type biosensor with an acclimated bacterial community". Biosensors and Bioelectronics, 47:50-55.
2. EUISMET-Belgium-September 27-28, 2012– "A. Kaur, J.R. Kim, R.M. Dinsdale, A.J. Guwy, G.C. Premier 2013. Microbial fuel cell type biosensor for volatile fatty acid with an acclimated bacterial community". Biosensors and Bioelectronics, 47:50-55.
3. 13TH World Congress on Anaerobic Digestion June 25-28, 2013. Spain –"A. Kaur, H. C. Boghani, J. R. Kim, I. Michie, R. M. Dinsdale, A. J. Guwy, G. C. Premier, Operating Strategies to Improve Performance and Coulombic Efficiency by Selecting Electrogens Over Methanogens in Microbial Fuel Cells".

4. World Renewable Energy Network/Congress WREN/WREC- 3-8 August 2014, - "A. Kaur, I. Michie, R.M. Dinsdale, A.J. Guwy, G.C. Premier-Improved dynamic response and range using poised potential from Microbial fuel cell based volatile fatty acid sensor". University of Kingston, London, United Kingdom.

11.3.3 Poster Presentations

1. Electrochemical Horizons:2012, Bath, United Kingdom – "A. Kaur, J.R. Kim, R.M. Dinsdale, A.J. Guwy, G.C. Premier 2013. Microbial fuel cell type biosensor for volatile fatty acid with an acclimated bacterial community". Biosensors and Bioelectronics, 47:50-55.
2. IWA world water congress and exhibition - Pusan, Korea 2012–"A. Kaur, J.R. Kim, R.M. Dinsdale, A.J. Guwy, G.C. Premier 2013. Microbial fuel cell type biosensor for volatile fatty acid with an acclimated bacterial community". Biosensors and Bioelectronics, 47:50-55.
3. KSBB Spring Meeting and International Symposium 2013, Korea - "A. Kaur, J.R. Kim, R.M. Dinsdale, A.J. Guwy, G.C. Premier 2013. Detection of volatile fatty acids using MFC type biosensor with an acclimated bacterial community" Biosensors and Bioelectronics, 47:50-55.
4. Biosensor 2014, Melbourne, Australia –May 27-30, 2014-"A. Kaur, J.R. Kim, R.M. Dinsdale, A.J. Guwy, G.C. Premier-Improved dynamic response and range using poised potential from Microbial fuel cell based volatile fatty acid sensor".
5. H2FCSUPERGEN-The Hydrogen and Fuel cell Hub, Birmingham, United Kingdom (December 16-18, 2013).