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Enhancement of The Start-up of an Up-flow Anaerobic Sludge Blanket Reactor Using Electrochemically Enriched Biofilms

By

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Mohamad Abdallah

ABSTRACT

With rising issue of wastewater discharge and resulting pollution, anaerobic wastewater treatment has emerged as a prominent technology to overcome these issues. A myriad of anaerobic treatment techniques has demonstrated appreciable results in treating high strength wastewaters, with high-rate digesters being the popular option. The up-flow anaerobic sludge blanket reactor (UASB) is a high-rate anaerobic digester capable of achieving high COD removal rates under high organic and hydraulic loadings. A critical feature of the start-up of an UASB reactor is the acclimation of the inoculated biomass through a process known as granulation. Granulation is characterized by the agglomeration of specific types of bacterial and methanogenic archaea known to possess electroactive properties which allow cell to cell attachment through a mechanism known as direct interspecies electron transfer (DIET). Recently, several species known to be DIET participants, labeled as electroactive microbes, have been selectively enriched in microbial electrolysis cells known as bio-electrochemical reactors (BER) through applying constant electrode potentials favoring their proliferation through catalytic metabolism of reactor substrates. In this framework, the following thesis aimed to enhance the granulation and performance of an UASB reactor fed with high strength wastewater through supplementation with electroactive species enriched using BERs. An anodic BER (ABER) was poised at +400 mV vs Ag/AgCl to enrich electroactive bacterial species at the working electrode whereas a cathodic BER (CBER) was poised at -700 mV vs Ag/AgCl to enrich electrotrophic methanogens. After 5 feeding phases, the biofilms were extracted from the working electrodes of both BERs, in addition to the counter electrodes. An UASB reactor, operated at a HRT of 48 hours and OLR of 1 g-COD/L.d, was operated in two phases: Phase 1 being the control run and Phase 2 supplemented with the BER enriched biomass. Electrochemical analyses were performed on the BERs which showed the development of electrode respiring microbial communities based on cyclic voltammograms and chronoamperometric scans. Better COD and VSS removal rates were achieved in UASB Phase 2 as compared to Phase 1. Phase 2 COD removal of 85% was achieved in 20 days,

whereas the same COD removal was achieved in 62 days for Phase 1. Settleability tests performed on the developed sludge in the UASB bed showed a highly granular sludge (SVI = 8.5 mL/gTSS) in Phase 2 compared to flocculant sludge (SVI = 33 mL/gTSS) in Phase 1 after 20 days of operation. The findings of this study prove that electrochemically enriched biofilms can be utilized in anaerobic digesters through direct supplementation of biofilms, rather than hybrid system integration, and can exhibit improved reactor performance.

Keywords: Up-flow Anaerobic Sludge Blanket (UASB), Anaerobic Digestion, Bioelectrochemical Reactors (BER), Direct Interspecies Electron Transfer (DIET), Cyclic Voltammetry (CV)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABER: Anodic Bio-electrochemical Reactor

CBER: Cathodic Bio-electrochemical Reactor

EAB: Electrochemically Active Biofilm

DIET: Direct Interspecies Electron Transfer

MET: Mediated Electron Transfer

UASB: Upflow Anaerobic Sludge Blanket

AD: Anaerobic Digestion

MBR: Membrane Bioreactor

BES: Bio-electrochemical System

BER: Bio-electrochemical Reactor

EETC: Exocellular electron transfer components

EPS: Extracellular Polymeric Substances

WE: Working Electrode

RE: Reference Electrode

CE: Counter Electrode

OCP: Open Circuit Potential

HAc: Acetic Acid

CHAPTER ONE

INTRODUCTION

1.1. Introduction to wastewater treatment and anaerobic digestion

In recent times, a major societal concern has been about the environmental impact of wastewater discharge, particularly those coming from industrial sources reaching estimated volumes of up to 1500 km^3/day, generated worldwide (Tauseef et al., 2013). Most of these wastewaters are discharged into water bodies and remain untreated which pose a critical issue regarding environmental safety and water scarcity (Corcoran, 2010; Saravanan et al., 2023). Therefore, attempts to mitigate the issue of wastewater discharge have mainly focused on developing technologies capable of treating wastewater to reduce its polluting impact and increase energy recovery potentials.

Wastewater treatment processes can in general be divided into two different concepts: aerobic and anaerobic treatment. Aerobic treatment is the process of treating wastewater in the presence of oxygen as the terminal electron acceptor during the breakdown of the organic substrates into simpler by-products. This concept of treating wastewater is less advantageous compared to the benefits of anaerobic treatment, as anaerobic treatment requires less energy (no need for aeration), more energy production (methane produced as biogas fuel), less sludge produced for disposal and ability to treat high strength wastes (Metcalf & Eddy, 2004). Anaerobic digestion (AD) is the process by which organic substrates are broken down in the absence of molecular oxygen as the terminal electron acceptor of the redox reaction processes (Mata-Alvarez et al., 2014). It is divided into 4 main phases: hydrolysis, followed by acidogenesis/fermentation, acetogenesis and finally methanogenesis. During the hydrolysis phase, the complex organic substrate proceeds to be broken into simpler compounds (long-chain fatty acids, amino acids and

monosaccharides), followed by acidogenesis where monomers are broken down into ethanol, hydrogen gas, carbon dioxide and volatile fatty acids (VFAs) such as propionate, acetate and butyrate. These compounds are then converted into acetate, CO_2 and H_2 in the acetogenesis phase which are finally converted into methane CH_4 during methanogenesis. The aforementioned digestion steps are categorized as biological processes undergone by a consortium of various micro-organism through syntrophic interactions. With the rising issue and growing interest in energy production through more green sources of energy, anaerobic digestion has proved to be an efficient technique for producing an important source of green energy in the form of biogas fuel, methane.

Among the different anaerobic treatment processes, high-rate anaerobic digestion processes have emerged as a popular option compared to other conventional technologies. High-rate anaerobic digesters are characterized by their ability to withstand high organic and hydraulic loads with high solids retention times due to their ability to retain biomass within the system while minimizing washout (Von Sperling & Chernicharo, 2005). High-rate anaerobic digesters include a variety of configuration which include the anaerobic contact process, up-flow anaerobic filter (UAF), downflow stationary fixed film (DSFF) and the upflow anaerobic sludge blanket (UASB) reactor. Despite the efficiency of high-rate anaerobic systems in treating wastewaters characterized by high COD loads, several drawbacks persist such as effluent discharge containing high concentrations of viable biomass or clogging of systems containing attached media reducing their favorability. Therefore, newer strategies and novel technologies had to be developed and incorporated into high-rate systems anaerobic systems to mitigate these issues and restore their efficiency.

1.2. Overview of bio-electrochemical systems

With the different microbial interactions discovered in anaerobic digesters and the prospects of utilizing these interactions to enhance digester performance, bio-

electrochemical reactors (BERs) can be a promising technology for enhancing anaerobic digestion. BERs are simply defined as electrochemical cells, either microbial fuel cells (MFC) or microbial electrolysis cells (MEC), where the system is made up of at least two electrodes immersed in a conductive solution containing microbial communities (Lewandowski & Beyenal, 2007). For such configurations to be successful, microbial communities must develop at the electrode surfaces and exhibit respiratory interactions with the electrodes. This can be observed in the form of electric currents or development of potential difference. Furthermore, different electrochemical techniques can be utilized to characterize the possible mechanisms by which these communities interact with electrodes, typically defined as redox proteins or conductive nanowires (B. H. Kim et al., 2004; Reguera et al., 2005).

Microbial communities that utilize solid electrodes as either an electron sink or source are defined as electrochemically active bacteria (EAB). The uniqueness of such organisms lies in their ability to exchange electrons (utilized in redox metabolic reactions) with redox sensitive minerals, conductive material or conductive surfaces, such as those of poised electrodes (Lee et al., 2016; Z. Zhao, Zhang, Wang, et al., 2015). These communities are capable of respiring using heterogenous electron acceptor, such as electrodes or Fe(III), through either conductive nanowires or expressed membrane-proteins (Beyenal & Babauta, 2015; B. Kim, 1999; B. H. Kim et al., 2004). One technology used to develop such communities are microbial fuel cells (MFC), where several bacterial species such as those of the genus *Shewanella* and *Geobacter* have been enriched in these cells. However, the main constraint in utilizing MFCs in microbial enrichment is the inability to control the applied potential on the electrodes, which is dictated by the amount of substate available for oxidation/reduction. A modification to the MFC technology is the MEC configuration which utilizes a potentiostat to either control the cell potential or electrode potential (with respect

to a reference electrode) at a fixed value. Studies utilizing anaerobic MECs for EAB enrichment have successfully grown variable microbial communities such as those of the genus *Geobacter*, specifically of the species *sulfurreducens* and *metallireducens*, in addition to *Clostridium* genus. Recently, methanogenic archaea have also been successfully developed in MECs such as those relating to the genus *Methanobacterium*, *Methanococcus* and *Methanospirillum*. Many of these enriched communities have been known to be found in anaerobic digesters where they syntrophically interact to degrade influent wastewaters.

Recent studies have given rise to several questions considering the mechanism by which these biofilms are able to interact with electrodes as their electron sink or source. Several studies aimed to elucidate such mechanisms which can be categorized as either through indigenous and exogenous redox shuttles, protein nanowires or direct transfer through membrane proteins (B. H. Kim et al., 2004; Lewandowski & Beyenal, 2007). Another aspect of BER-enriched microbial communities that needs to be addressed is their ability to operate syntrophically in anaerobic digesters, independent of any attached surfaces or imposed electrochemical conditions.

CHAPTER TWO

LITERATURE REVIEW

2.1. The Up-flow Anaerobic Sludge Blanket (UASB) Technology

2.1.1. Introduction to the UASB configuration and its advantages over other anaerobic treatment technologies

Among the different anaerobic wastewater treatment technologies, the UASB technology is considered one of the most efficient and robust configurations in terms of treatment performance, sludge stabilization, land requirements and ultimately methane production (Chong et al., 2012; Henze, 2008). A high-rate treatment process, the UASB technology is characterized by the ability to treat high strength wastewaters, in addition to municipal wastewaters, under high loading rates and short hydraulic retention times, making it a popular option from an operational perspective compared to other anaerobic treatment technologies (Del Nery et al., 2008; Dutta et al., 2018; Singh & Viraraghavan, 1998). Several studies have reported COD removal efficiencies of up to 92% with HRT as low as 6 hours and OLRs as high as 44.9 kg-COD/m^3.d in UASBs treating industrial scale wastewaters (Del Nery et al., 2008; Dutta et al., 2018; Lettinga et al., 1980; Musa et al., 2019; Vadlani & Ramachandran, 2008).

The uniqueness of the UASB configuration lies in its compact design featuring a dual biological treatment (known as the reaction zone) and settler system (known as the separation zone) within the same reactor. The reaction zone is the compartment where the substrate metabolism and biomass is retained, typically composed of a bed (heavy biomass accumulation) and blanket zone (flocculant biomass dispersion); the settler system is present above the reaction zone and is characterized by a gas-liquid-solids (GLS) separation assembly (Haandel & Lubbe, 2019; Tchobanoglous et al., 2014). The GLS structure is

designed in such a way that the gas produced in the reaction zone is trapped inside the conically shaped separator so that no gas escapes in the effluent. Furthermore, the settling compartment is designed to ensure a decrease in upflow velocity through this section (outside the gas capture compartment) due to increasing cross sectional area, which can allow the gravitational settlement of solid agglomerates (Von Sperling & Chernicharo, 2005). Wastewater flows through the reactor in an upflow manner which, in addition to the produced biogas, allows for efficient mixing of the biomass and wastewater, negating the need for any mechanical mixing (Lettinga & Hulshoff Pol, 1991).

The treatment process in an UASB occurs in biological granules formed of a consortium of microbes capable of performing the different steps involved in the anaerobic digestion process (hydrolysis, acidogenesis, acetogenesis and methanogenesis). UASB granules are typically consolidated in a layer called “bed” at the bottom of the reactor and are characterized by their settleability and immobilization allowing the system to operate without the risk of sludge washout (Hulshoff Pol et al., 1983). With proper startup and operation, granules of mean size ranging between 1 to 5 mm and solids content ranging between 3 to 10% (equivalent to 3 to 10 g-TSS/L) can be maintained in the bed with up to 2 kgCH_4-COD/kg-VSS.d methanogenic activity (Hulshoff Pol et al., 1983). Furthermore, granule settleability can be qualified through settling tests such as the sludge volume index (SVI), for which granular sludge falls in the range of 10 to 20 mL/g-TSS (Ghangrekar et al., 2005; Lettinga et al., 1980).

2.1.2. The concern with UASB start-up and obstacles hindering its performance

A major drawback of the UASB technology that hinders it from being a popular option for anaerobic wastewater treatment is the long start-up periods which could take up to 8 months (Vlyssides et al., 2008). From an operational perspective, an UASB start-up is considered complete once the wastewater organics removal reaches a stable state with no

significant sludge washout. However, due to wastewater variability in terms of composition and the hydraulic and volumetric loading rates at which these reactors are operated, the start-up period may be prolonged and even reactor failure may occur. Another concern is the reactor's response to variable loading rates which may instigate instability in terms of COD removal and CH_4 production (Rizvi et al., 2015; Singh & Viraraghavan, 1998). These issues are primarily related to the concept acclimatization of the reactor biomass to imposed loads, which requires long periods for this configuration.

UASB acclimatization mainly refers to the granulation of the suspended biomass within the reactor. Granular formation within the reactor's bed is a critical feature for efficient start-up and subsequent operation characterized impacted by physical, chemical and biological factors. Heavy research was done to elucidate how granules are formed and identify the factors that may enhance their agglomeration. The granulation process as reported by Schmidt & Ahring (2000) is defined a four step phenomenon consisting for biomass approach to an inert material, reversible adsorption by cells, irreversible adhesion to the inert material and proliferation of the biomass. Nevertheless, the size, activity and granulation are dependent on the operational conditions and could not be generalized.

2.1.3. Operational aspects to enhance UASB start-up

The operational parameters of an UASB reactor, especially during the start-up phase, can play an essential role in ensuring proper reactor operation and sludge granulation. To a great extent, researchers have investigated reactor start-up procedures which included modifying parameters such the hydraulic and organic loading rate, sludge loading rate and inorganic components within influent wastewater.

The substratum on which the granules formed are typically divalent and trivalent cations that can act as an attraction surface for the negatively charged cells. Studies using synthetic feeds have looked into amending the influent wastewaters with ions such as

Calcium (Ca^{2+}), Magnesium (Mg^{2+}), Aluminum (Al^{3+}) and Ferric (Fe^{3+}) (Y. Chen et al., 2008). The main goal was to determine the dosage of each and the effect on speeding up the granulation process. Yu (2001) investigated the role of calcium in enhancing granulation in a UASB reactor fed with synthetic waste and concluded that an optimum concentration of 150 to 300 mg/L of calcium ions within the influent are beneficial, while exceeding this range can lead to detrimental performance. Another study by Schmidt & Ahring (1993) for an UASB operated under thermophilic conditions concluded that opting for Mg^{2+} composition of up to 720 mg/L can yield satisfactory granulation for that particular condition.

On the contrary, other studies have attempted to look into more practical aspects of enhancing UASB start-up which included experimenting with the HRT, OLR or SLR and monitor reactor performance. A study performed by Ghangrekar et al. (1996) explored the effects of a new operational parameter, the sludge loading rate (SLR), which specifies the organic loading in influent wastewaters normalized with respect to UASB biomass. The authors have shown that starting up an UASB reactor with synthetic feed at SLRs less than 0.2 g-COD/g-VSS.L.day can shorten stat-up times to 30 days with treatment efficiency of up to 95%. Studies utilizing municipal wastewaters in psychrophilic (Singh & Viraraghavan, 1998) and mesophilic (Rizvi et al., 2015) conditions have shown that a start-up HRT of 48 hours can allow for proper granulation before increasing organic loading.

Despite the reported progress achieved in terms of enhancing start-up, most of the mentioned parameters do not provide a standard guideline as the performance seems to be heavily dependent on specific conditions such as temperature and wastewater composition. Consequently, enhancing UASB operation may include the need of incorporating hybrid systems (Chong et al., 2012).

2.1.4. The microbiology of UASB biomass

Microbial communities that develop in UASB reactors play a vital role in its proper performance, reflected in the form of efficient COD removal, stable effluent quality (VFA and alkalinity composition) and biogas production, specifically methane. The immobilized granules in the UASB bed are composed of a consortium of microbial communities that perform the different steps involved in anaerobic digestion, whereby the by-products by one community are transferred to another (Nnaji, 2014). Granular formation among the different communities has given rise to research that aim to probe the mechanisms by which the constituent microbes interact and agglomerate.

The initial mechanism though to dominate in methanogenic ecosystems is mediated electron transfer (MET) using external redox mediator such as hydrogen or formate. This mechanism occurs through transfer of electrons from metabolically oxidized substrates to mediators which serve as electron shuttles; afterwards, these mediators are utilized as electron donors by syntrophic partners within the granular community (Stams & Plugge, 2009). Recent research has given rise to an alternative mode of electron transfer in syntrophic communities known as direct interspecies electron transfer (DIET). This mode of electron transfer is made available through cell to cell conductive attachments expressed as either conductive nanowires or filaments (known as pili) or extracellular expressed proteins in the form of multiheme cytochromes (Morita et al., 2011; Shrestha et al., 2014; Shrestha & Rotaru, 2014). The hypothesis behind electron exchange through conductive pathways was tested by Morita et al. (2011) whereby methanogenic aggregates from a brewery fed UASB reactor exhibited metallic-like conductivities probably contributed to by a number of microbial species.

Wastewater feed, in addition to operational conditions, can play a critical role in determining the microbial species that proliferate in the system. Studies performed in UASB

reactors fed with different types of brewery wastewater showed the development of granules (up to 2 mm in size) with variable conductivity, having the genus *Geobacter* as mostly dominant (Shrestha et al., 2014). Granular conductivity in these samples could be attributed to the degree of structural pilin protein expression affected by the environmental conditions in the UASB (Malvankar et al., 2012). Using wastewaters containing more complex organics (compared to ethanol from breweries) yielded more diverse microbial communities in the granules. For example, Zhang et al. (2021) obtained a consortium of various bacterial communities while treating municipal sewage with *Geobacter* being insignificant comparatively. On the other hand, a control UASB fed with synthetic wastewater (sucrose and dichloronitrobenzen acting as the organic source) showed a higher relative abundance of *Planctomyces*, *Prosthecobcter* and *Phenylobacterium* (H. Chen et al., 2017).

2.1.5. Novel technologies to improve UASB start-up and subsequent efficiency

With the current trend in microbiological research in UASBs being riveted towards the mechanisms by which involved communities interact syntrophically, specifically through DIET, attempts to capitalize on these findings comprised of enriching such microbial communities. Many studies have attempted to utilize novel strategies to enhance start-up and performance in UASB reactors. These strategies include, but are not limited to, adding conductive material or beneficial microbial communities into the reactor in addition to utilizing electrochemical techniques in hybrid UASB systems. Supplementing anaerobic digester sludge with conductive materials has shown positive results in terms of enhancing performance through promoting DIET-involved microbial communities (Baek et al., 2021; Lee et al., 2016; Martins et al., 2010). UASB supplementation with conductive materials such as biochar, graphite or carbon-cloth have led to enhanced methane production rates while shifting the microbial communities to DIET-involved microorganisms (Zhang et al., 2021; Z. Zhao, Zhang, Woodard, et al., 2015; Z. Zhao et al., 2016).

Hybrid electrochemical systems with UASB reactors can provide a useful technology to enhance UASB start-up due to the possible electroactivity of UASB granules. UASB systems that implemented electrochemical systems such as microbial fuel cells, or constant voltage/current application yielded promising performance results (H. Chen et al., 2017; Yang et al., 2019; Zhen et al., 2017). The main characteristic in these systems is the development of electroactive biofilms attached on the electrodes which contributed to the enhanced performance.

On a different note, enhancing the performance of UASB reactors without any internal modifications is rarely reported despite being a more feasible approach for large-scale UASB implementation. The biological aspect of the UASB treatment process suggests that manipulating the sludge communities may play a role in enhancing UASB start-up. For example, Keyser et al. (2003) utilized the bacterial species *Enterobacter sakazakii* in a UASB reactor fed with winery effluent which resulting in significant reduction in start-up time accompanied by up to 90% COD removal.

2.2. Bio-electrochemical systems (BES) for enhanced anaerobic digestion

2.2.1. Oxidative bacteria development in anodic BERs

Through operating BERs at specific potentials capable of catalyzing biological oxidation of substrates, certain bacterial species known as exoelectrogenic bacteria can be specifically enriched at these electrodes which may play an essential role in anaerobic digestion. Through applying a potential at the biofilm electrode (i.e. working electrode) greater than the substrate electrochemical equilibrium potential with respect to a specified reference electrode, exoelectrogens can be selected and attached on these electrodes which can catalyze substrate metabolism and exchange electrons with the electrode (Beyenal & Babauta, 2015). Among the different bacterial communities found on ABERs, the genus *Geobacter spp.*, which relates to the class of *Deltaproteobacteria*, was the most prominent

and heavily examined exoelectrogens due to its role in anaerobic digester biomass (Lee et al., 2017; P. Liu et al., 2020). Several studies have highlighted the contributions of Geobacters, specifically developed electrochemically, in enhancing performance and treatment efficiency in anaerobic digesters (Lee et al., 2017; J. Zhao et al., 2017; Zhu et al., 2014). A study performed by Zhao et al. (2015) showed that applying a constant voltage of 0.6 V in a controlled cell potential configuration to electrodes inserted in an anaerobic digester lacking *Geobacter* species, selectively enriched the *Geobacter* genus (both attached on the electrode and in the suspended sludge) and specifically *Geobacter metallireducens.* The bioelectrochemical-anaerobic digester setup led to approximately a 30% enhancement in methane production compared to no applied voltage. Furthermore, the setup used created a medium suitable for DIET-involved methanogens such as *Methanosaeta* known to grow in UASB granules and contribute significantly to its stable performance (Rotaru et al., 2014). Another study conducted in an acetate fed anaerobic bioelectrochemical reactor showed a higher rate of methane formation with a different set cell potential of 0.39 V and led to proliferation of *Geobacters*, mainly affiliated with the *anodireducens* species (Lee et al., 2017).

Experimental conditions in bioelectrochemical reactors can affect the EABs developing on the working electrode and subsequent effect on anaerobic digestion performance. Several factors can affect the development of EABs on anodes which include the substrate used (Jung & Regan, 2007; Kiely et al., 2011; Xing et al., 2009; Zhu et al., 2014), BER configuration and potential applied at the working electrode (Kiely et al., 2011; Torres et al., 2009) in addition to inoculum type (specific cultures or wild species). Experimental studies utilizing simple substrates such as acetate (Kouzuma et al., 2013; Zhu et al., 2014), or more complex electron donors such as glucose (Xing et al., 2009), lactate (Jung & Regan, 2007), wasted activated sludge (Z. Zhao, Zhang, Wang, et al., 2015) or

potato wastewater (Kiely et al., 2011) have yielded different EABs and subsequent performance. Most of the EABs reported in these studies belong to the genus *Geobacter*. This suggests that the substrate used for EAB enrichment can select different species in accordance with complexity in terms of biodegradation (hydrolysis and fermentation) and availability as a potential electron donor for electroactive communities that can participate in anaerobic digesters. Furthermore, despite ABERs exclusively enriching exoelectrogens on the electrode surface, thicker biofilms can form which may include communities that can directly interact with exoelectrogens such as methanogens.

Heavy emphasis was recently placed on elucidating the mechanisms through which these oxidative bacteria exchange electrons with electrodes and possibly with syntrophic companions. Electrochemical analyses along with pyrosequencing and gene expression analysis were performed to identify cell compartments such as conductive nanowires (e-pili) or multi-heme proteins (*c*-type cytochromes) that may be responsible for DIET with research mainly focusing on the genus *Geobacter* involvement in electroactive responses. Chronoamperometric response, which is current development due to polarization of the WE in an ABER, shows a respiratory mechanism of electroactive biofilms (Parot et al., 2008). Evidently, cyclic voltammograms (CV) in such cells yield sigmoidal shape responses mainly in acetate-fed mediums which indicate catalyzed metabolism of acetate through electron exchange with an electrode. To further probe involved components in such activities, CVs ran in substrate-depleted conditions have shown peaks related to possible extracellular electron transfer components (EETCs) known as c-type cytochromes (e.g. OmcZ, OmcS and OmcB) that can facilitate external electron transfer. Conductivity tests performed on such communities have shown that pilA expression was fund in *Geobacter sulfurreducens* and *Geobacter metallireducens* in BERs operated at various potentials. The capability of EETC expression in BERs and their associated microbial communities were

mainly reported in acetate fed mediums whereas response to more complex feed sources warrants more research (Zhu et al., 2014).

However, studies involving ABERs have not placed sufficient focus on the possible communities developing on counter electrodes. Counter electrodes in ABERs act as electron sources to complete the circuit in a BER. The reaction most reported to occur on CE is the abiotic production of hydrogen gas (H_2) which can either be utilized by hydrogenotrophic methanogens (e.g. *Methanospirillum*, *Methanobacterium* or *Methanococcus*), or hydrogen producing bacteria (e.g. *Clostridium*) to be recycled within the BER system. Nevertheless, columbic efficiency computations and methane production data showed discrepancies in terms of the expected production rates and electric current observations. With the recent discovery of methanogens capable of directly utilizing electrons from electrodes for direct reduction of CO_2 into CH_4, further emphasis on such communities and mechanisms is warranted to understand the capabilities of developing methanogens, in addition to oxidative bacteria, in BER systems that aim to enhance anaerobic digestion. As the applied potentials in the BERs can allow hydrogen evolution on the counter electrode, several studies have observed a development of methanogenic communities utilizing hydrogen as an electron source on the cathode. However, no sufficient evidence was found to exclude the development of methanogens capable of electro-methanogenesis on the cathodes. Furthermore, the methane production recorded in such BERs could be traced to multiple sources which may include methanogenic biofilms growing on anodiphiles, planktonic methanogens in addition to hydrogenotrophic or electro- methanogens.

2.2.2. Methanogenic archaea development in cathodic BERs

To elucidate the capabilities of methane production in bioelectrochemical cells, a different configuration of the BER system, the cathodic bio-electrochemical reactor (CBER), can be used to promote methanogenesis, or in other word electro-methanogenesis,

in anaerobic BERs. Through controlling the applied potential of the WE (vs RE) up to a limit where it is negative enough to catalytically reduce CO_2 to CH_4 but not cause abiotic hydrogen evolution, methanogenic communities can develop and attach on these electrodes (Beese-Vasbender et al., 2015). Methanogenic involvement in BER systems were typically confined to either planktonic biomass,, attached cells on EABs such as *Methanothrix* (previously known as *Methanosaeta*) (Zhao et al., 2015) or hydrogenotrophic methanogens (Lee et al., 2017; Mayer et al., 2019). Some of the reported methanogens capable of electro-methanogenesis where previously thought to participate in hydrogen mediated methane production while those capable of acetoclastic or DIET-involved methanogenesis were scarcely reported in cathodic BERs (Zakaria & Dhar, 2019; Zheng et al., 2020).

Like ABERs, the conditions through which electromethanogenesis is promoted affect the community development and performance of CBERs. Electrode material can play a significant role in determining how methane production in CBERs can occur in terms of reducing the overpotential for either hydrogen production or methane production catalysis (Siegert et al., 2014). Cell configuration can play an essential role in how biocathodes can operate, specifically due to the soluble substrates available for biomass present in the cell (Cheng et al., 2009; Jiang et al., 2013; Siegert et al., 2015). Several studies performed using inoculum containing wild species have yielded satisfactory results in terms of enhanced methane production and methanogenic development. Cheng et al. (2009) successfully developed a biocathode with up to 80% electron capture on which species such as *Methanobacterium*, *Methanospirillum* and *Methanoregula*. Linear sweep voltammetry and gas production measurements in this study show no abiotic hydrogen production in these cells, proving that electrons were directly used for biologically catalyzed methane production. Another study by De Vrieze et al. (2014) using a complex substrate (molasses) and electrodes in a failing anaerobic digester, polarized at potentials up to -800 mV vs

Ag/AgCl resulted with proliferation of *Methanosaeta* and *Methanosarcina* with no apparent effect due to the applied potential. However, no electrochemical analyses were performed to clarify whether the mentioned archaea are electrotrophic. Furthermore, despite being heavily reported in methanogenic biomass in anaerobic digester, *Methanosaeta* and *Methanosarcina* species (which are known to be DIET-participants in anaerobic digesters) were rarely reported on biocathodes (Zakaria & Dhar, 2019). With complex feeds in single chambered MECs inoculated with wild species, a wide consortia of cathodic biofilms can develop which may include hydrogenotrophs, homoacetogens (bacterial species capable of reducing CO_2 with H_2 to produce acetate) and the newly discovered electrotrophic methanogens (Zakaria & Dhar, 2019).

The mechanisms through which these communities interact with electrodes is still a matter of heavy debate. Studies on electrotrophic methanogens in terms of cell-to-electrode interactions have been limited compared to their anaerobic digester oxidative counterparts, mainly of the genus *Geobacter*. Some studies have attributed the direct exchange mechanism with electrodes to direct attachment to a conductive medium rather than utilizing extracellular proteins or conductive nanowires (Beese-Vasbender et al., 2015; Cheng et al., 2009). One study showed the possibility of expression of cytochromes B and C by a *Methanobacterium* strain through redox peaks at -0.135 and -0.14 V vs SHE obtained by a CV scan in a double-chamber CBER polarized at -600 mV vs SHE (Beese-Vasbender et al., 2015). The authors of this study did not rule out the possibility of EETCs being expressed by the *Methanobacterium* strain used to catalyze methane production, but the thin biofilm formed suggests a direct attachment mechanism. Nevertheless, biologically catalyzed hydrogen production by hydrogenotrophic methanogens is also a possibility in biocathodes evidenced by lower overpotentials for hydrogen production (Villano et al., 2010).

However, these communities were particularly confined in bioelectrochemical applications and mostly were not tested in anaerobic digester to understand their performance in an altered condition. As hydrogen gas is a by-product used in anaerobic digestion, low hydrogen production rates may indicate direct hydrogen up-take by cathodic communities in addition to direct electron up-take (Baek et al., 2021). Thus, the possibility of enriched methanogenic genera requires testing with other syntrophic companions in anaerobic digesters for example to further inspect their capabilities for direct accept electrons from bacterial communities and affect reactor performance without the need for attached media.

CHAPTER THREE

AIM AND OBJECTIVES

3.1 Research aim

The aim of this research work was to speed up the start-up of a lab-scale UASB reactor treating synthetic wastewater and enhance the effluent quality and biogas production rates. This study also aimed to enrich specific electroactive and electrotrophic microbial communities using two lab-scale BERs poised at favorable potentials for usage of the biofilms in the enriched-UASB run. Additionally, it was intended to perform a thorough investigation of the microbial communities in both UASB granules for comparison of proliferated microbial communities and gene expressions involved to the granulation and electrolysis process.

3.2 Research objectives

1. Operate and asses BER overall performance

1.1. Enrich electroactive communities and electrophic methanogens in anodic and cathodic BERs, respectively, in different feeding cycles

1.2. Evaluate the electrogenic communities electroactivity using electrochemical techniques

1.3. Evaluate substrate removal in each BER

2. Evaluate the performance of the UASB reactor supplemented with enriched biomass versus a control UASB

2.1. Assess the methane production rate and yield

2.2. Monitor the effluent quality in terms of COD removal and VFA content in addition to any sludge washout

2.3. Visualize the formation of granules in the UASB bed and assess their characteristics and settleability

2.4. Compare the different performance parameters to check if there is any significant improvement

3. Determine the factors that may lead to enhanced performance in a supplemented UASB

3.1. Perform microbial community analysis to probe the microbes developed on the BER electrodes

3.2. Perform microbial community analysis (qualitative and quantitative) for the UASB bed granules in both rectors at different periods to monitor any trends with time between the two reactors

3.3. Determine the gene expression of possible extracellular components that can play a role in UASB granular formation and biofilm-electrode interactions

3.3 Scope of work

The scope of this research work mainly focused on operating a single UASB reactor (10.8-L reaction zone volume) at mesophilic conditions (37°C) in two phases; the first phase was a control run to observe the required time to achieve proper start-up, and the second phase was operated with enriched biomass from two BERs to monitor any decrease in the start-up period. During both phases, synthetic wastewater was used as the main substrate to simulate real life high strength wastewater as the present work is a proof of concept, and thus, minimizing the variability of actual wastewater was needed to properly track obtained results. The effluent was monitored continuously to check for sludge washout. Effluent quality was measured every other day to detect any enhancement in total COD removal (which incorporates the soluble and particulate material such as biomass) and soluble COD removal (which strictly includes substrate material). Only one hydraulic/organic loading setting was chosen for both phases to minimize heavy washout risks and allow proper start-

up. Sludge measurements, including solids concentration and settleability tests were done on a weekly basis due to the limited volume of sludge available. These measurements highlighted the development of immobilized granular sludge.

Two BER configurations were used in this study to enrich both anodic and cathodic biofilms along with the biofilms developed on the auxiliary/counter electrode. One voltage was chosen to operate each configuration such that it allows for the development of the desired biofilms while minimizing the risk of either abiotic hydrogen evolution or hindered electroactivity due to high absolute overpotentials. An Ag/AgCl reference electrode was used in the electrode potential controlled system for comparable results and conditions with similar studies. Working electrode was made of carbon fabric to allow efficient cell attachment whereas counter electrode material was carbon felt which allows for a higher surface area (less limiting current conditions). Feed medium used in both BERs was similar to that used for the UASBs to possibly minimize variability between the two systems in addition to providing a complex growth medium. Each BER was operated for 5 phases to grow a thick enough enriched biofilm for UASB inoculation. Sparging with N_2 (ABER) and N_2/CO_2 (CBER) was performed to mix the medium and secure anaerobic conditions; the continuous sparging; however, would not allow biogas measurements for H_2 and CH_4.

Inoculum material used for the two UASB phases and the BERs was active anaerobic sludge from an anaerobic treatment facility. Providing the same inoculum for all reactors allowed the full adaptation and selection of microbial communities that would be involved in the respective treatment configuration, demonstrating a more rigorous start-up process rather than using pre-adapted sludge.

Microbial analysis was performed through next generation sequencing to identify microbial communities up to the species level to elucidate any changes in the consortia during both UASB phases. Quantification through real time PCR of different bacteria,

archaea and expressed genes was performed to highlight any proliferation or diminishment of available communities in addition to possible interactions between syntrophic companions.

CHAPTER FOUR

METHODOLOGY

4.1. UASB configuration and operation

A lab-scale UASB reactor was used for the two proposed runs. The entire reactor assembly consisted of the reactor along with the separator compartment with the GLS separator and clamped to ensure no air leakage (Armfield Ltd, Hampshire, England). The reaction zone had a total volume of 12 liters and consisted of a total height of 30 cm (conical bottom inclusive), a diameter of 25 cm and a conical shaped bottom (of height 10 cm) through which bed zone sampling and influent feeding is performed. The separator compartment is made up of deflector for gas bubble directing (angle of 45° with respect to the horizontal) and an inverted cone structure and gas collection box to act as the GLS with an angle of 30° (with respect to the horizontal) and total height of 15 cm. The effluent port is located 5 cm above the GLS bottom and is connected to a U-bend to ensure no air intrusion.

The UASB reactor was seeded 12-L of anaerobic sludge obtained from Bkassine anaerobic WWTP (Saida, South Lebanon) and was inoculated in the laboratory using 3 5-L CSTRs (CHEMGLASS, New Jersey, USA) until stable pH and methane production was observed (See Appendix A for detailed procedure of sludge inoculation pre-UASB start-up). The reactor was operated under mesophilic conditions at temperature of 35°C using the UASB water jacket and a circulation bath (Cole Parmer, Court Vernon Hills IL, USA). Once inoculated, the reactor was sparged with 100% N_2 (99.9999% purity), and the sludge was left for 24 hours to acclimate before starting the feeding process.

The UASB reactor was operated in two consecutive phases. Phase1 which consists of a control UASB sludge inoculated with only anaerobic sludge whereas Phase 2 consisted

of inoculation with the same anaerobic sludge in addition to enriched electro-active biomass obtained from the working and counter electrodes of the anodic and cathodic BERs. During both phases, the reactor treated synthetic feed prepared with modification in accordance to Fang & Chui, (1993) (composition in Appendix A) having a COD of 2000 mg/L to mimic high strength wastewaters. Influent was pumped into the reactor using a peristaltic pump (MasterFlex Avantar, Provo UT, USA). The start-up phases of the reactor was operated at a HRT of 48 hours and OLR of 1 g-COD/L.d. The sludge loading rate (SLR) during the start-up was 0.2 g-COD/g-VSS.d for an inoculum VSS of 7500 mg/L in accordance with Ghangrekar et al. (1996). The sludge retention time (SRT) was maintained at 330 days through minimizing sludge wastage. Reactor pH was maintained within the optimum range of 6.8 to 7.2.

Table 1. Formulation for synthetic wastewater composition normalized per one gram of COD

Substrate Component	Mass content (mg) per 1 gCOD
Milk powder	186
Sucrose	633
Citric acid	50.6
Sodium bicarbonate	1000
Ammonium chloride	260
Potassium mono-hydrogen phosphate	75
Potassium di-hydrogen phosphate	30
Magnesium sulphate	25
Calcium chloride	75
Ammonium molybdate	0.8
Sodium tetraborate	0.44
Nickel Sulfate	16
Copper (II) Chloride	0.65
Zinc Chloride	1.26
Ferric Chloride	12.45
Manganese (II) Chloride	2.22

4.2. BER configuration and operation

The bio-electrochemical reactors (BERs) were designed and constructed in accordance with the schematics presented in Appendix A. The setup is composed of a three-electrode microbial electrolysis cell composed of a working electrode, counter electrode and reference electrode along with two ports for sparging (with a bubbler), a sampling port and gas release port. The working electrode (WE) material was carbon fabric while that of the counter electrode (CE) was carbon felt (acquired from WSU, Pullman WA, USA). Two titanium wires (Ti) were placed securely on each electrode and soldered to a copper electric wire to which the potentiostat is connected. Resistance was measured for each wire and the electrode using a multimeter; a resistance less than 1 Ω was considered acceptable (sufficient conductivity). The two electrodes were separated by a distance of 2 cm using plexiglass frame with spacers and insulating cloth to ensure minimal contact which may lead to short-circuiting. The reference electrode (RE) was placed at a distance of 3cm away from the WE to reduce solution resistance (and ohmic drop effects) as much as possible. The RE used was an Ag/AgCl electrode (+0.199 V vs SHE) composed of saturate KCl solution (4 Molar) and Ag wire with porous frit (Gamry Instruments, USA). The electrodes were connected to potentiostat (Gamry Instrument, Warminster PA, USA). The reactors were operated under mesophilic conditions using a water bath through which water is recirculated to maintain constant temperature.

Two separate BERs were used for biomass enrichment: the first BER was designate as anodic (ABER) and the second as cathodic (CBER). Both BERs were inoculated with anaerobic sludge and synthetic feed (1/8 v/v) such that 1 L of the inoculum was added in addition to 8 L of 1 gCOD/L synthetic feed. The synthetic feed was prepared in accordance with Table 1 The BERs were operated in controlled electrode potential mode whereby the WE potential was set at a constant potential with respect to the RE. The ABER WE was

polarized at a potential of +400 mV vs Ag/AgCl and was sparged continuously at a rate of 2000 L/min with N_2 gas to ensure efficient mixing and no air intrusion. The CBER WE was polarized at -700 mV vs Ag/AgCl and was sparged continuously with a mixture of N_2/CO_2 (80% to 20%) at 2000 mL/min to ensure efficient mixing, no air intrusion and constant supply of CO_2 as an electron acceptor. The anodic and cathodic BERs were operated in 5 and 4 phases, respectively, whereby the synthetic feed medium was replaced once COD was 90% removed and was consequently replaced with 8-L of synthetic feed (1 gCOD/L) in addition to 1-L of leftover medium. The leftover medium is recycled to maintain planktonic biomass presence to aid in the treatment. In each BER, pH was maintained around 7 through supplementation with PBS for the ABER (Composition in Appendix A) or bicarbonate buffer ($NaHCO_3$ addition) for the CBER.

4.3. BER-enriched biomass inoculation

The electrode-attached biomass in the BERs was extracted for enrichment and downstream analysis once the 5^{th} operational phase ended. The extraction procedure was done under anaerobic conditions to ensure no oxygen exposure risk was present which may compromise the developed biomass. Using a sterilized spatula and scalpel, the attached biomass was removed from the electrodes (WE and CE from both BERs) and stored in 15 mL tubes. Approximately, 10 mL of biomass were collected from each electrode, which were then added to Phase 2 inoculum to commence the enriched UASB start-up.

4.4. UASB effluent quality testing

Effluent water quality was monitored on a regular basis for several parameters. Colorimetric tests were measured using HACH DR3900 spectrophotometer by selecting the designated test method. The chemical oxygen demand (COD) was tested every other day for both total and soluble basis according to HACH method 8000 (USEPA Reactor

Digestion Method). The soluble fraction of the effluent was obtained through centrifugation (Thermo Scientific Multifuge X1R) at 13000 rpm followed by filtration using a syringe and Nylon micro-filters having pore size of 0.22 µm (Kinesis KX Syringe Filters). Volatile fatty acids (VFAs) were also measured in the soluble fraction using HACH TNT 872 Volatile Acids kit and following the manufacturer's procedure to quantify the total VFAs as acetic acid whereas the individual composition (acetate, propionate and butyrate) was analyzed using ion chromatography (Metrohm 882 Compact IC plus; using column 250/7.8 for measuring organic acids). Alkalinity, total suspended solids (TSS) and volatile suspended solids were analyzed following APHA methods 2320 and 2540, respectively (American public health association et al., 2012). Measurements for pH were performed using an AD1020 pH meter (Adwa Instruments, HU).

4.5. UASB bed and profile analysis

To monitor the progression and stabilization of the UASB reactor, a profile analysis of the bed and blanket zones is performed. Analyses are done at different heights within the reactor (measured from the influent port) as follows: Bed Zone (0 cm at inlet port), Blanket Zone 1 (6 cm), Blanket Zone 2 (18 cm) and Blanket Zone 3 (25 cm). Analyses are performed on a weekly basis and include total and soluble COD, pH, and TSS/VSS measurement.

Profiling of the UASB reactor is performed based on the VSS measurements at different levels in the UASB reactor to visualize the settlement of biomass. Total VFA analysis is tested in the Bed zone only to monitor sludge stability. To measure sludge settleability and estimate whether sludge is granular or flocculant, the sludge volume index (SVI) test is performed for the Bed Zone biomass using a modified procedure known as the diluted sludge volume index (DSVI). The test was done in accordance with (Von Sperling & Chernicharo, 2005) such that the sludge was diluted 32 times (dilution with a factor of 2) with tap water or reactor effluent. Once the sludge is added, a 30-minute waiting period

begins after which the suspended solids volume is noted as (SS_{30}). The DSVI is then calculated as follows:

$$\text{DSVI}\ (\text{mL/g-TSS}) = \frac{SS_{30}(\text{mL})}{\text{TSS(g/L)}} \times \text{DF} \quad (1)$$

Where SS_{30} is the solids settled volume after 30 minutes (mL), TSS is the total suspended solids of the sludge sample used (g-TSS/L) and DF is the dilution factor (32 in this case). The DSVI value is then converted to equivalent SVI according to equation (2) (Mines, Jr. & Vilagos, 2000):

$$\text{DSVI} = 3.1796 \times (\text{SVI})^{0.7355} \quad (2)$$

4.6. UASB biogas collection and analysis

Biogas produced in the UASB was collected in a 10-L Tedlar gas bag connected to the gas box of the GLS compartment. Another 1-L bag was used to collect headspace biogas (escaped biogas particles in the headspace) so that no pressure would build-up in the headspace. Biogas samples were taken from the GLS gas bag and collected in a 1-L Tedlar gas bag for composition analysis. Gas collection was performed daily, and collection time was recorded daily rates computations.

Biogas composition was analyzed using gas chromatography (GC) (7890B Agilent Technologies, USA) with thermal conductivity detector (TCD) to detect CH_4 and CO_2 composition. The front detector was set at a temperature of 250°C, and the oven and injector at 90°C. Analysis time was set at 25 minutes during which peaks corresponding to the desired gases were detected at different retention times. The carrier gas used was helium (He) at a flowrate of 45 mL/min, set pressure of 75 psi. A packed column system consisting of 4 columns (DC200, UCW982, HayeSepQ and Mol sieve 13X) was used.

Theoretical methane production was computed based on the influent COD using the following equation (Nielfa Gonzalez et al., 2014):

$$\text{Expected } CH_4 \text{ (L/d)} = \frac{\text{COD (g/d)} \times \text{R (L.K.atm/mol)} \times \text{T (K)}}{64 \text{ (g/mol)} \times \text{P (atm)}} \quad (3)$$

Where R is ideal gas constant (0.082 L.K.atm/mol), T is headspace temperature (293K assumed as room temperature), P is pressure (assumed as 1 atm) and COD the influent mass rate (g/d). Furthermore, methane yield rate is calculated as the methane production rate with respect to treated influent COD mass.

4.7. BER treatment analysis

The BERs were routinely monitored and analyzed to check their performance and stability. Total and soluble COD and total VFA were analyzed every other day using the methodology previously described for the UASB effluent water quality. The redox potential (ORP) of each BER was analyzed using an ORP probe (Adwa Instruments, HU) such that a 10 mL volume was extracted and sparged with the gas used for its respective BER to mimic experimental conditions. Daily monitoring of pH was performed for each BER. The counter-electrode potential was measured daily using a multimeter set at a maximum reading of 2 V with the black terminal connected to the RE and the red terminal to the CE electric wires.

4.8. Electrochemical analysis

To test the electrochemical activity of biofilms developing on the electrodes, several electrochemical tests were performed. The open circuit potential (OCP) was measured using the OCP function in the Gamry Framework software (Gamry Instruments, PA, USA) to check the oxidative state at the electrode surface. Polarization of the electrodes was done using the Chronoamperometry function in the Framework software through setting the desired potential for each BER, and the electric current response is recorded.

To analyze the electroactivity of the biofilms, cyclic voltammetry (CV) was performed routinely on the WE in the same BER using the cyclic voltammetry function. The scan rate chosen was 5 mV/s over a potential range of -900 mV to +600 mV vs

Ag/AgCl. Four sweep cycles were performed to eliminate any background current interference and the most stable scan (4^{th} cycle) was reported. Once Phase 5 was complete and the WE were removed, a clean carbon felt electrode was placed and a CV scan for the CE (now set as the WE) was performed.

4.9. Biomass imaging analysis

To observed attached biomass on the electrode and granular formation in the UASB, imaging analysis was performed through scanning electron microscopy and lens microscopy. The lens microscopy consisted of extracted bed biomass and dispersing the sludge using distilled water. Then, using a magnifying lens, images were taken using a high-quality camera of the granules formed to measure their size.

For morphological imaging, SEM was performed on electrode and bed samples. For the BER samples, a 1x1 cm portion of the electrodes were cut using sterile scissors and rinsed with distilled water to remove any stuck planktonic phase biomass. The samples were then fixated with 3% glutaraldehyde at 4°C for 24 hours. The samples were then washed three times for 15 minutes in a PBS buffer solution (0.1M, pH 7.4) followed by dehydration in an ethanol gradient solution using the following sequence, 50%, 70%, 90%, 100% and 100% for 10 minutes each. The samples were then immersed in a mixture series of ethanol and HMDS using the following composition (percentage reported with respect to HDMS): 33%, 50%, 66%, 100% and 100% for 10 minutes each. Samples were then incubated for 24 hours in a desiccator and shipped for SEM imaging.

CHAPTER FIVE

RESULTS AND DISCUSSION

5.1. Overall BER performance

The two BER configurations (anodic and cathodic) were operated at the same time with the constantly poised potential being restarted every week or when a cyclic voltametric analysis was performed. Both BERs treated synthetic wastewater (composition as per Table 1) at a loading of 1 g-COD/L, equating to a total of COD mass loading of 8 g-COD. Each feeding cycle was operated for approximately a month to two months until the feed in the medium was depleted to allow non-turnover conditions at the end of the phases.

5.2. Anodic BER (ABER) performance

5.2.1. ABER treatment performance and resulting current densities

The first phase of the ABER run was conducted using 1-L of anaerobic sludge and 8-L of substrate (8g-COD), the BER was spared with N_2 to ensure mixing conditions and avoid any air intrusion. The chronoamperometric scans have shown an increasing trend in current density after 5 days of constant potential at the working electrode reaching a maximum current 61.5 mA (Fig. 1). The current density trend observed was reported for electroactive bacteria enriched in anodic BERs under batch mode conditions which represent the microbial metabolism and respiration using the electrode as a terminal electron acceptor. Furthermore, the increasing trend, followed by a stable current represent the exponential growth of attached biofilms at the electrode surface (Beyenal & Babauta, 2015). In subsequent phases (Fig. 2-5), the medium was replaced with 8-L of growth medium (1g-COD/L) in addition to 1-L of the inoculum medium. Current density results have shown a lower maximum current response compared to phase 1, with a maximum current reaching

as low as 15 mA in phase 5 (Fig. 5). Interestingly, the period at which the maximum current plateau was maintained extended for a longer period of time during the phases succeeding phase1 before dropping off due to media depletion. This response could be attributed to the formation of thicker biofilms at the electrode which can impose mass transport limitations for the biofilms at the electrode surface, and thus, limit the maximum possible current attainable. Hysteresis observed in the chronoamperometric data is attributed to the replacement of the sparging tank whereby no mixing was occurring.

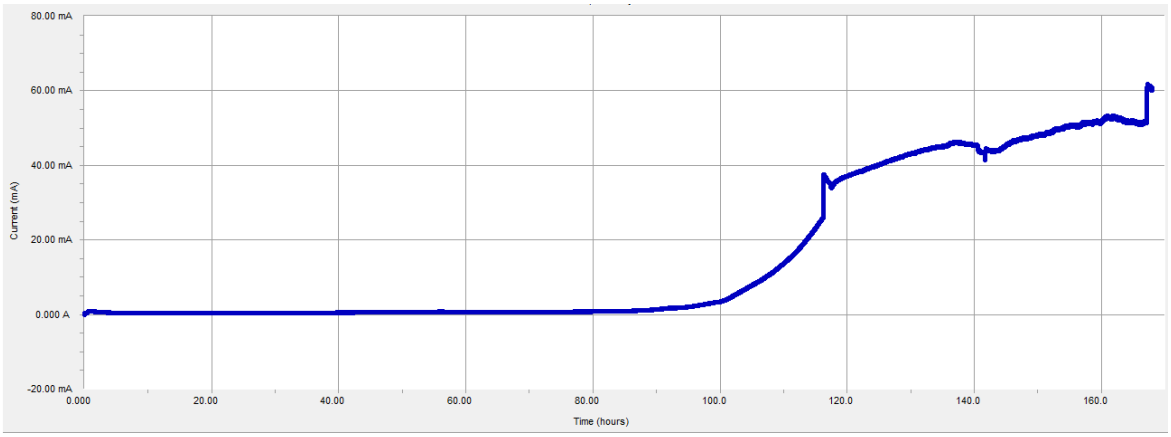

Figure 1. Current production from a chronoamperometric scan for ABER during Phase 1, obtained over the first 6 days of constant applied potential

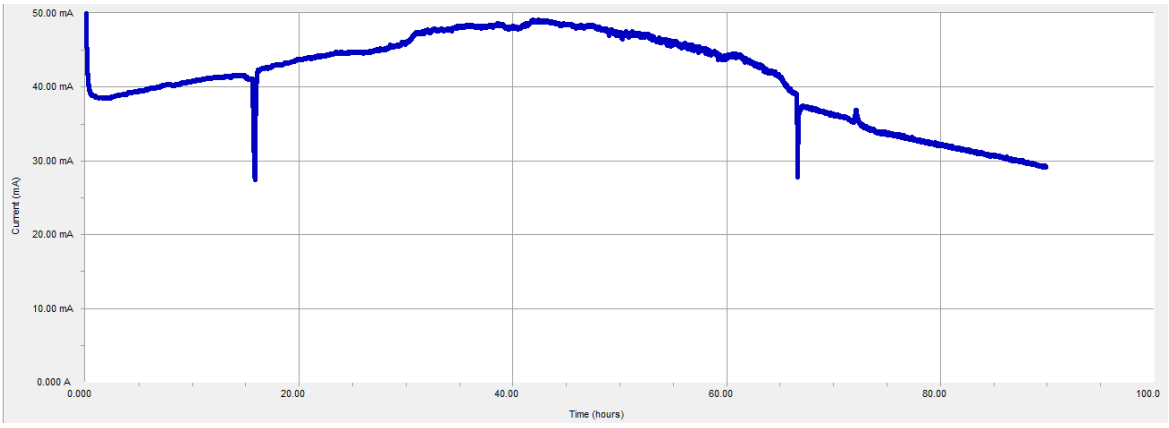

Figure 2. Current production from a chronoamperometric scan for ABER during Phase 2 over 11 days of constant applied potential

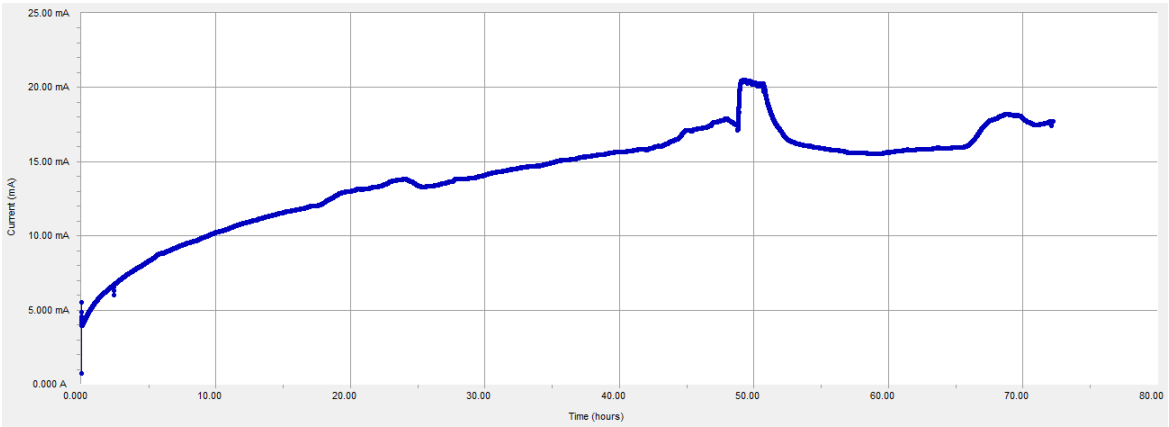

Figure 3. Current production from a chronoamperometric scan for ABER during Phase 3 over 7 days of constant applied potential

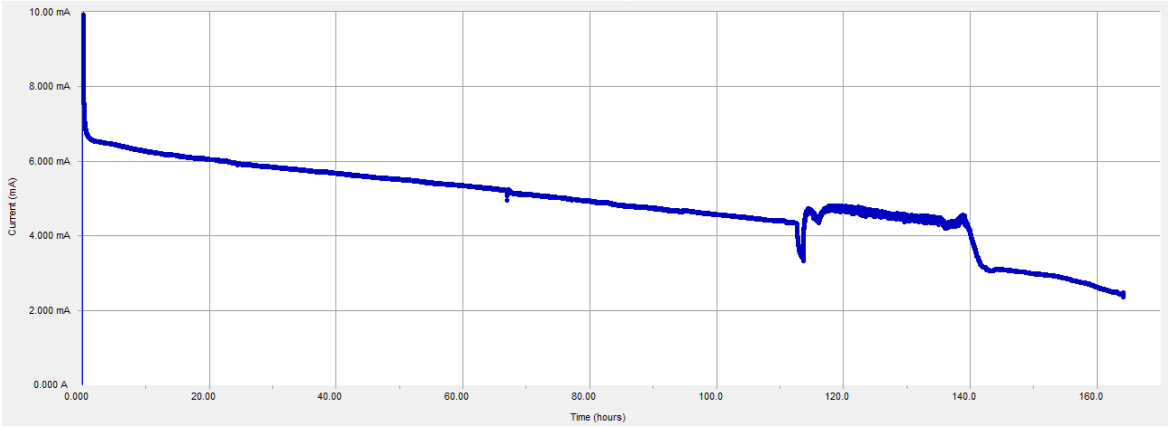

Figure 4. Current production from a chronoamperometric scan for ABER during Phase 4 over 19 days of constant applied potential

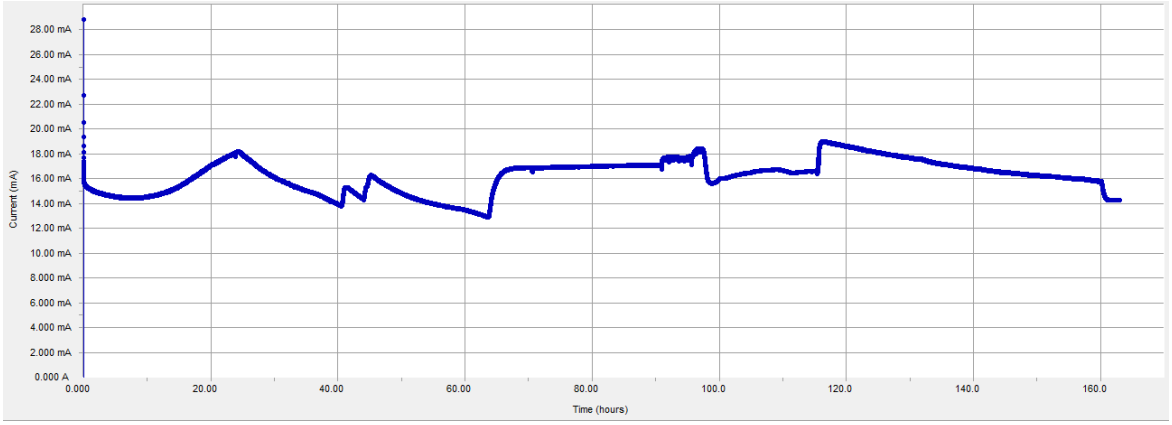

Figure 5. Current production from a chronoamperometric scan for ABER during Phase 5 over 13 days of constant applied potential

On the hand, the counter electrode potential was also assessed in the ABER to monitor variations in the electrode potential. CE potential (as opposed to WE potential), is a parameter controlled by the potentiostat, rather than the user, in order to ensure closed

circuit conditions at the CE (a cathode/electron source in the case of ABER). Variations in such potential tend to ensure either abiotic or biotic activity at the CE. Fig. 6 shows the temporal variations for the CE potential (starting from Phase 2). At the start of Phase 2, potentials lower than -1.00 V and as low as -1.2 V vs Ag/Ag/Cl were attained which may indicate abiotic hydrogen production at the electrode, much lower than the thermodynamic equilibrium potential of -0.6 mV vs Ag/AgCl for $2H^+/H_2$ due to exchange current density limitations by the carbon felt electrode (Bard & Faulkner, 2012). However, the potential increases to the -800 mV limit which may correspond to biological reduction activities at the counter electrode. For phases 4 and 5, the CE potential nearly stabilizes around the -800-mV limit which may be indictive of the development of viable electroactive cathode biofilms.

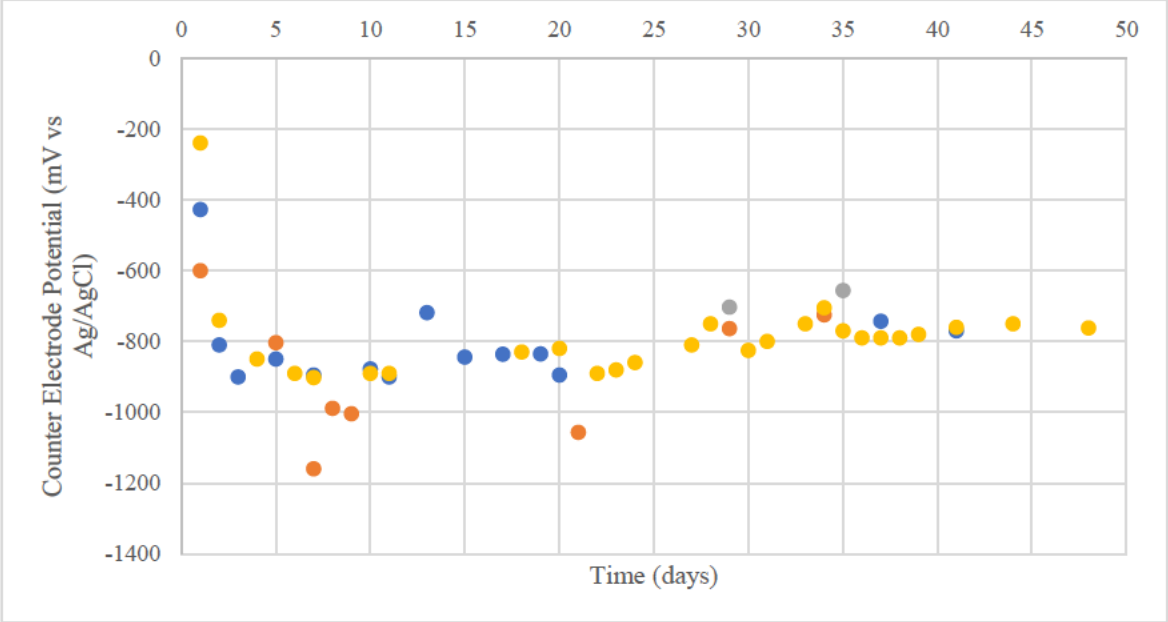

Figure 6. Variation of counter-electrode potential (Ag/AgCl) for ABER throughout the phases (except Phase 1): Phase 2 (grey), Phase 3 (orange), Phase 4 (blue), Phase 5 (yellow)

5.2.2. ABER electrochemical analysis

In order to examine the electroactivity of the microbial species developed at the working electrode and counter electrode, CVs were performed at a scan rate of 5 mV/s on a

wide potential spectrum (-0.9 to +0.6 V vs Ag/AgCl) (Fig. 8-14). Figure 7 shows a CV in the BER medium with no inoculum material to serve as a control CV. CVs were performed at two distinct conditions: turnover and non-turnover. Turnover condition CVs are performed throughout the phase run when substrate (measured as COD) is available while non-turnover condition refers to depleted media with no available substrate. Turnover CVs (Fig. 8-12) were performed at 3 different points during each phase to monitor electroactive development under different COD availability. The CVs obtained for the ABER during the start of all phases shows an increased current at a potential around -0.25 V vs Ag/AgCl with an oxidative peak observed in within the range of -0.1 to +0.3 V vs Ag/AgCl. An oxidative peak corresponds to the presence of redox components which are oxidized through microbial catalysis at the working electrode. These peak are typically observed in microbial electrolysis cells operated at anodic potentials which may correspond to acetate metabolism or another oxidation process for the complex wastewater fed (Kiely et al., 2011).

Shifts in the oxidation peaks may be a result of ohmic drop within the BER due to the large electrode volumes and spacing between the RE and WE, in addition to solution conductivity (Beyenal & Babauta, 2015). The CVs performed at the start, mid and end of phase shows changing trend to observed CVs during the first two phases (Fig. 8 and 9) which may correspond to microbial community shifts in response to applied potential. Fig. 10 and 11 show a similar trend in CV development, with more visible peaks (at higher currents) as the phase progresses. In phase 5 (Fig. 12), the CVs show similar trend with time, but lower peaks are observed as the medium becomes depleted. Nonetheless, the CVs show the presence of electroactive organisms that developed on the WE similar to the trends obtained for *Clostridium* grown in an MFC (H. S. Park et al., 2001).

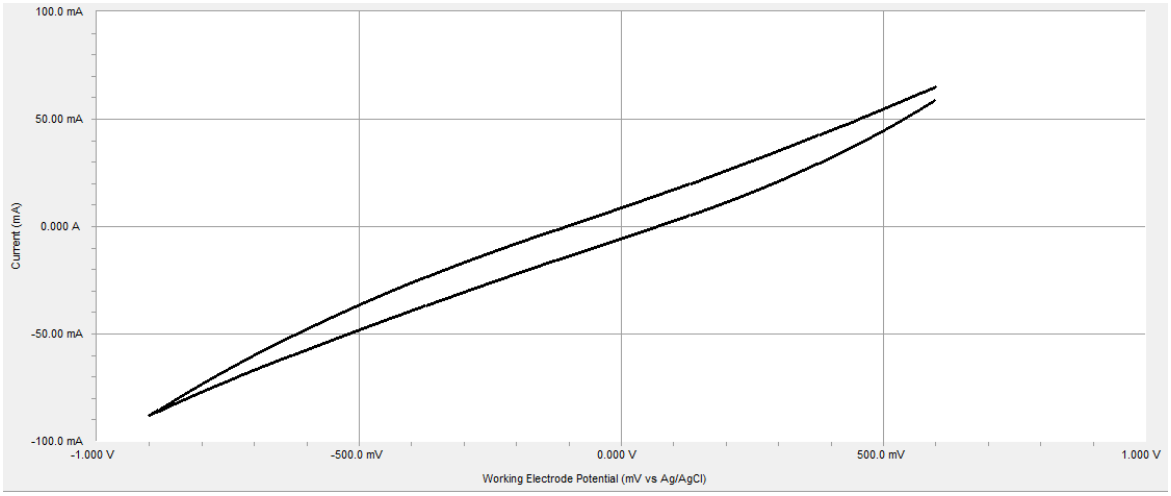

Figure 7. Cyclic voltammetry at the working electrode under control conditions (no present anaerobic sludge)

Figure 13 shows a CV obtained for the CE in the ABER at the end of phase 5 to characterize possible electroactivity. A noticeable reduction peak is observed at -0.35 V vs Ag/Ag/Cl along with two oxidation peaks at -0.35 and 0.00 mV vs Ag/AgCl. These results may indicate that electroactive communities have developed at the CE in the form of cathode oxidizing organisms with similar peaks observed by (Lee et al., 2016).

A non-turnover CV (Fig. 14) was performed at the end of phase 5 once COD reached 49 mg/L and current was as low as 100 µA. Non-turnover CVs are performed in BERs to observe redox peaks that may characterize extracellular electron transfer components which allow electroactive organisms to exchange electrons directly with electrodes. Figure 14 shows the development of several oxidation peaks at -0.42 and -0.39 V vs Ag/AgCl in addition to significant peak observed at -0.15 V vs Ag/AgCl. The peaks may correspond to outer cell proteins expressed by the attached biofilm, namely *c*-type cytochromes such as

OmcZ and OmcB at the potential range around -0.42 to -0.39, while the peak observed at -0.15 V corresponds to redox peak reported at non-turnover conditions but is still unknown.

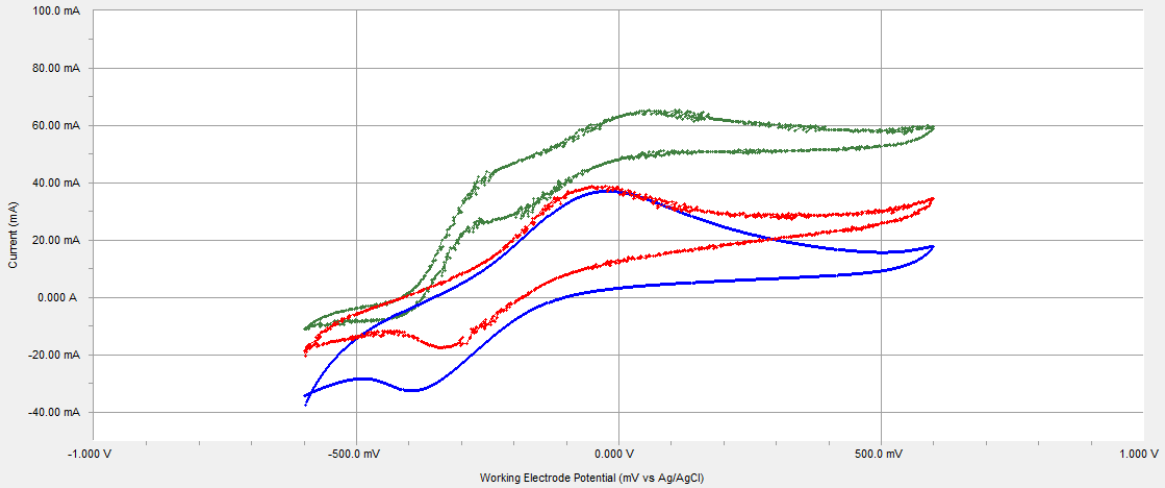

Figure 8. Cyclic voltammetry at the ABER working electrode at different times during Phase 1: Day 9 (green), Day 25 (red), Day 30 (blue)

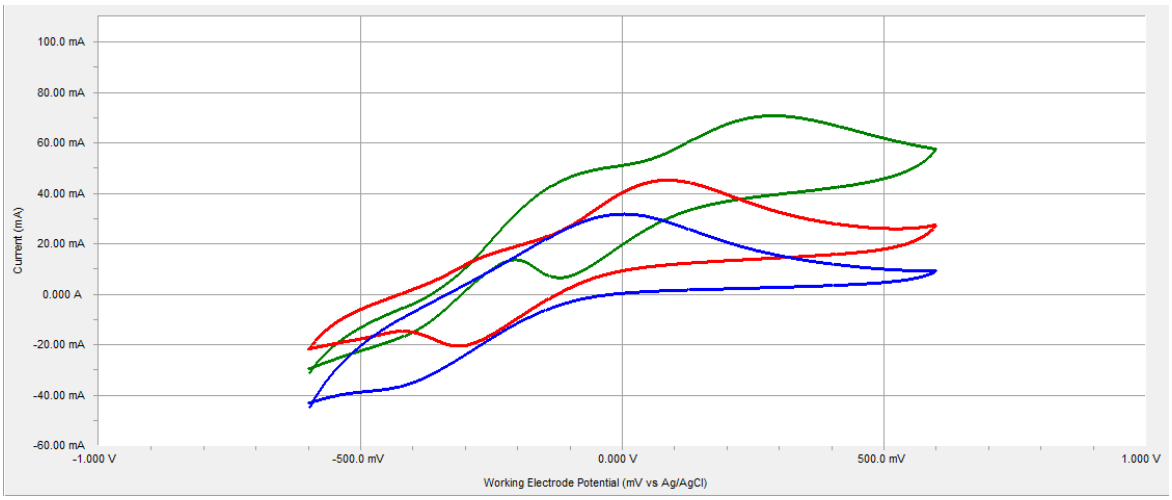

Figure 9. Cyclic voltammetry at the ABER working electrode at different times during Phase 2: Day 6 (green), Day 14 (red), Day 35 (blue)

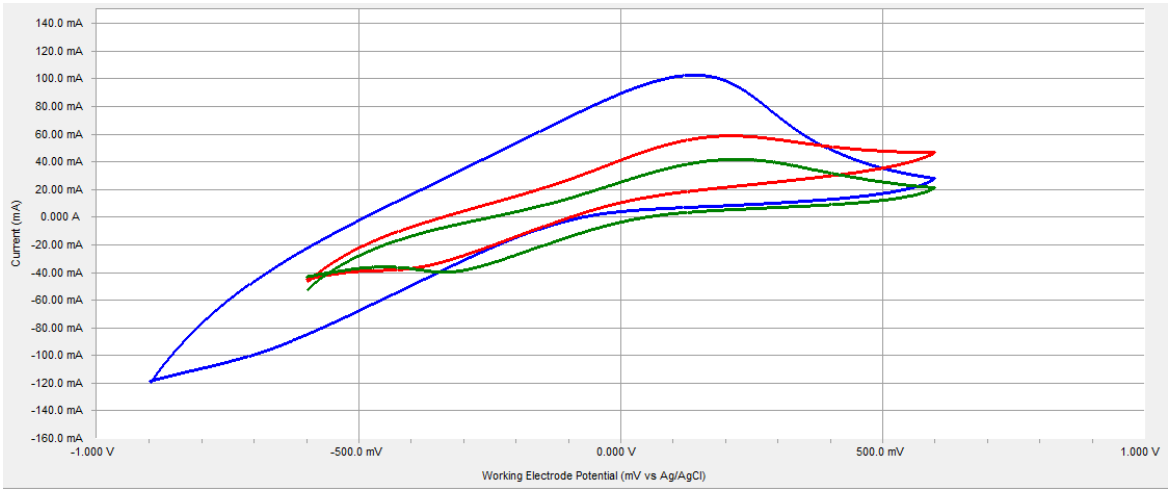

Figure 10. Cyclic voltammetry at the ABER working electrode at different times during Phase 3: Day 5 (green), Day 15 (red), Day 50 (blue)

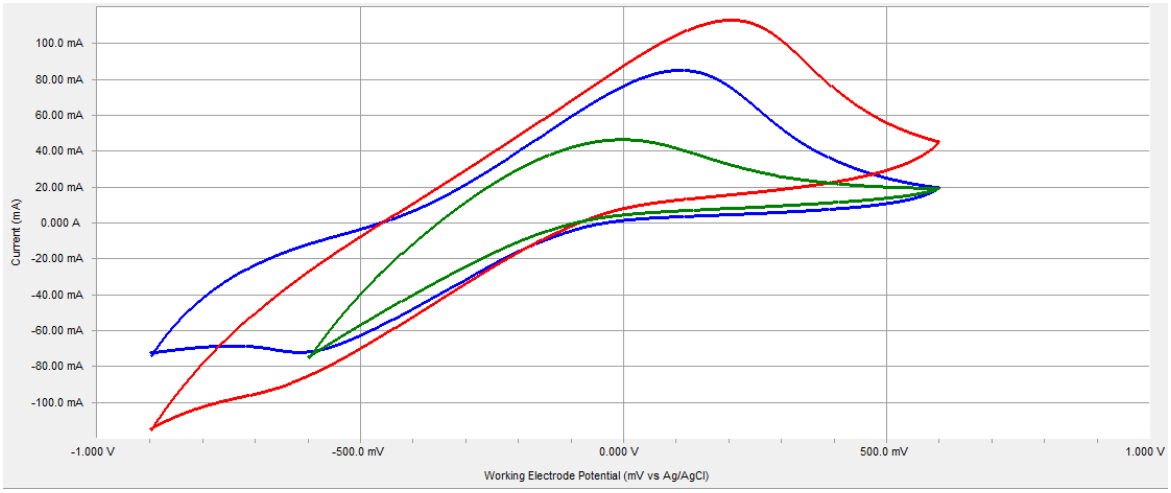

Figure 11. Cyclic voltammetry at the ABER working electrode at different times during Phase 4: Day 7 (green), Day 20 (red), Day 46 (blue)

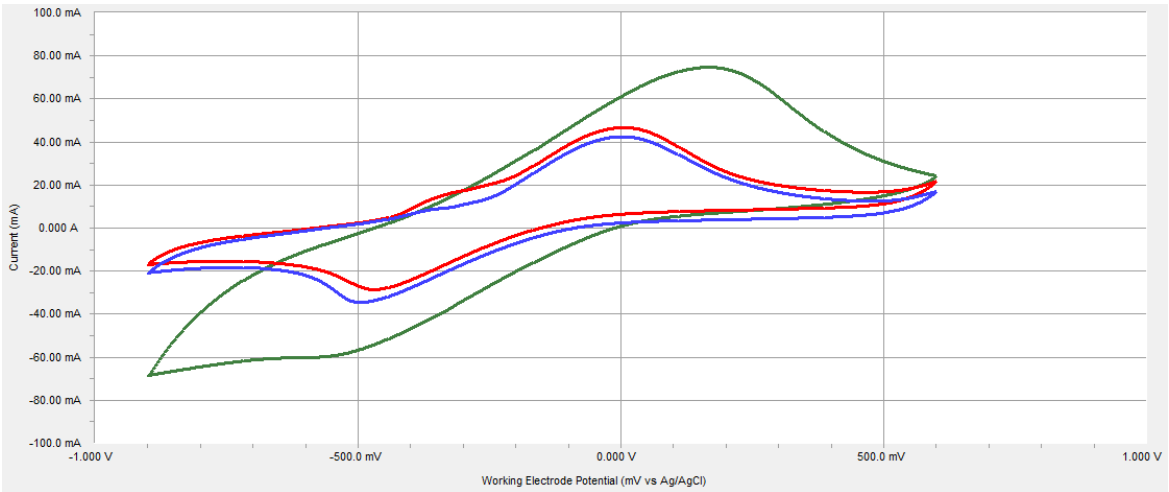

Figure 12. Cyclic voltammetry at the ABER working electrode at different times during Phase 5: Day 1 (green), Day 28 (red), Day 36 (blue)

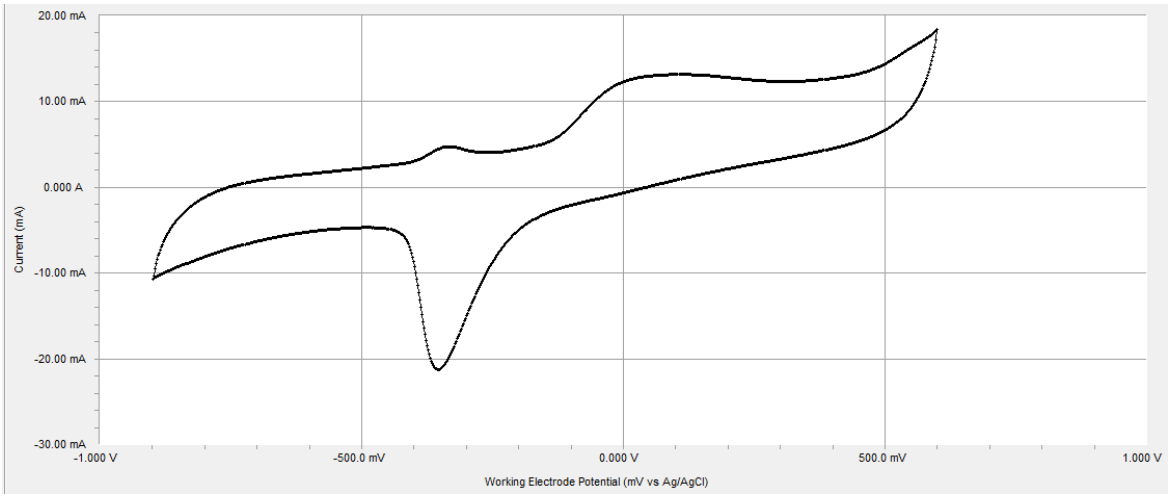

Figure 13. Cyclic voltammetry at the ABER counter electrode (now a working electrode) performed at the end of Phase 5 using a clean carbon felt electrode as the counter electrode

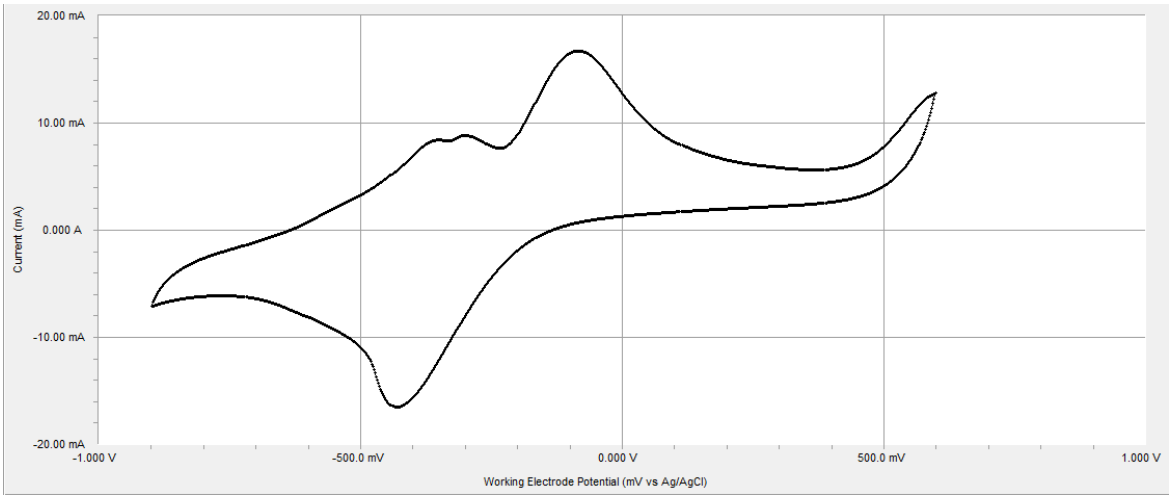

Figure 14. Non-turnover cyclic voltammetry at the ABER working electrode at the end of Phase 5 (when sCOD = 49 mg/L assumed to be depleted growth medium)

5.3. Cathodic BER (CBER) performance

5.3.1. CBER treatment performance and resulting current densities

The cathodic BER was operated for four consecutive phase at an applied potential of -700 mV vs Ag/AgCl to induce electroactivity at the WE. The potential of the CBER was set at sufficient negative potential to allow the development of biofilms of the electrode for microbial catalysis and electrode oxidation rather than abiotic production of hydrogen gas (Beese-Vasbender et al., 2015). The current density data has shown observable negative

currents indictive of electrode oxidation and subsequent microbial respiration using the electrode. The currents; however, had a different trend than that observed for ABER (Fig. 15-18). Maximum currents took longer periods of time to be achieved which could be attributed to the slower growth kinetics of methanogenic archaea growing on electrodes of CBERs.

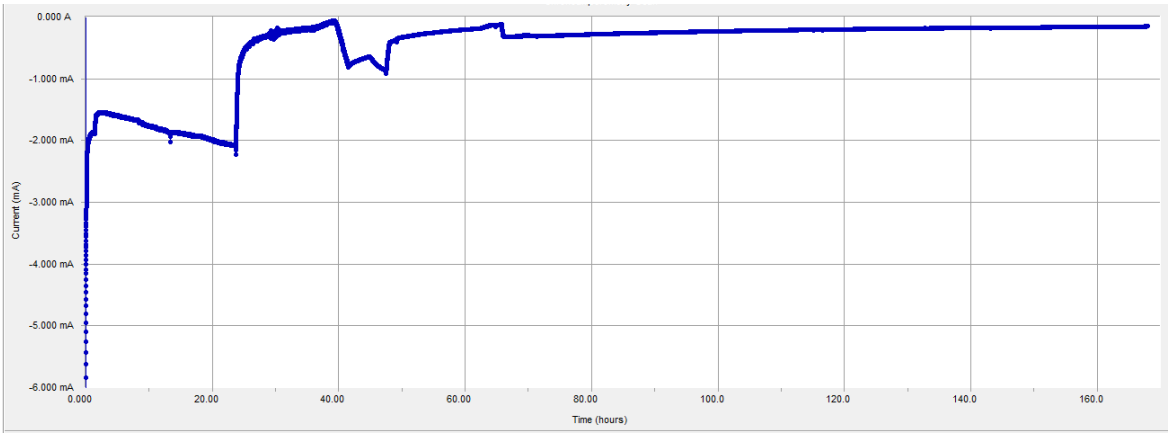

Figure 15. Current production from a chronoamperometric scan for CBER during Phase 1, obtained over the first 7 days of constant applied potential

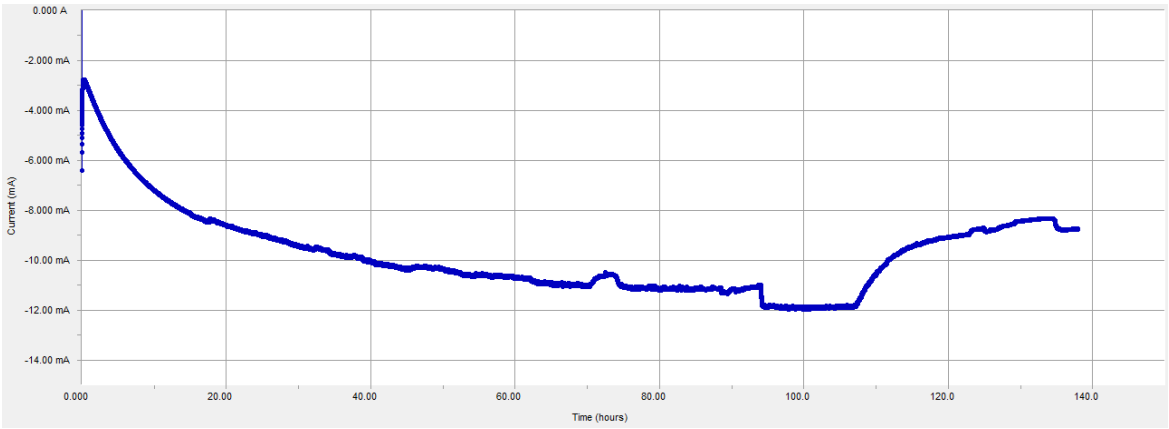

Figure 16. Current production from a chronoamperometric scan for CBER during Phase 2, obtained over the first 7 days of constant applied potential

During the first phase (Fig. 15), the electric current stabilized at around -200µA despite showing at first currents as low as -2 mA during the phase beginning. In subsequent phases, however, an appreciable current was observed reaching as low as -12 mA in phase 2 (Fig. 16), followed by lower currents (Fig. 17 and 18; absolute values) in phase 3 and 4 (-

8 and -4 mA, respectively). Similar to the ABER currents, these values could be attributed to the development of thicker biofilms with phase progression.

Another noticeable feature of the observed currents is its relative stability compared to the ABER in spite of COD consumption in the CBER. Wit a constant flux of sparging gas containing 20% CO_2, the cathodic community may have utilized the influent CO_2 as a terminal electron acceptor by electrophic communities as observed in BERs lacking organic growth medium (Beese-Vasbender et al., 2015; Siegert et al., 2015). Another probability is hydrogenotrophic methane production through bio-hydrogen production at the cathode through development of hydrogenotrophic methanogens or hydrogen synthesizing anaerobic bacteria (Deng et al., 2017; Lin et al., 2007; J.-G. Park et al., 2019).

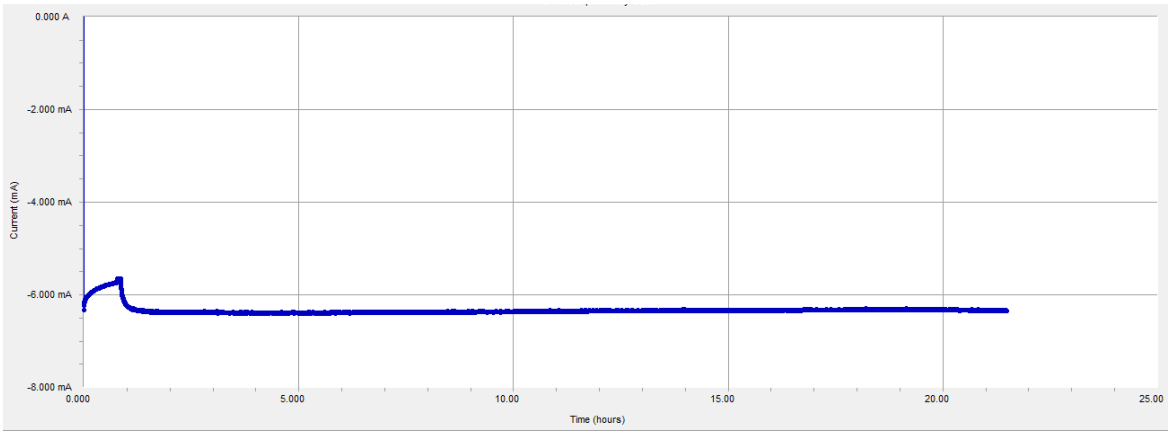

Figure 17. Current production from a chronoamperometric scan for CBER during Phase 3, obtained over the first day of constant applied potential

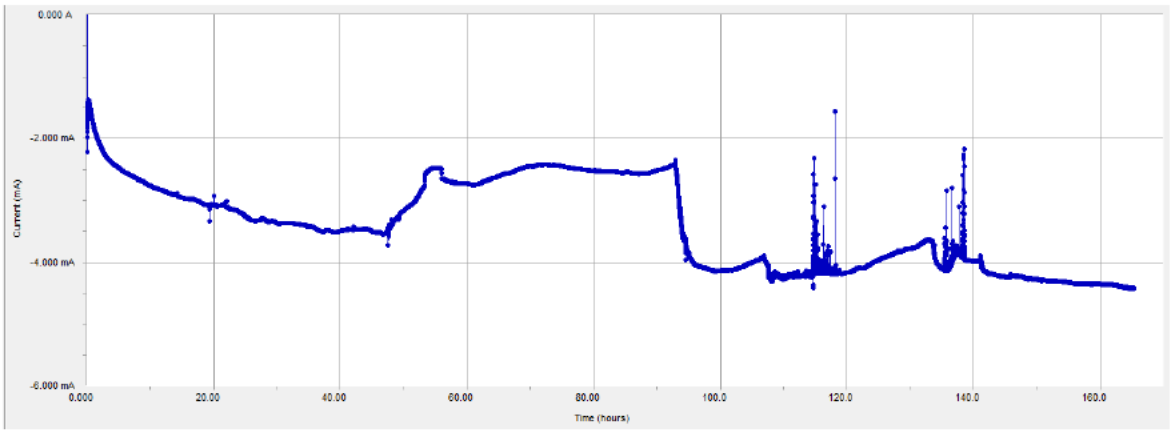

Figure 18. Current production from a chronoamperometric scan for CBER during Phase 4, obtained over the first 14 days of constant applied potential

The data shown in Fig. 19 corresponds to temporal variations of the CE (anode in the case of CBER) during the different operational phases (except phase 1). For all 3 reported phases, the CE potential was maintained around -400 mV vs Ag/AgCl at the start of the phase when COD availability was high. After about 15 days of operations, the CE potential shifted to much positive potentials (around +800 mV vs Ag/AgCl) as the COD media was being depleted. These data may indicate the possible development of oxidative communities at the CE using higher positive overpotentials to overcome energy barriers (Torres et al., 2009).

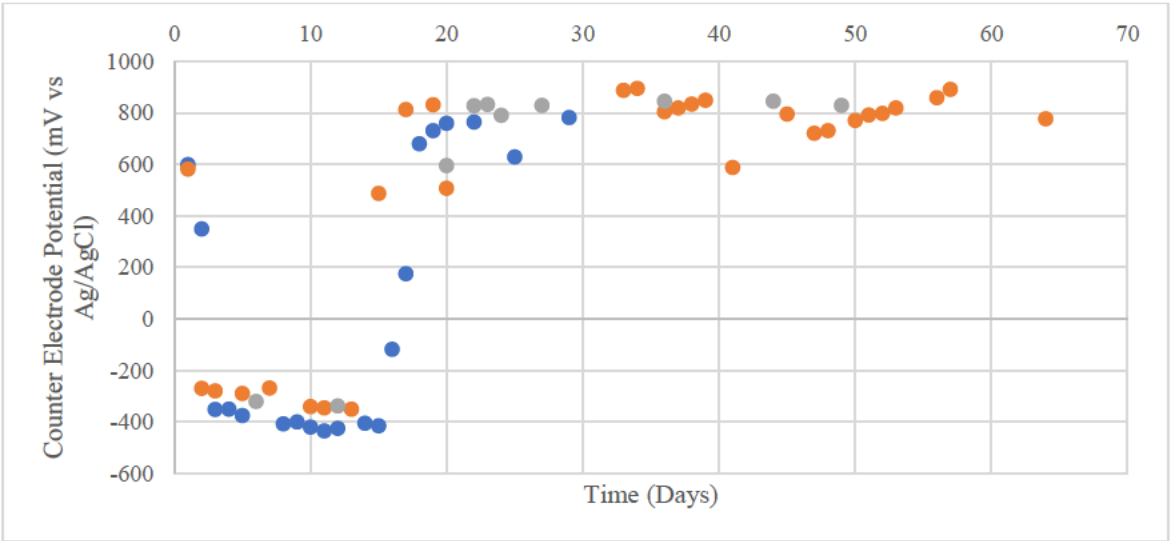

Figure 19. Variation of counter-electrode potential (Ag/AgCl) for CBER throughout the phases (except Phase 1): Phase 2 (grey), Phase 3 (orange), Phase 4 (blue)

5.3.2. CBER electrochemical analysis

CV scans were run at a scan rate of 5 mV/s during the four phases of operation with 3 scans reported at various periods in each phase (Fig. 20-23). The CV data shows nearly similar redox peaks during phase 1 and throughout phases 2 to 4. During phase1 (Fig. 20) as shift in voltammogram shape was observed between the start and end of phase which may signify the selective change in microbial communities present. Nonetheless, the redox peaks observed during all phases showed similar trend with decreasing peak current values as the COD became depleted in the medium.

Figure 21, 22 and 23 shows the development of a visible reduction peak at a value around -400 mV vs Ag/AgCl. This reduction peak could be attributed to the catalyzed reduction of CO_2 to CH_4 by a facilitated mechanism through a poised electrode (Beese-Vasbender et al., 2015). Two oxidation peaks were also observed at -0.35 mV and +0.15 mV vs Ag/AgCl in all phases of operation (Lee et al., 2016; H. Liu et al., 2005; M. Strycharz et al., 2011). Cyclic voltammograms of similar redox nature were previously reported by (Beese-Vasbender et al., 2015) for the methanogenic archaeon *Methanobacterium IM1*. The peaks that develop in CBER communities may be attributed to redox active components expressed by the attached biofilms through which electrons are externally received, though methanogens are believed to exchange electrons through direct cell attachment rather than extracellular genetic expressions.

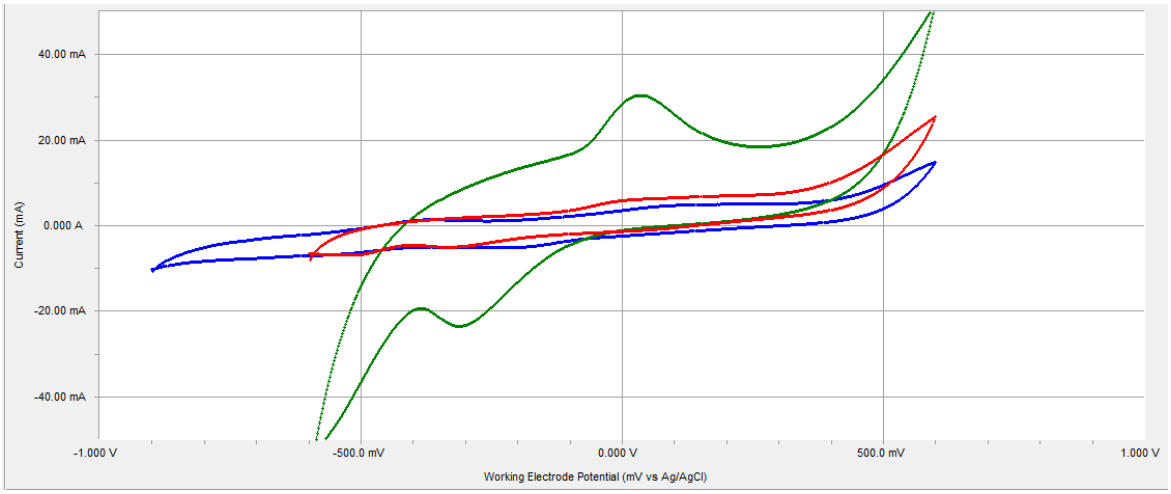

Figure 20. Cyclic voltammetry at the CBER working electrode at different times during Phase 1: Day 7 (green), Day 21 (red), Day 36 (blue)

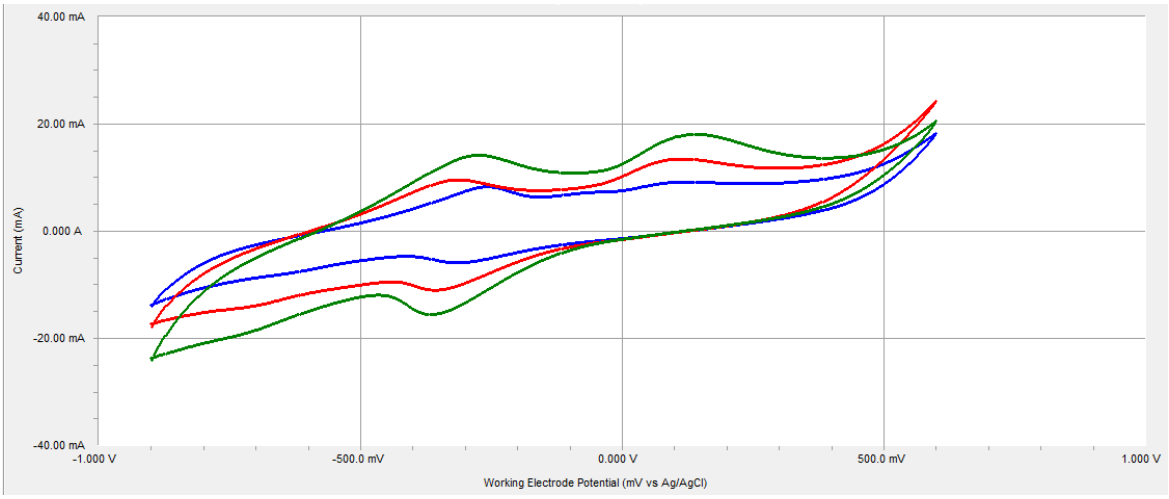

Figure 21. Cyclic voltammetry at the CBER working electrode at different times during Phase 2: Day 12 (green), Day 20 (red), Day 65 (blue)

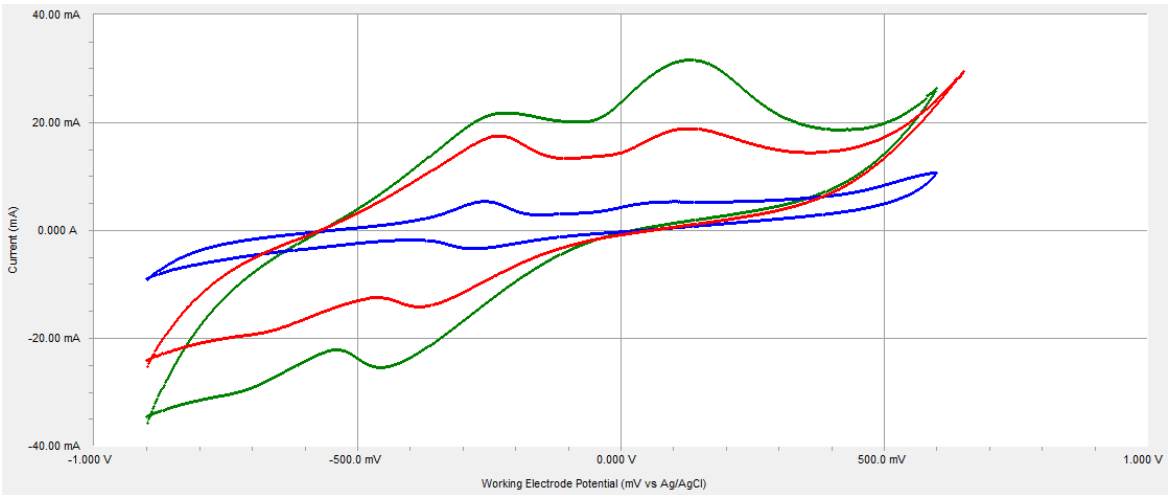

Figure 22. Cyclic voltammetry at the ABER working electrode at different times during Phase 3: Day 13 (green), Day 27 (red), Day 46 (blue)

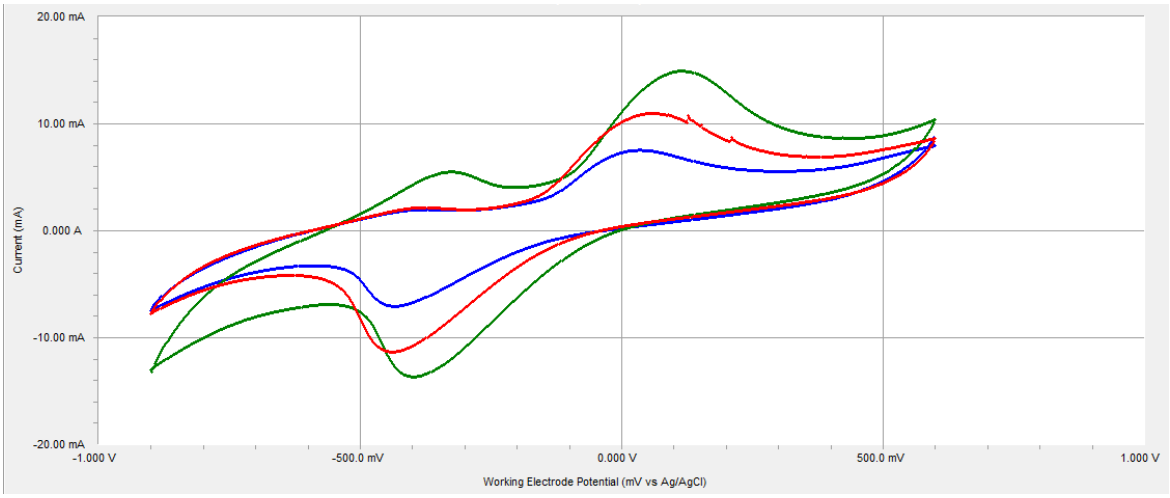

Figure 23. Cyclic voltammetry at the ABER working electrode at different times during Phase 4: Day 3 (green), Day 17 (red), Day 25 (blue)

A CV scan was also performed at depleted conditions (soluble COD reaching about 53 mg-COD/L) during phase 4 (Figure 24). Peaks obtained were similar to those obtained in Figure 23. This phenomenon could be explained by the continuous presence of CO_2 despite the lack of any organic source to be oxidized at the counter electrode of the CBER. To maintain constant current production, decay of possible communities present at the electrodes, in addition to the planktonic phase, may have occurred. However, due to the constant flux of CO_2 to serve as a TEA, the redox peaks displayed similar shapes and electric current values to those observed throughout the phase.

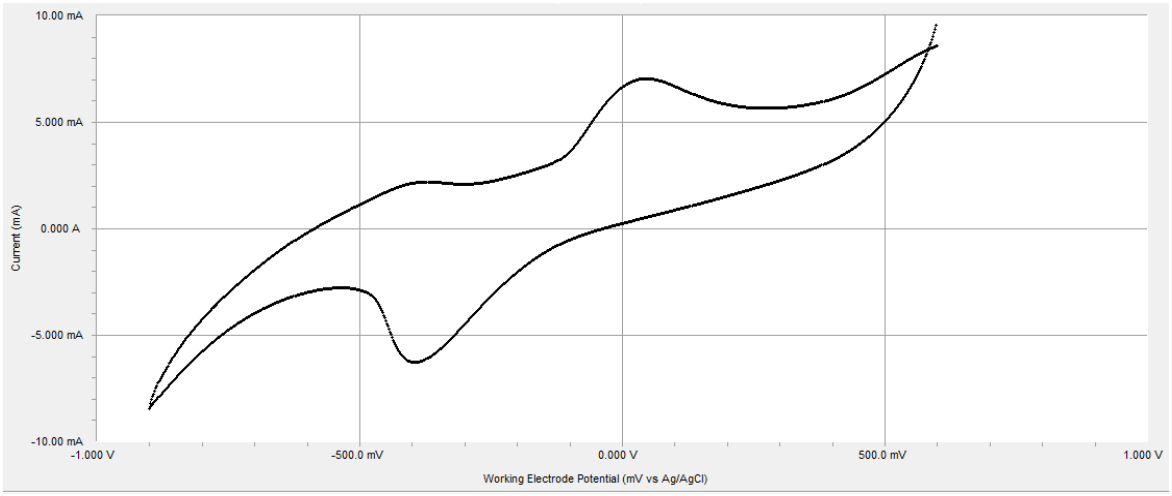

Figure 24. Non-turnover cyclic voltammetry at the CBER working electrode at the end of Phase 4 (when sCOD = 53 mg/L assumed to be depleted growth medium)

5.4. Overall UASB reactor performance

5.4.1. Biogas production and gas composition

The UASB reactor was run in to consecutive phases with the first run being a control run and the second run supplemented with biomass enriched from the BER electrodes at a loading rate of 1 gCOD/L.d and HRT of 48 hrs. UASB start-up is characterized by a slow progression from unstable performance characterized by low COD removal and high sludge washout until stable treatment and biogas production is obtained. For this study, the start-up period was achieved once a stable tCOD removal of 85% was achieved along with VSS removal greater than 90%. After about 5 days of continuous operation, both phases started producing biogas containing CO_2 and CH_4 (Fig. 26). Figure 25 shows the methane composition of the produced biogas in both phases with Phase 2 reaching the 50% threshold after 4 days and remaining stable throughout operations whereas Phase 1 reached that point after 19 days. The results show nearly similar methane composition (around 50%) in both phases. Biogas composition could be attributed to the organic contents of the substrate, namely the carbohydrate protein and lipid content. Substrates with higher carbohydrate content typically result in lower methane composition having equal composition of both CO_2 and CH_4 which was observed in the obtained results (Henze, 2008).

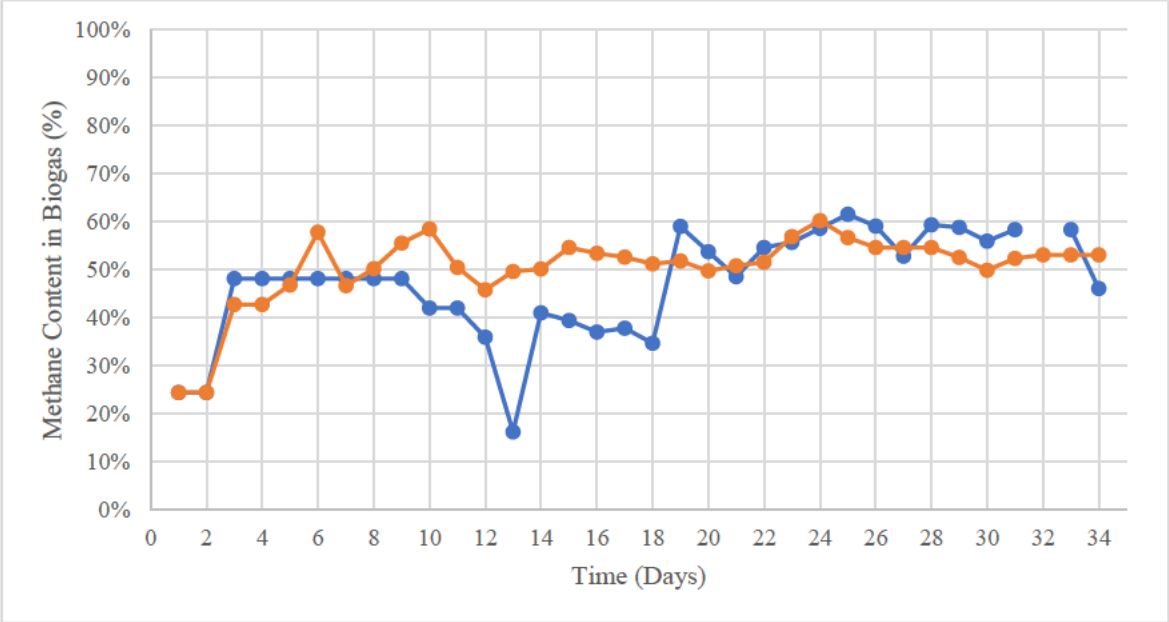

Figure 25. Composition of methane in the biogas produced by the UASB in both runs, Symbols: Phase 1 (blue) and Phase 2 (orange)

Another parameter assessed was the daily volumetric methane production in both phases (Figure 26) reported in terms of the methane produced daily normalized to the UASB effective volume. Methane production in the enriched-UASB (Phase 2) showed a much higher methane daily production, with rates greater than 100 mL/L/day obtained in 6 days while the control run (Phase 1) required 22 days to each similar results. Furthermore, the methane yield with respect to the amount of treated COD was computed to measure methanogenic efficiency (Fig. 27). Phase 2 achieved stable yields greater than 150 mL-CH_4/g-COD treated in about 5 days of operation, while Phase 1 required 21 days to achieve this threshold. (Singh & Viraraghavan, 1998) reported nearly similar methane yield results for an UASB reactor treating municipal wastewater with a total COD removal of 85%. The methane production results show a successful and sped up methanogenesis in Phase 2 as compared to Phase 1, which may indicate that supplementation with active bacterial and methanogenic communities directly to the UASB may have facilitated the anaerobic digestion process.

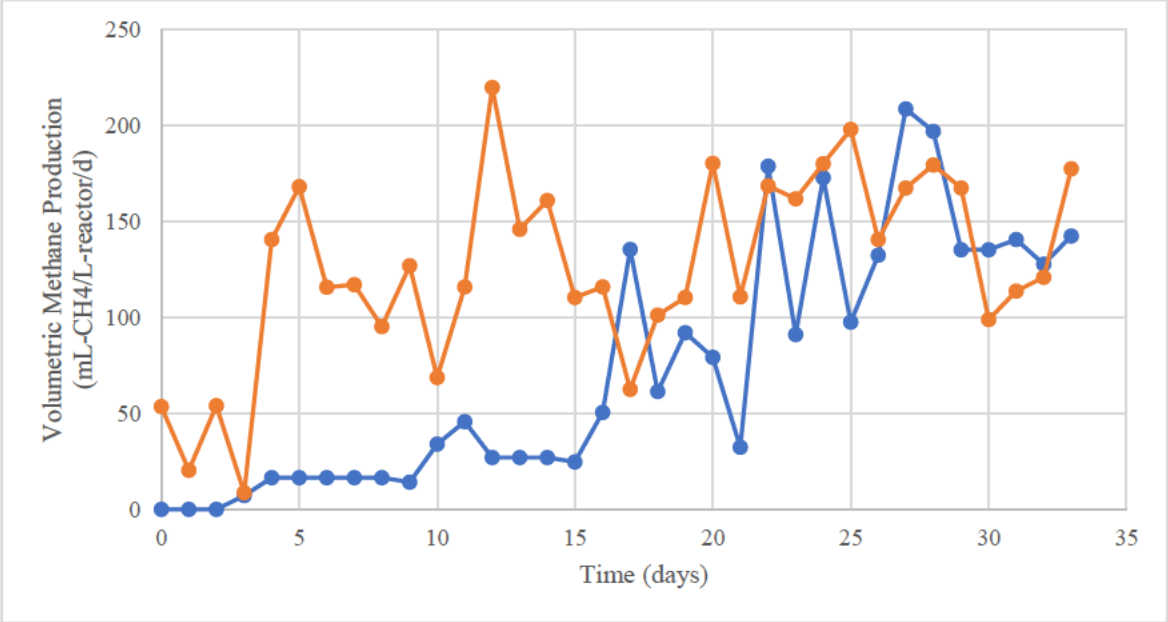

Figure 26. Daily methane production rate for both UASB runs: Phase 1 (blue), Phase 2 (orange)

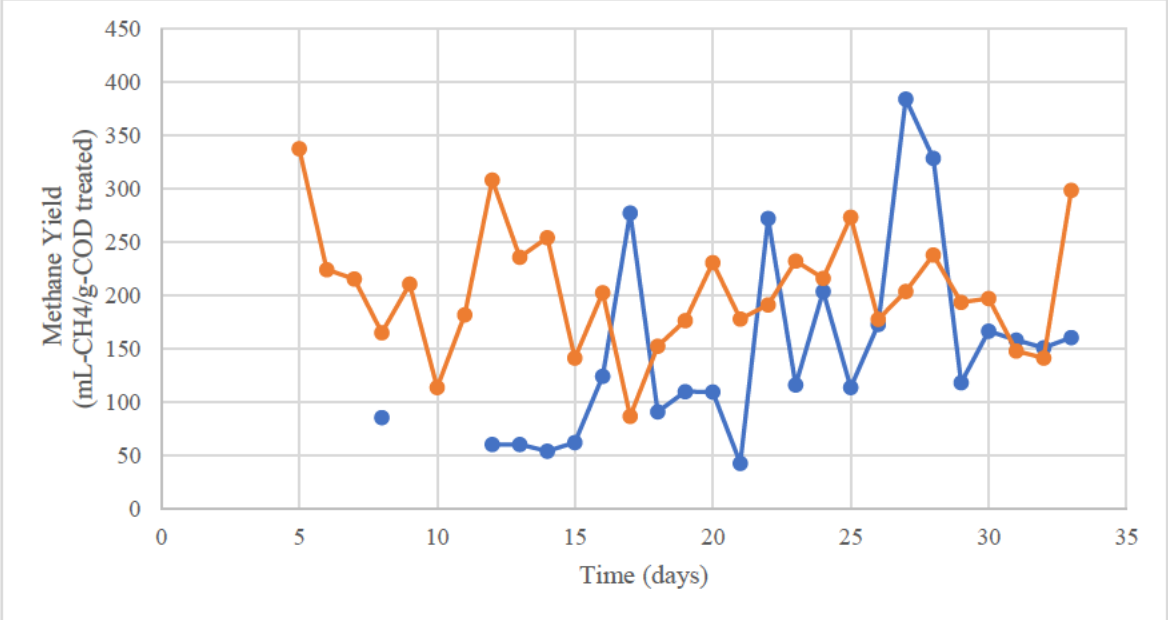

Figure 27. Daily methane yield variation with respect to treated COD for the two UASB phases: Phase 1 (blue), Phase 2 (orange)

5.4.2. COD removal and sludge washout assessment

Effluent quality and treatment efficiency were evaluated in both phases to monitor enhancement in the treatment process. Figures 28 and 29 demonstrate a significant improvement ($P < 0.05$) in the total COD removal in both total and soluble forms between the two phases. Start-up could be considered complete after nearly 22 days in the enriched

UASB run as compared to the control run which remained below the total COD removal threshold over 34 days of operation. Since total COD incorporates both the soluble component (which is mainly the influent wastewater) and particulate material, the soluble COD removal was also assessed in Fig. 29. Phase 2 achieved 85% removal of soluble COD after 13 days as compared to Phase 1 (27 days), which indicates an efficient removal of wastewater components in Phase 2 in a less period.

Sludge washout was another critical parameter evaluated in both UASB phases. Sludge ceased to be detected in the enriched run after nearly 3 days of operation (70% VSS removal), indicating the development of well adapted and settleable sludge whereas the control run had required 17 days to reach 70% VSS removal rates, with heavy washout observed within the first 2 weeks (Fig. 30).

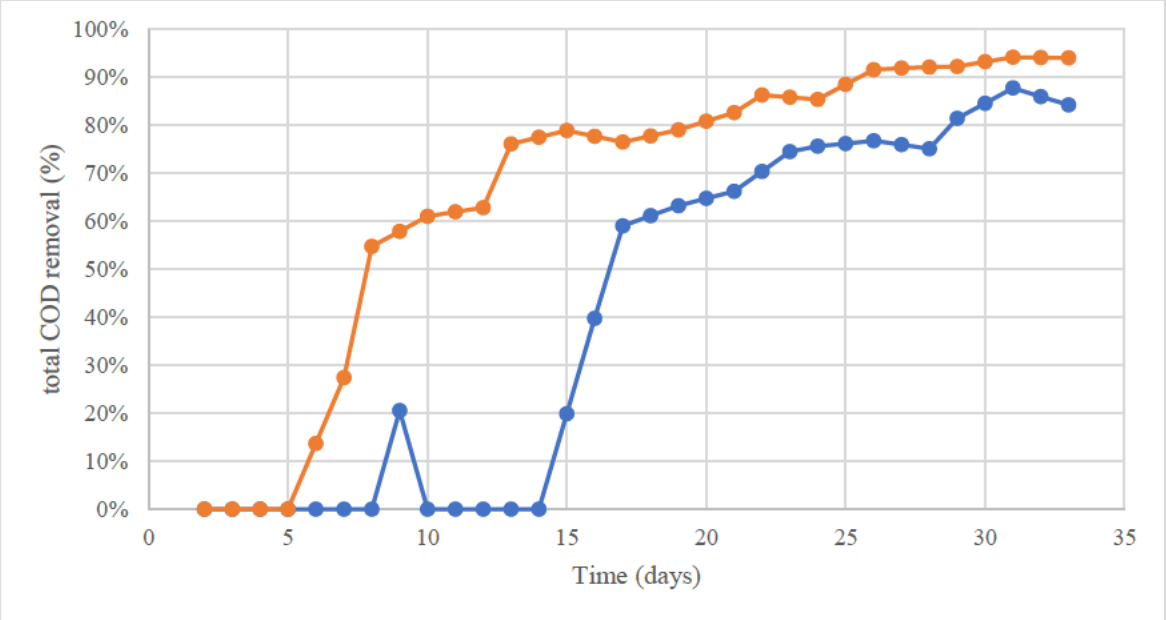

Figure 28. Temporal variation in total COD removal for both UASB runs, Symbols: tCOD removal-Phase 1 (blue) and tCOD removal-Phase 2 (orange)

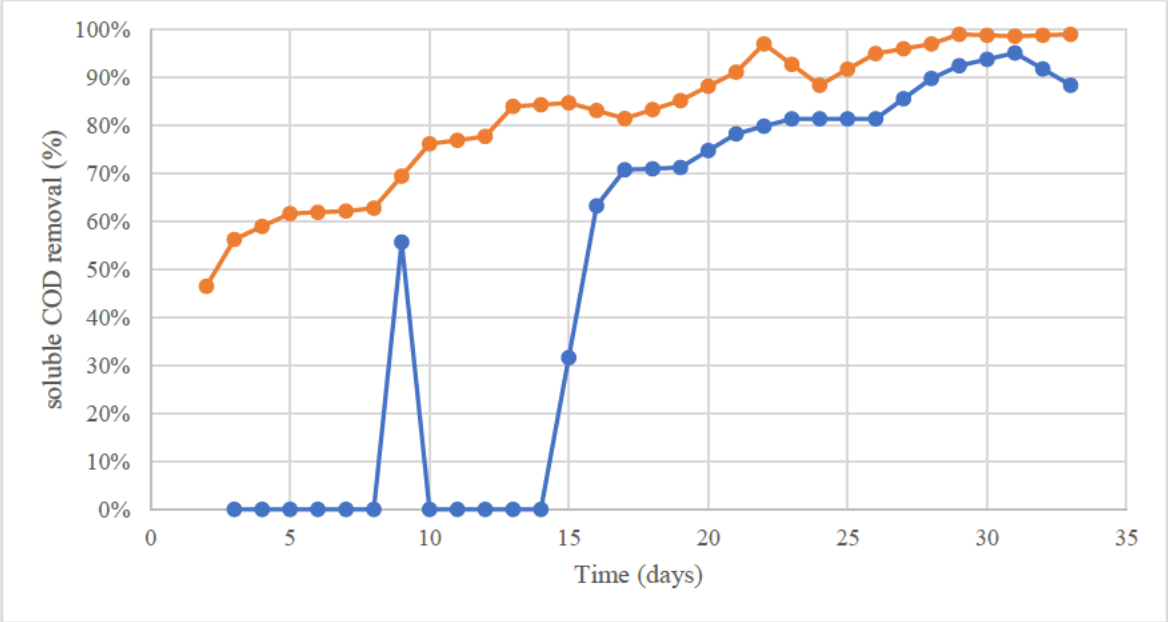

Figure 29. Temporal soluble COD removal variation for both UASB runs, Symbols: sCOD removal-Phase 1 (blue) and sCOD removal-Phase 2 (orange)

The following results demonstrate that the amendment of an UASB reactor with viable biomass (enriched using BERs), independent of attached or conductive media, can aid in enhancing its performance and treatment efficiency. Similar results in terms of enhanced start-up were reported for an UASB reactor treating winery effluent only through supplementation with a specific bacterial species (*Enterobacter sakazaki*), successfully yielding high treatment efficiency (Keyser et al., 2003).

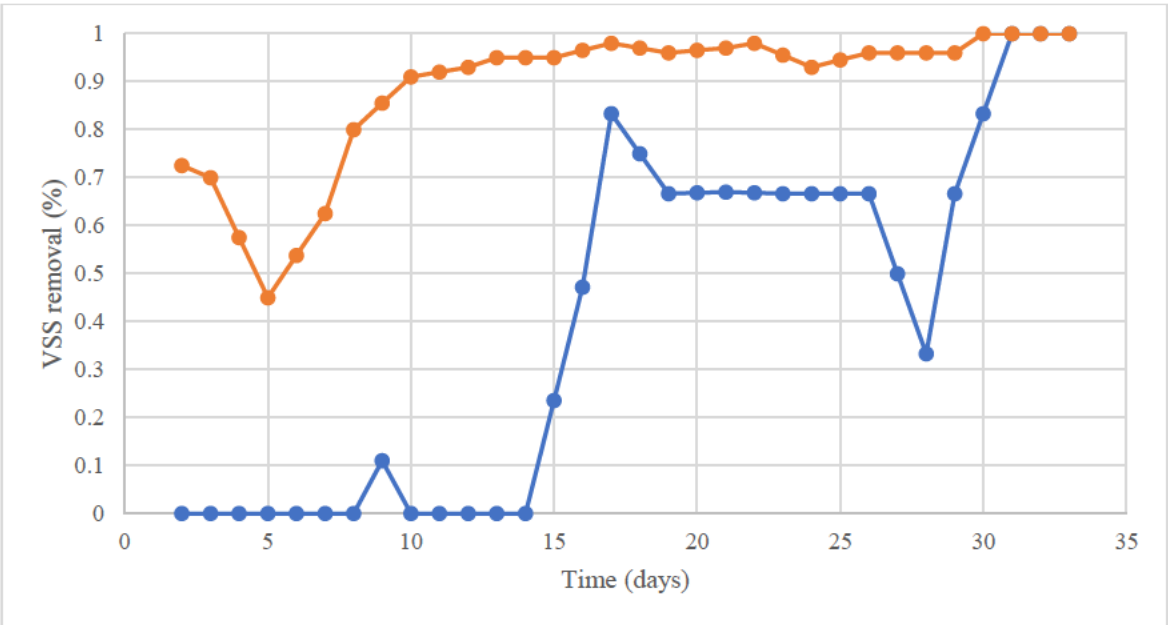

Figure 30. Temporal VSS removal variation: VSS removal-Phase 1 (blue) and VSS removal-Phase 2 (orange)

5.4.3. Granule sludge formation and settleability assessment

To assess the differences between the sludge developed in the UASB bed during phase 1 and 2 (Table 2 and 3, respectively), several tests were performed to characterize the developed biomass. Bed biomass was tested on a weekly basis while settleability tests were performed every other week in order to limit the amount of sludge sampled from the bed as the quantity present was limited. During phases 1 and 2, the pH remained within favorable methanogenic ranges over 4 weeks of operations (7.31 ± 0.77 and 7.19 ± 0.47, respectively). To measure biomass growth, the VSS of the bed was measured and showed an increase over the course of 4 weeks, reaching 28222 ± 509 mg/L in Phase 1 whereas Phase 2 had a lower VSS concentration of 25667 ± 0.00 mg/L. VSS/SS ratios also increased throughout the two phases (0.75 and 0.87 in Phase 1 and 2 by week 4, respectively) which shows the development of more stable biomass within the sludge. As a measure of sludge stability, the total VFA (tVFA) was measured which showed relatively high values within the bed sludge (1025 and 1092 mg/L as acetic acid in Phase 1 and 2 by week 4, respectively); such values may promote the development of flocculant sludge due to shifts in methanogenic communities from granule-favoring Methanosaeta to floc-favoring Methanosarcina (Ghangrekar et al., 1996).

Table 2 - Weekly progression of bed biomass characteristics during UASB Phase 1

	Week 1	**Week 2**	**Week 3**	**Week 4**	**Week 5**
pH	8.44	7.15	6.82	6.84	6.55
VSS (mg/L)	7111 ± 192	29333 ± 2028	30333 ± 333	28222 ± 509	26555 ± 2009
VSS/TSS	0.58	0.69	0.85	0.75	0.86
tVFA (mg-HAc/L	835	637	305	1025	1249

Table 3 - Weekly progression of bed biomass characteristics during UASB Phase 2

	Week 1	**Week 2**	**Week 3**	**Week 4**	**Week 5**
pH	8.44	6.75	7.21	6.96	7.23
VSS (mg/L)	8000 ± 0.00	23167 ± 1179	22667 ± 333	25667 ± 0.00	27334 ± 472
VSS/TSS	0.71	0.85	0.87	0.87	0.86
tVFA (mg-HAc/L	835	500	868	1092	631

The sludge volume index was used to assess the settleability; and therefore, the degree of granulation in the bed sludge. Results in Fig. 31 show the variation in the SVI between the two phases over two points of operation. Despite having relatively similar SVI during week 1 (61 and 66 mL/g-TSS for Phase 1 and 2, respectively), the values seemed to drop sharply by week 4 which highlights the development of more consolidated sludge mass as compared to the conventional anaerobic sludge with which the UASB was inoculated with. By week 4, the SVI had dropped to approximately 33 mL/g-TSS for Phase 1 (which corresponds to the flocculant sludge range of 20 to 40 mL/g-TSS) while Phase 2 had an SVI of 8.3 mL/g-TSS which falls within the range of granular sludge (less than 20 mL/g-TSS) as reported previously (Ghangrekar et al., 2005; Lettinga et al., 1980). These results shows that despite having lower VSS than the control run, the enriched UASB successfully developed granular biomass with high settleability coupled with efficient reactor performance and subsequent start-up.

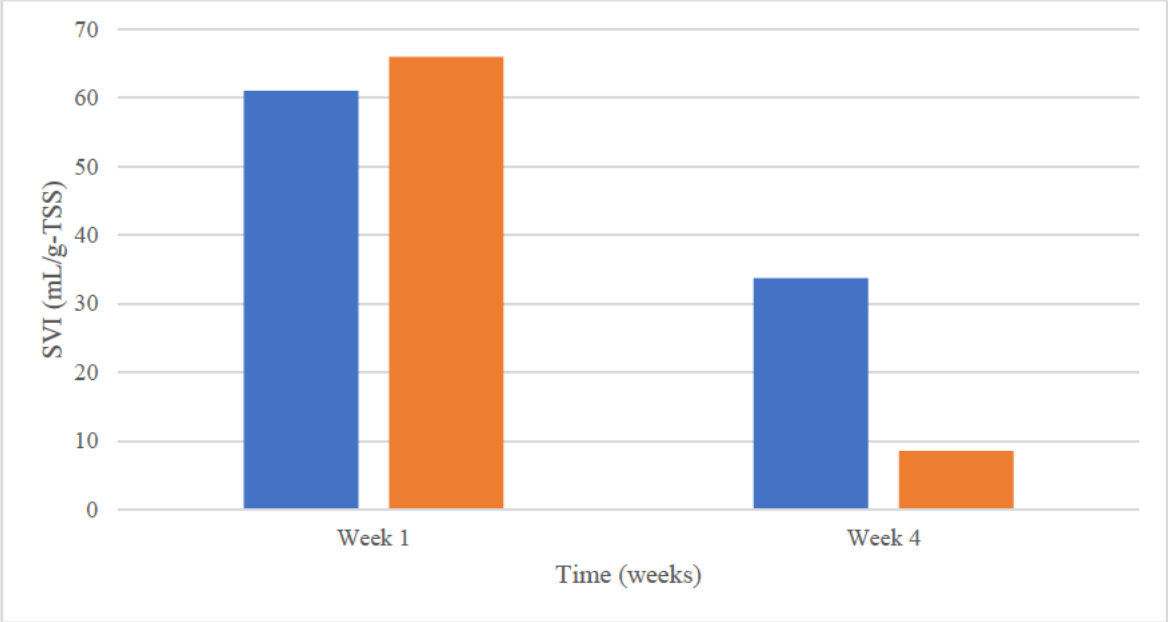

Figure 31. Sludge volume index (SVI) weekly variation for the UASB sludge bed between the two phases: Phase 1 (blue), Phase 2 (orange)

Imaging analysis was performed on the granular sludge in the UASB bed through SEM and through a microscope lens (attached to phone) as can be seen in Fig. 32. SEM imagery performed on the granular sludge of the Phase 1 UASB show the development of granules with nearly spherical morphology and a diameter of approximately 0.2 mm (Fig. 32a) with sizes reaching up to 1 mm. Another image taken using a microscope lens during the UASB Phase 2 also showed the development of 1 mm granules within 20 days of operation. Similar granular sizes were obtained using similar synthetic feed as to that reported by Fang & Chui (1993) after successful UASB start-up. Previous studies have reported variable sizes of sludge granules operated in UASBs operating under different conditions, with sizes ranging from 0.2 mm to 5 mm (Del Nery et al., 2008; Hulshoff Pol et al., 1983; Singh & Viraraghavan, 1998). Variability in granular sizes could be attributed to environmental and operational conditions rather than performance reasons. These conditions are directly correlated to the hydrodynamics within the UASB system, affected by physical parameters such as upflow velocities and shear stress caused by influent flow

which can help determine agglomerate sizes to suite the applied conditions (Y. Liu & Tay, 2002).

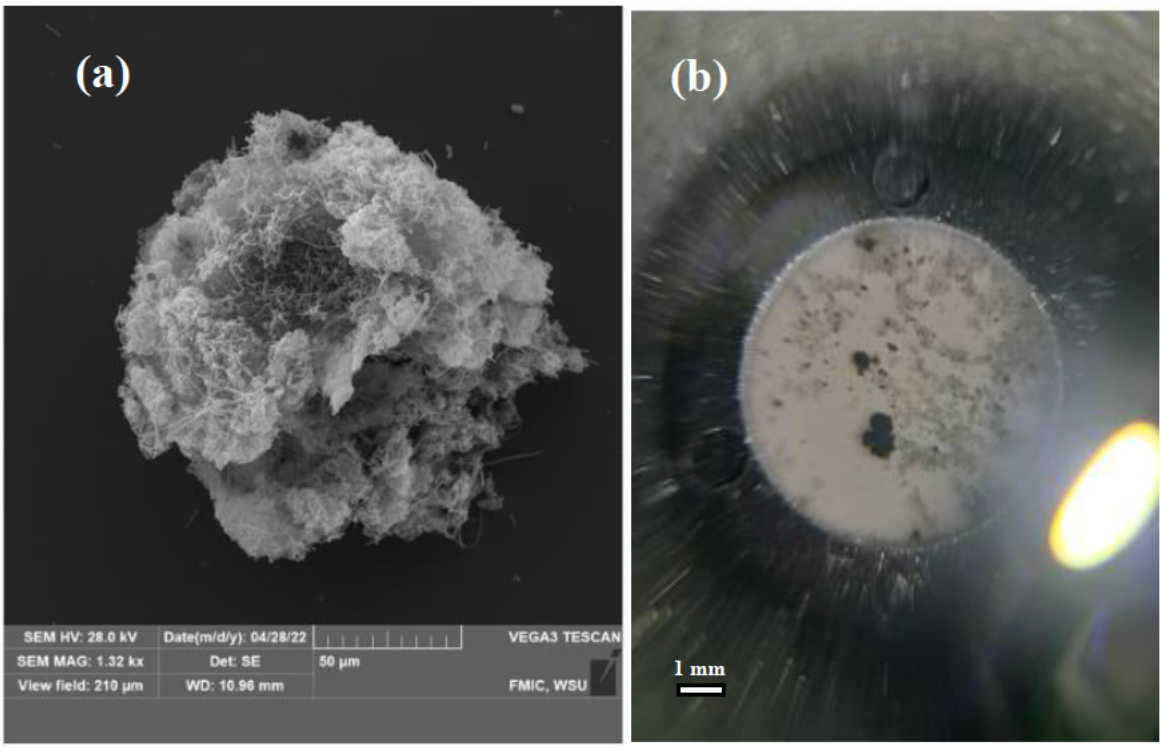

Figure 32. Microscopic images of the granules formed in the UASB sludge bed: (a) SEM of a granule developed during Phase 1 (b) photograph captured by high resolution lens for a granule developed during Phase 2

CHAPTER SIX
CONCLUSIONS

6.1. Summary of works and conclusions

The UASB technology is a mature and effective configuration for the efficient treatment of high strength wastewaters in addition to significant methane production rates. UASB start-up plays an important role in achieving the aforementioned performance efficiency which requires finding innovative means to overcome the slow start-up process and promote the UASB as a favorable option for wastewater treatment. This work focused on overcoming the slow start-up of UASB treating high strength wastewater through supplying it with enriched biomass capable of inherently forming dense granules composed of microbial species capable of anaerobic digestion. Biomass was enriched using BERs set at favorable potentials for cell to electrode attachment and proliferation, with their electroactivity observed through electrochemical tests such as cyclic voltammograms and chronoamperometric curves. The enriched UASB yielded higher volumetric daily methane production, methane yield and significant COD removal. Observable methane production was noticed almost immediately in the enriched run compared to the control run which required 22 days to achieve stable methane yields greater than 100 mL-CH_4/g-COD treated. Furthermore, the enriched UASB run achieved stable COD removal (85% total and soluble COD removal) in a shorter period of time as compared to a control UASB run which needed longer time to adapt and stabilize. Furthermore, sludge settleability tests showed the formation of a granular sludge bed within 4 weeks for the enriched run (SVI = 8.5 mL/g-TSS) while the control sludge bed showed a relatively flocculant bed (SVI = 33 mL/g-TSS). These observations highlight the ability of inoculating an UASB with enriched electroactive biofilms to speed up and enhance the UASB start-up.

6.2. Future works

Despite the apparent enhancement in UASB performance with supplementation of enriched media, additional experimental procedures could be performed to elucidate the reasons behind the enhanced performance. These may include microbial community analysis of the sludge granules and the communities developed on the BER electrodes. Microbial analyses can include high throughput sequencing and real-time PCR analysis to investigate the communities that developed on the electrodes which could be correlated to the CVs obtained and microbial growth with phase progression. Furthermore, these analyses can show the changes in microbial communities of the UASB granules at different points of operation. These results can further show whether the electrode biofilms proliferated in the UASB and resulted in different microbial composition compared to the control run. Genetic expressions can also be performed to understand the possible extracellular electron transferred mechanisms utilized at the electrodes and within sludge granules which could contribute to electroactive responses and possible cell agglomeration facilitation.

To make the results obtained in this study more applicable to real life applications, utilizing different wastewaters and operational conditions can be performed. In order to unlock the UASBs full operational potential, applying higher loading rates (organic and hydraulic) may provide insight into the adaptability of the enriched-UASB system to different environmental conditions, in addition, utilizing more complex wastewaters which may include industrial wastewaters, can demonstrate the ability of such system to sustain the variabilities of influent wastewaters.

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