Enhanced methane yields from anaerobic digestion of *Arthrospira maxima* biomass in an advanced flow-through reactor with an integrated recirculation loop microbial fuel cell

Alister. E. Inglesby and Adrian. C. Fisher*

Received 18th March 2012, Accepted 14th May 2012
DOI: 10.1039/c2ee21659k

A semi-continuously fed continuous stirred tank reactor (CSTR), advanced flow-through anaerobic reactor (AAR) and advanced flow-through anaerobic digester with an integrated recirculation loop microbial fuel cell (ADMFC) were investigated for the production of methane using *Arthrospira maxima* as the sole feedstock. The study demonstrated that the inclusion of a recirculation loop MFC increased the methane yields and so energy efficiency of anaerobic digestion of *Arthrospira maxima* biomass. The semi-continuously fed CSTR operating under a hydraulic retention time (HRT) of 10 days and an organic loading rate (OLR) of 0.5 g DW per L\(^{-1}\) d\(^{-1}\) concluded a maximum methane yield of 90 ± 19 mL CH\(_4\) per g VS with an energy efficiency of 17.1 ± 0.8%. The continuous phase AAR and ADMFC units were operated with HRTs ranging from 2 to 4 days and organic loading rates varying from 0.5 to 3 g DW L\(^{-1}\) d\(^{-1}\). The maximum methane yield and energy efficiency concluded from the ADMFC unit was 173 ± 38 mL CH\(_4\) per g VS and 29.7 ± 6.8% respectively, whilst that of the AAR was 136 ± 16 mL CH\(_4\) per g VS and 22.1 ± 2.6%.

Introduction

*Arthrospira maxima* filamentous cyanobacteria biomass has been targeted as key natural resource in the post-fossil fuel era due to its high growth productivity of up to 27 tonnes annual biomass per hectare in outdoor mass culture,\(^1\) low cost of cultivation (buoyant filaments enable easy filtration harvest) and application as both a foodstuff and for high value product production.\(^2\) Along with this it is an attractive biomass as it can grow to high cellular densities at fast rates (maximum quantum yields of 2.8-9.4% solar-to-biomass energy conversion).\(^3\) *A. maxima* grows optimally in alkaline water (pH 9.5 to 11) at high concentrations of sodium (0.4-1.4 M), so it grows with little or no parasitic microbial contamination in the wild environment.\(^3\) In order to create a self-sustainable process regardless of the intended product, the biomass itself can be used to derive energy be it directly or indirectly. *A. maxima* has previously been shown to be a potential feedstock for the anaerobic digestion (AD) process aimed at methane (CH\(_4\)) production. When used as a substrate for AD, *A. maxima* resulted in a specific methane yield (SMY) of 0.3-0.37 m\(^3\) CH\(_4\) per kg volatile solid (VS), with energy.
efficiencies (EE) up to 59%. AD of micro- and macro-algae as well as cyanobacteria has become a resurgent field of work in the renewable energy arena. Studies conducted through life cycle analysis, techno-economic investigation, theoretical modelling and experimental research; have all suggested the technology as a high potential bioenergy production process.\textsuperscript{9–12} Until very recently\textsuperscript{13,14} lab-scale anaerobic digestion of algal or cyanobacterial biomass has been investigated in simple batch or semi-continuously fed continuous stirred reactors (CSTRs). These reactors are limited in performance. High rate anaerobic reactors such as upflow anaerobic sludge blanket (UASB), anaerobic filter (AF), anaerobic membrane bioreactors (ANMBR) and anaerobic fluidised bed reactors (AFBR) allow for biomass to be digested without significant dewatering as well as with decreased hydraulic retention times (HRT). Solid retention times (SRTs) are significantly higher in the advanced digesters due to immobilisation techniques as well as three phase separators most are installed with.\textsuperscript{14} In this study three different systems are proposed for comparison. A simple CSTR, advanced anaerobic reactor (AAR: improved solid retention and mixing) and an advanced digester with an integrated microbial fuel cell (ADMFC: AAR plus a recirculation loop MFC) were all investigated.

Since \textit{A. maxima} has a significant protein content (>65%), digestion of this nitrogenous matter results in the release of high amounts of ammonia into solution (lit.,\textsuperscript{7} up to 7000 mg L\textsuperscript{–1}). Ammonia exists in two phases namely free ammonia (NH\textsubscript{3}) and ammonium ions (NH\textsubscript{4}\textsuperscript{+}), the former being inhibitory to methanogens at concentrations above 1.7 g L\textsuperscript{–1}.\textsuperscript{15} A number of different techniques have been investigated for ammonia removal from AD systems such as: natural zeolites for ion exchange (removing ammonium ions), stripping, precipitation and codigestion with carbon rich streams, amongst others.\textsuperscript{5,16–19} In this study it is proposed that a recirculation loop microbial fuel cell (MFC) be implemented for online ammonium ion removal through the cation exchange membrane of the MFC. Effective ammonium recovery can be achieved by ion flux through the cation exchange membrane to the cathode chamber, driven by the electron production from degradation of organic substrate in the anode chamber of a MFC.\textsuperscript{20} Kuntke and colleagues reported that the charge transport was proportional to the concentration of ions however; a concentration gradient did influence the charge transport. Furthermore, a charge exchange process could influence the charge transport and therefore the recovery of specific ions.\textsuperscript{20}

The combination of AD and MFC technology has been researched for target specific applications. Weld and Singh (2011) investigated a hybrid AD–MFC system aimed at improved wastewater treatment.\textsuperscript{21} The work showed that the addition of electrogenic bacteria \textit{via} an MFC positioned in a recirculation loop provides an alternative route for the removal of volatile fatty acids that bypass the functionality inflexible methanogens which are the key microbes driving AD. Jeong \textit{et al.} (2008) reported that the removal of volatile fatty acids in anaerobic digestion of organic wastes could accelerate their eventual decomposition to CO\textsubscript{2} and H\textsubscript{2}O using a recovery of electric energy by a microbial fuel cell.\textsuperscript{22} The researchers implemented a simple design of a fermenter with a recirculation loop MFC to generate these results. De Schamphelaire and Verstraete (2009) investigated the concept of AD followed by a downstream polishing MFC with a mixed consortium of \textit{C. vulgaris} and \textit{S. obliquus} algae as the feedstock.\textsuperscript{23} Both of those species are known to be highly resistant to biodegradation.\textsuperscript{12} The results reported by De Schamphelaire and Verstraete reiterated this with low methane yields and electricity generation in their process.

The objectives of this study were: (1) to demonstrate the importance of advanced systems in laboratory scale anaerobic digestion of \textit{A. maxima} biomass; (2) vary the feed rate and biomass concentration to note important trends for optimisation purposes; (3) determine the importance of introducing a recirculation loop MFC into the AD process and finally (4) discuss the opportunities for the integrated anaerobic digestion and microbial fuel cell system.

\section*{Experimental}

\textbf{Cultures}

\textit{Arthrospira maxima}. Stock cultures of \textit{Arthrospira maxima} cyanobacteria (obtained from the Culture Collection of Alga and Protozoa (CCAP), Scotland) were maintained at ambient temperature in 500 mL Duran bottles, continuously mixed with a magnetic stirrer and illuminated with three fluorescent bulbs from one side of the flask. All cultures were grown on Zarrouk’s media comprising of: nutrients (18 g L\textsuperscript{–1} NaHCO\textsubscript{3}, 2.5 g L\textsuperscript{–1} NaNO\textsubscript{3}, 0.5 g L\textsuperscript{–1} K\textsubscript{2}HPO\textsubscript{4}, 1 g L\textsuperscript{–1} K\textsubscript{2}SO\textsubscript{4}, 0.04 g L\textsuperscript{–1} CaCl\textsubscript{2}, 2H\textsubscript{2}O, 1 g L\textsuperscript{–1} NaCl, 0.2 g L\textsuperscript{–1} MgSO\textsubscript{4} \textsubscript{7}H\textsubscript{2}O, 0.01 Fe\textsubscript{2}O\textsubscript{3}, 7H\textsubscript{2}O and 0.08 g L\textsuperscript{–1} EDTA), metal solution A5 (2.86 g L\textsuperscript{–1} H\textsubscript{2}BO\textsubscript{3}, 1.81 g L\textsuperscript{–1} MnCl\textsubscript{2}, 4H\textsubscript{2}O, 0.22 g L\textsuperscript{–1} ZnSO\textsubscript{4} \textsubscript{7}H\textsubscript{2}O, 0.08 g L\textsuperscript{–1} CuSO\textsubscript{4} \textsubscript{5}H\textsubscript{2}O and 0.0124 g L\textsuperscript{–1} Na\textsubscript{2}MoO\textsubscript{4}) at a concentration of 1 mL L\textsuperscript{–1} and metal solution B6 (56.6 mg L\textsuperscript{–1} K\textsubscript{2}CrO\textsubscript{7}, 47.8 mg L\textsuperscript{–1} NiSO\textsubscript{4} \textsubscript{7}H\textsubscript{2}O and 4.2 mg L\textsuperscript{–1} CoSO\textsubscript{4} \textsubscript{7}H\textsubscript{2}O) at a concentration of 1 mL L\textsuperscript{–1}. The cultures were further inoculated into bubble columns (5 L) for generation of the required biomass, which was dried (80 °C) and stored for later use.

\textbf{Anaerobic microbes (mixed consortia)}. The anaerobic consortia inoculated into the various units studied in this work originated from a 1 L stock digester maintained on \textit{Arthrospira maxima} as a substrate. The stock was initially inoculated with 20% (v/v) activated sewerage sludge (obtained from Cranfield University Water Treatment Laboratory) and 80% of 2 g L\textsuperscript{–1} \textit{A. maxima} dried biomass diluted in a basal medium (2 g L\textsuperscript{–1} NaHCO\textsubscript{3}, 3.2 g L\textsuperscript{–1} K\textsubscript{2}HPO\textsubscript{4}, 2.4 g L\textsuperscript{–1} KH\textsubscript{2}PO\textsubscript{4}, 0.04 g L\textsuperscript{–1} CaCl\textsubscript{2}, 2H\textsubscript{2}O, 1 g L\textsuperscript{–1} NaCl). The stock digester was operated in fed batch mode with intermittent loading of media. Gas production and composition were monitored to ensure that the digester was operating efficiently. The method of inoculation utilised in this study ensured a relatively consistent initial population of microorganisms in each digester.

\textbf{Semi-continuous operation of continuous stirred tank reactor (CSTR) anaerobic digester}

\textbf{Fabrication}. The CSTR digester utilised this study was specifically designed and constructed in-house. The reactor consisted of a 6 mm thick Perspex tube (220 × 78 (ID) mm) fitted with an overflow port 200 mm from the base of the tube. The positioning of the overflow port allowed for a 1 L liquid volume...
and 100 mL headspace volume. The Perspex tube was machined and glued into a solid Perspex base (100 × 100 × 10 mm). The top of the tube was flanged such that a lid could be screwed on with 4 butterfly nuts and bolts. The lid was sealed with a 90 mm outer diameter rubber gasket, which was placed in a special groove that had been machined into the base of the lid. The lid was fitted with four ports. The first port had a nylon tube (200 × 8 mm) running through to a ball valve (10 bar specified) for substrate feeding. The second port was fitted with a 3.18 mm ball valve (3 bar cracking pressure) to serve as the safety relief valve. The third port was fitted with a 3.18 mm pressure gauge (4 bar rating) for headspace pressure readings. The fourth port was fitted with a 3.18 mm ball valve (10 bar specified) and a 4 mm non-return valve for gas collection and sampling. The digester was operated under a 100 mL feed/withdraw process, which resulted in a 10-day HRT. The reactor was fitted with a heat jacket and maintained at 35 °C via hot water circulation. Continuous mixing was also supplied using a magnetic stirrer. The digesters were run in duplicate to obtain an accurate dataset.

Start up and operation. The CSTRs (duplicate) were initially inoculated with 200 mL of stock culture and loaded with 0.5 grams of fresh Arthropora maxima biomass in 800 mL of basal media. The microbial consortia were subsequently given time to acclimatise to the substrate. Since the initial reactor seed had already been acclimatised to A. maxima, the acclimatisation period only continued for 5 days (significant biogas production observed) at which point daily feeding of 0.5 g DW L⁻¹ d⁻¹ of pre-dried Arthropora maxima biomass commenced. The retention time was kept constant at 10 days by feeding and removing 100 mL daily. The operating conditions of the CSTR digester were selected based on conclusions and recommendations made by Samson and LeDuy (1986) and Inglesby (2011). Since the SRT was equal to the HRT in the CSTR reactors, this could not be as quick as the more advanced designs (AAR and ADMFC) where the HRT and SRT were uncoupled due to improved solids retention. After 15 days of operation the digesters (duplicate) were deemed to be in steady state (based on constant CH₄ production within 10% for 5 days) and all data are reported as averages from this point on (50 days of steady state data), equivalent to five HRT periods.

Fabrication of the advanced flow-through anaerobic reactor (AAR)

The AAR utilised in this study was specifically designed and constructed in-house. The reactor consisted of two segments. Firstly a 6 mm thick Perspex (180 × 78 (ID) mm) chamber operated as the major liquid volume. The Perspex tube was machined and glued into a solid Perspex base (100 × 100 × 10 mm). A feed port was tapped into the base of the tube. The second segment of the reactor operated as a three-phase-separator. It was constructed from a Perspex tube with an ID of 60 mm and a height of 60 mm. A gas cap was positioned inside the tube. Below the opening of the gas cap, a circular baffle was used to deflect gas to the gas-cap opening. A temperature probe was fitted into the reactor lid for temperature monitoring. The sides of the tube were tapped such that it could be fitted with two effluent ports. A ball valve (10 bar specified) was fitted into the each port, one for recirculation and one for the final effluent. The top of the tube was capped with a Perspex lid and fitted with a 3.18 mm ball valve (10 bar specified) and a 4 mm non-return valve for gas collection and sampling. The feed media (A. maxima-rich stream), together with the recirculation stream was pumped into the reactor via the feed port. The re-circulation stream helped to mix the contents of the reactor. The total liquid volume (including recirculation piping) of the AAR was 1 L. Nitrogen gas was used to continuously sparge the feed medium in order to create an anaerobic environment. The operation temperature was kept constant at 35 °C via a heating jacket positioned around the reactor tube. For the AAR and ADMFC system, the total liquid flow rate (Q) was controlled at 100 mL min⁻¹ using peristaltic pumps (Watson Marlow 101U peristaltic pump).

Fabrication of the recirculation loop flat plate microbial fuel cell (RMFC)

The RMFC used in this study was constructed using 10 × 120 × 160 mm Perspex frames. Individual frames with a specific design were cut using an HPC 3060 S 40 W laser cutter and engraver. Rubber gaskets matching the design of the frame were inserted between frames. The entire unit comprised of six Perspex frames, six rubber gaskets and an ion exchange membrane all bolted together using 5 mm stainless steel threaded rod. The anode and cathode chambers comprised of three frames each. One frame was a closing support frame whilst the other two frames were designed to allow for a serpentine flow pattern across the chamber. A proton exchange membrane (120 × 160 mm, Nafion® 117, DuPont) separated the anode and cathode chamber. The 5 mm diameter feed and effluent ports were positioned on one of the frames to allow for counter current flow of the anolyte relative to the catholyte. Both anode and cathode chambers were loaded with 100 g of graphite granules (0.2–0.7 diameter, DHart graphite). This allowed for a total working volume of 120 mL in each chamber. Inserting an 180 × 10 mm stainless steel strip into each segment of the serpentine designed frames allowed for an electrical connection. The five strips of each chamber were further connected using a stainless steel mesh to provide the electrical point of connection for the anode/cathode. The operation temperature was kept constant at 35 °C via a heating jacket positioned around the MFC.

Start-up and operation of the AAR and ADMFC system

The two systems were operated in continuous mode. The anaerobic digestion only system (AAR) was operated as an individual digester with a recirculation loop to induce a well-mixed system. In the hybrid ADMFC system the RMFC was positioned in the recirculation loop of the AAR unit (Fig. 1). The AAR and ADMFC units were run simultaneously and start-up was as follows. Both AARs were initially inoculated with 100 mL of inoculum in 900 mL basalm medium (2 g L⁻¹ glucose) and operated in batch mode for 6 days to acclimatise the consortia to the carbon substrate. After this, they were switched to a continuous mode, and fed with a basal medium (2 g L⁻¹ glucose), with a designated hydraulic retention time (HRT of 4 days). The reactors operated in this mode for 12 days until a steady state was
the basal media diluent. anode and cathode chamber. The pH and conductivity were constantly refreshed with basal media containing 50 mM ferri cyanide. The basal media was used as the diluent instead of it was integrated into the recirculation line of the AD to create the ADMFC unit. The cathode chamber of the RMFC was connected to the external resistors, across the external resistors, 1. The pressure in the column did not exceed 2000 psi. The methane, carbon dioxide, oxygen, hydrogen sulphide and hydrogen contents of the biogas were determined by analysis using a Gas Data Ltd GFM 416 biogas analyser. The analyser was calibrated with a standard gas calibration. The feed concentrations were manipulated such that HRTs of 4 and 2 days were evaluated with feed concentrations \((C_0)\) of 2 and 6 g \(A.\ maxima\) per L yielding OLRs of 0.5, 1, 1.5 and 3 g DW L\(^{-1}\) d\(^{-1}\). The flow rate and feed concentration for the ADMFC system were manipulated accordingly to take into account the extra volume of the RMFC unit. All flow was controlled using peristaltic pumps (LKB Microperpex® peristaltic) (Table 1).

The RMFC was initially loaded with 20 mL of effluent from a polishing MFC (PMFC) downstream of the semi-continuous CSTR digesters and 100 mL of the basal medium. The PMFC had been polishing digester effluent for over half a year and had a well acclimatised consortia. The RMFC was operated in batch mode until a stable voltage output was recorded. Following this it was integrated into the recirculation line of the AD to create the ADMFC unit. All flow was controlled using peristaltic pumps (LKB Microperpex® peristaltic) (Table 1).

The RMFC was subsequently operated at this optimum external load resistance within a range from the maximum of 1 k\(\Omega\) to a minimum of 4 \(\Omega\). The voltage output was recorded using a Picolog AD24 (16 channel) data logger. A stable output voltage was acquired prior to an external load step change. Polarisation curves enabled the optimum external resistance needed to acquire the maximum power output to be determined. The RMFC was subsequently operated at this optimum external load (260 \(\Omega\)).

**Analytical techniques**

Ammonium ion (\(\text{NH}_2\)) concentration was analysed using test kit LCK 303 (Dr Lange, HACH, Loveland, Colorado, USA) in a spectrophotometer HACH XION 500 (HACH, Loveland, Colorado, USA). All COD measurements were carried out using the AQUANAL reagent test protocol and a Thermo Scientific 8000 spectrophotometer (Thermo Scientific, Colorado, USA). A full volatile fatty acids (VFA) analysis was conducted to quantify the concentration of acetic, propionic, iso-butyric, butyric, iso-valeric and valeric acids present in all digesters over the duration of digestion. The concentration of each VFA was determined using HPLC on a Hewlett Packard 1045 system equipped with a Hamilton PRP-X300 Organic Acid and Alcohol column (7 μm 4.1 × 250 mm) and a UV (210 nm wavelength) detector. The system was run isocratically using a mobile phase of 20 : 80 tert-butanol : 10 mM potassium monobasic at a flow rate of 1 mL min\(^{-1}\). The pressure in the column did not exceed 2000 psi. Sample injection volumes of 100 μL were used. Total solids (TS), volatile solids, conductivity and pH were measured using the standard methods (APHA). The methane, carbon dioxide, nitrogen, hydrogen sulphide and hydrogen contents of the biogas were determined by analysis using a Gas Data Ltd GFM 416 biogas analyser. The analyser was calibrated with a standard gas containing 1% \(\text{CH}_4\), \(\text{H}_2\), \(\text{CO}_2\), \(\text{O}_2\) v/v in nitrogen.

**Electrochemical methods**

Polarisation curves were produced by variation of the external resistance within a range from the maximum of 1 k\(\Omega\) to a minimum of 4 \(\Omega\). The voltage output was recorded using a Picolog AD24 (16 channel) data logger. A stable output voltage was acquired prior to an external load step change. Polarisation curves enabled the optimum external resistance needed to acquire the maximum power output to be determined. The RMFC was subsequently operated at this optimum external load (260 \(\Omega\)).

**Performance calculations**

**RMFC power and current.** The volumetric powers \((P)\) and currents \((I)\) of the RMFC were calculated using the equations \(P = V^2(k \times R)\) and \(I = V(k \times R)\) where \(V\) (volts) is the voltage across the external resistors, \(R\) (\(\Omega\)) is resistance of each external resistor, and \(k\) (m\(^3\)) is the operating volume of the RMFC anode chamber.
**Energy efficiency.** The energy from methane production relative to the energy in the amount of substrate fed was used to calculate the energy efficiency of the digestion (eqn (1)).

\[
EE_{AD} = \frac{P_{\text{methane}} \cdot E_{\text{methane}}}{\Delta H_{A \text{maxima}} m_{\text{added}}}
\]

(1)

For the ADMFC system, the energy recovered from the RMEFC unit was added to the energy from methane and normalised to the energy stored within the *A. maxima* based feed as follows:

\[
EE_{\text{ADMFC}} = \frac{P_{\text{methane}} \cdot E_{\text{methane}} + E_{\text{MFC}} \cdot I_a}{\Delta H_{A \text{maxima}} m_{\text{added}}}
\]

(2)

where: \( P_{\text{methane}} \) = productivity of methane (L CH₄ per L d); \( E_{\text{methane}} \) = 35.8 kJ L⁻¹ CH₄; \( E_{\text{MFC}} \) = steady state cell voltage (V); \( I_a \) = steady state current (A per L day); \( \Delta H_{A \text{maxima}} \) = heat of combustion of *A. maxima* (22 kJ g⁻¹ VS) and \( m_{\text{added}} \) = volatile solids in *A. maxima* added to system (g VS L⁻¹ d⁻¹).

**Statistical analysis**

A one-way analysis of variance (ANOVA) test was conducted to determine the significance of differences between two or more groups of experimental results. Significant differences were reported at a *p* value of 0.05.

**Results and discussion**

**Substrate properties owing to digestibility**

The most important characteristics of the *A. maxima* substrate are summarised in Table 2. The composition of the substrate is important in understanding its digestibility.²⁸ Quantifying the carbohydrate, lipid and protein content facilitates an improved understanding of the system’s progress and explanation of the formation of certain products that may impact digester efficiency.²⁶ The C/N ratios for *A. maxima* (lit.⁷,⁲⁷,⁴⁴; 4.5–5.3) may be used to predict the ammonia release into the system.²⁹ The ratio is low for *A. maxima* due to the high protein content (>60%) and low lipid content (<10%), suggesting that ammonia accumulation may be an issue. Optimum ratios for AD are between 20 and 30.²⁹ *A. maxima* has a VS content in excess of 80%, which makes it an attractive substrate for AD. The alkalinity for the *A. maxima* slurries would be high due to the release of intracellular components and from residual media on the unwashed biomass.²⁷ The increased alkalinity can act as a buffer to stabilise the pH and reduce the possible inhibition by VFAs and NH₃.⁷

The filaments of *A. maxima* tend to break open, releasing the intracellular contents more easily than for small, rigid cells.²⁷ It has been reported recently that the species of microalgae/cyanobacteria is critical for digestibility; moreover that *Arthrospira* spp. is a good substrate.¹³,²⁹ *A. maxima* have been used a primary carbon substrate for axenic culture *R. palustris*.³⁰ In their study, Inglesby and colleagues showed how the bacteria (present in many waste waters)³¹ could consume the solid substrate and maintain healthy growth rates.³⁰

Since *A. maxima* cells were removed from a high saline environment (Zarrrouk’s media) to a non-saline environment (AD content) the cells faced osmotic shock and so rapid lysis of the cells did occur. This was confirmed in previous studies on the mechanical disruption of *Arthrospira* spp.²⁷ The lysis of cells speeds up the hydrolysis stage of digestion, which has shown to be limiting in the AD of algal based feeds.²⁵ Zamalloa et al. (2011) reported a similar finding when removing *Phaeodactylum tricornutum* from its growth media (high salinity) and placing it into a digester. This property of the biomass was reported as significant in contributing towards higher methane yields compared to digestion of rigid *Scenedesmus* spp. biomass.

**Biodegradation performance of the AD systems**

The progress of organic degradation across the system was monitored through measuring solid and soluble COD concentrations and then adding them to determine total COD changes. The COD of the solid biomass represented oxygen demand by complex organics comprising the cell wall and intracellular content of the cyanobacteria. The soluble COD represented the demand for oxygen from organic compounds in the aqueous phase of the particular stream. For all digestion systems (CSTR, AAR and ADMFC) a decrease in solid COD was accompanied by an increase in soluble COD, indicating that hydrolytic enzymes liberated insoluble biomass components. *Arthrospira maxima* has a soft cell wall made of complex sugars and proteins, unlike the cellulosic walls of most microalgae, making it more easily disrupted and digested.³⁰ In fact, the cell walls of most algae are composed of highly resistant, non-hydrolysable aliphatic biopolymers.³² The polyether nature of these algenans makes them highly resistant to degradation.³² The cell walls also contain large amounts of hemicellulose, which is characterised by slow hydrolysis.³² The lack of these degradation resistant compounds was confirmed by the significant decrease in solid COD in the effluent of the digestion units (Table 3). Biomass production, due to microbial growth (acidogens and methanogens), contributed to an increase in solid COD.

COD removal efficiency increased with increasing HRT for all AD systems. The ADMFC orientation resulted in the greatest COD removal of 69 ± 6% at an HRT of 4 days and OLR of 0.5 g DW L⁻¹ d⁻¹. With a longer HRT the bacteria were afforded more time to consume the solid substrate. Moreover, there was a longer period for hydrolysis of the solid to soluble organics, which were easier consumed by the microflora. The removal
efficiency observed in the ADMFC unit was significant compared to previous AD studies with *A. maxima* or microalgae based feeds. Samson and LeDuy (1986) had a maximum removal efficiency of 50% when they operated their semi-continuously fed CSTR under a HRT of 20 days and with an OLR of 1.2 g VS L\(^{-1}\) d\(^{-1}\). More recently Zamalloa et al. (2012) had COD removal efficiencies of 50% when they digested a *Phaeodactylum tricornutum* (marine microalgae) in a unique flow-through hybrid digester. The relative error in effluent TCOD concentrations was high (up to 27%) in some cases. This was a result of large fluctuations in the amounts of solids in the effluent. Zamalloa et al. (2011b) reported as similar result in their hybrid digesters when fed microalgae. The fluctuations originate from the inconsistency in solid substrate hydrolysis and the three-phase separation units.

With high OLRS and longer HRTs the increased degradation results in digesters being more susceptible to possible inhibition from free ammonia (due to increased degradation of nitrogenous matter). In the AAR reactor system with increased OLRS an increase in ammonium ion concentration was observed (300–650 mg L\(^{-1}\)). The pH of all the digestion systems was maintained at 7–7.5, mainly by the buffer in the basal medium. This was low relative to the pK\(_a\) of ammonia (9.23). This minimised the non-ionised ammonia (NH\(_3\)) concentration in the aqueous phase of the digester and maximised the ionised ammonia (NH\(_2^+\)) concentration. Even with the average higher COD destruction observed in the ADMFC system, the ammonium ion concentration was maintained equal to or less than the AAR unit, for the equivalent operating conditions. This resulted in the ratio of ammonium ion concentration to total removed COD being lower for the ADMFC effluent compared to that of the AAR system. An ANOVA analysis done on the difference in value for the ratios of the AAR and ADMFC systems proved that this difference was statistically significant (\(p < 0.05\)) for all parameters tested (OLRs 0.5 to 3 g DW L\(^{-1}\) d\(^{-1}\)). This indicated that the RMFC successfully removed a significant amount of ammonium ions and so provided a more stable environment for the methanogenic culture of the anaerobic digester segment to the ADMFC unit. Improved microbe and solids retention in the ADMFC system, due to attachment to the carbon electrode and solid–liquid separation contributed to the increased total COD removal efficiency. It also contributed to improved methane yields in the digester (discussed in detail below). Increased SRT has previously been shown to favour the growth of slow-growing microorganisms such as methanogens and also increase the fraction of fermented organic matter.

Volatile fatty acid degradation across MFCs is improved over anaerobic digestion due to the more stable microflora. Pind et al. (2003) indicate that iso-valeric acid is a metabolite from amino acid (leucine) digestion and degrades into acetate and hydrogen. With *A. maxima*’s high protein content, it can be expected that, when digested, there will be high concentrations of valerate present. An accumulation of this acid is consistent with methanogenic inhibition, as reduced consumption of acetate allows for accumulation of iso-valerate. The maximum amount of iso-valerate present during the digestion of *A. maxima* was 1020 ± 60 mg L\(^{-1}\) in the CSTR system and was only present in trace amounts during AAR and ADMFC operation (shorter HRTs and so less chance of VFA accumulation). This suggested that there was an accumulation in the CSTR reactors and so decreased activity of the methanogens hence low methane yields (discussed below). Jeong et al. (2008) used a recirculation loop MFC for VFA removal from their digestion unit. The RMFC used in the current study contributed to VFA removal and the effluent of the ADMFC contained on average contained less VFAs as well as soluble COD (Table 3). Low VFA and soluble COD concentrations in the AD effluent streams were a sign of an efficient microbial consortia. The aqueous phase metabolites were dominated by acetic acid in both the AAR and ADMFC units with the concentration of acetic acid contributing to in some cases more than 50% of the soluble COD available. Samson and LeDuy (1986) commented on the possible use of anaerobic digestion of *A. maxima* biomass for organic acid production due to high residual contents observed in their digester effluent.

**Methane production from the AD reactors**

The feed concentration (g DW L\(^{-1}\)) and HRT (d) across the AAR and ADMFC were varied such that the units operated under four different organic loading rates (0.5, 1, 1.5 and 3 g DW L\(^{-1}\) d\(^{-1}\)).

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**Table 3** Biodegradation of the *A. maxima* based feed across the three reactor systems

<table>
<thead>
<tr>
<th>Operational condition</th>
<th>Effluent HAc (mg L(^{-1}))</th>
<th>Effluent ACOD (mg L(^{-1}))</th>
<th>Effluent SCOD (mg L(^{-1}))</th>
<th>Effluent TCOD (mg L(^{-1}))</th>
<th>TCOD removal efficiency (%)</th>
<th>Ratio [NH(_2^+)] : [TCOD]removed</th>
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<td><strong>CSTR</strong></td>
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<td>294 ± 13</td>
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<td>394 ± 60</td>
<td>509 ± 119</td>
<td>859 ± 217</td>
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<td>169 ± 30</td>
<td>376 ± 40</td>
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<td>3255 ± 491</td>
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<td>6</td>
<td>2</td>
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<td>341 ± 29</td>
<td>482 ± 40</td>
<td>567 ± 102</td>
<td>4832 ± 308</td>
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<td><strong>AAR</strong></td>
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<td></td>
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<tr>
<td>2</td>
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<td>2</td>
<td>3</td>
<td>396 ± 19</td>
<td>433 ± 15</td>
<td>481 ± 89</td>
<td>2546 ± 260</td>
</tr>
</tbody>
</table>

* ACOD aqueous phase/soluble COD; SCOD solid phase COD; TCOD total COD (sum of soluble and solid COD); HAc acetic acid concentration in effluent.
The organic loading rate (OLR, g DW L\(^{-1}\) d\(^{-1}\)) is an important factor in optimising methane production due to its effect on activity of both the acid forming and methane-producing microorganisms (Fig. 2).\(^7\)

The specific methane yields (SMY, mL CH\(_4\) per g VS) of A. maxima showed a reverse correlation with OLR for the AAR and ADMFC systems. The AAR-SMY decreased from 136 ± 16 to 45 ± 8 mL CH\(_4\) g\(^{-1}\) VS as the OLR increased from 0.5 to 3 g DW L\(^{-1}\) d\(^{-1}\) according to the equation:

\[
\text{AAR-SMY} = 89.30 \times \text{OLR}^{-0.629}
\]

Whilst the ADMFC-SMY decreased from 173 ± 38 to 65 ± 10 mL CH\(_4\) g\(^{-1}\) VS as the OLR increased from 0.5 to 3 g DW L\(^{-1}\) d\(^{-1}\) according to the equation:

\[
\text{ADMFC-SMY} = 119.3 \times \text{OLR}^{-0.541}
\]

This trend was effectively demonstrated using an inverse transformation of OLR (\(R_{\text{AAR}} = 0.979, R_{\text{ADMFC}} = 0.981\)) (Fig. 3). Samson and LeDuy (1986) reported a similar trend with decreasing yields at higher feed VS concentrations. Samson and LeDuy, investigated very high feed concentrations (up to 10 g VS L\(^{-1}\)) but over much longer retention times (up to 40 days) and so similar range of OLR (up to 6 g VS L\(^{-1}\) d\(^{-1}\)). The aim of this study was to use the advanced flow-through systems to allow for shorter HRT and higher feed concentrations, whilst maintaining high SRTs.

By reducing OLR, the SMY of the ADMFC could reach the maximum value of 173 mL CH\(_4\) per g VS (Fig. 3). For all OLRs the SMY from the ADMFC system was higher than that of the AAR (maximum 136 mL CH\(_4\) per g VS) and CSTR systems. An ANOVA analysis indicated that the difference in datasets for methane yields of the AAR, ADMFC and CSTR systems were statistically significant (\(p < 0.05\)) for OLRs of 1–3 g DW L\(^{-1}\) d\(^{-1}\).

This indicates more optimum performance from the improved ammonium ion mitigation, VFA removal (and so pH buffering) and increased SRT due to the integration of the RMFC unit. Moreover, the semi-continuous fed CSTRs showed the lowest performance, indicating the importance of advanced reactor designs in maximising efficiency of digestion. The absolute values of the yields obtained in this study were lower than previously recorded using and A. maxima based feedstock. (lit.,\(^2\)) maximum yield 350 mL CH\(_4\) per g VS. The pre-drying of substrate has shown to decrease the yield of methane by up to 20%\(^2\). This results from the loss of volatile organics during the drying process.

The methane content of the biogas was on average 5% higher (\(p < 0.05\) for difference in % for OLRs of 1–3 g DW L\(^{-1}\) d\(^{-1}\)) in the ADMFC system than the AAR system (Table 4) and increased with increasing OLRs. This suggested improved methanogenic activity, and is postulated to be a result mainly of the ammonium ion and VFA removal by the RMFC. With the inclusion of the RMFC, higher OLR can be used as the impact of nitrogenous matter digestion and so release of ammonium ions will be diminished by the exchange across the MFC membrane. At OLRs much greater than 3 g DW L\(^{-1}\) d\(^{-1}\) the methane content of the biogas will start to decrease without efficient ammonium ion and VFA removal.\(^7\) It should be noted that the two-chamber MFC system was a sealed vessel, however the fresh ferricyanide stream passing through the cathode chamber was not oxygenated and so small (micro-concentrations) of oxygen (O\(_2\)) may have diffused through the membrane into anode chamber and so the digester content. Micro O\(_2\) concentrations have recently been shown to improve digestor performance mainly due to the improved hydrolysis, sulfide removal and increased acetoclastic methanogen activity.\(^6\) Further studies on oxygen content in the digester should be done to confirm whether or not the RMFC does in fact result in micro aerobic conditions.

**Current and power generation from the recirculation loop MFC**

The recirculation loop MFC (RMFC) produced a steady state current under the varying operating conditions of the AD unit. Polarisation curves (data not shown) revealed Ohmic loses...
dominated the flat plate designed MFC under all the conditions tested. The consortia inoculated into the RMFC originated from a downstream polishing MFC (PMFC) of similar design. The PMFC operated for over half a year on A. maxima AD effluent as the primary substrate and so the consortia were very stable under the conditions of this study, consequently steady state current outputs were achieved quickly and corresponded to changes in AD operating conditions. The constant replenishing of fresh ferricyanide into the cathode chamber resulted in a stable cathode reduction reaction meant that the anodic oxidation was the controlling reaction in power production. Since the RMFC was a sealed unit gas production was not analysed, but was assumed to escape in the biogas.

The conductivity of the ADMFC contents increased slightly from 7.2 ± 0.1 mS cm⁻¹ (OLR 0.5 g DW L⁻¹ d⁻¹) to 8.7 ± 0.2 mS cm⁻¹ (OLR 3 g DW L⁻¹ d⁻¹). The relative stability in the conductivity derives from the constant basal media used as well as the proportional flux of ions across the membrane. The conductivity of the ADMFC contents was not significantly different than that of the AAR unit contents (p > 0.05).

Increased loading rates to the digester resulted in higher amount of available organics passing through the RMFC unit. Since the HRT across the RMFC was constant one would expect the increased OLR to result in higher volumetric current and powers from the RMFC. This was the case with a slight increase in power (W m⁻³) and current (A m⁻³) from 7.8 ± 0.9 to 10.2 ± 1.3 W m⁻³ and 16.8 ± 0.9 A m⁻³ to 18 ± 0.9 A m⁻³ with an increase in OLR to the AD unit of 0.5 to 3 g DW L⁻¹ d⁻¹ (Fig. 4). Previous studies on complex organics fed to MFCs reported that increased OLRs resulted in lower Coulombic efficiencies (the ratio of total charge actually transferred to the anode from the substrate relative to the maximum possible charge if all the substrate removed produced current). With further increase in OLR to the digester and so RMFC, the power and current reach a maximum and plateaued out.

In comparison to the results reported by De Schampheelaere and Verstraete (2009), the RMFC produced a higher volumetric power and current (10 W m⁻³ and 18 A m⁻³ vs. 0.167 W m⁻³ and 0.045 A m⁻³). The improved results can be attributed to the characteristics of the available organic compounds. A. maxima, an easily degradable substrate not characterised by a cellulosic cell wall, was converted into available intermediates across the digestion phase of the integrated process. Due to the short HRT of the digester there was a high residual organic content available for further conversion in the MFC. Velasquez-Orta et al. (2009) indicated that when feeding C. vulgaris directly into a MFC, the complexity of the substrate (particulate containing macromolecules) required more energy for the degradation processes (hydrolysis and fermentation), which limited the power output of the fuel cell. The degradation resistant nature of C. vulgaris due to complex biopolymers was highlighted in this study by the low conversion of substrate over the operating period (<50% COD).

Mussgnug et al. (2011) demonstrated via light microscopy that after 30 days of digestion, whole cells were still present in digester contents for the degradation resistant species, whereas for Arthrospira platentis there was no resemblance of whole cells (complete disintegration). Further confirmation of the improved RMFC operation was achieved when comparing the volumetric

![Fig. 4 Volumetric current (A m⁻³) and power (W m⁻³) of the RMFC unit with varying OLR in the AD unit of the ADMFC integrated system.](image-url)

Table 4 Energy capture efficiency of the three reactor systems

<table>
<thead>
<tr>
<th>Operational condition</th>
<th>C₀ (g DW L⁻¹)</th>
<th>HRT (d)</th>
<th>OLR (g DW L⁻¹ d⁻¹)</th>
<th>Methane (%)</th>
<th>MPR (mL CH₄ L⁻¹ d⁻¹)</th>
<th>SMY (mL CH₄ g⁻¹ VS)</th>
<th>Methane efficiency (%)</th>
<th>Energy efficiency (%)</th>
</tr>
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<tbody>
<tr>
<td>CSTR</td>
<td></td>
<td></td>
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<td>5</td>
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<td>70 ± 2</td>
<td>41 ± 7</td>
<td>90 ± 19</td>
<td>26 ± 5</td>
<td>17.1 ± 0.8*</td>
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<tr>
<td>AAR</td>
<td></td>
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<td></td>
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<tr>
<td>2</td>
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<td>71.1 ± 1.5</td>
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<tr>
<td>2</td>
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<td>71.5 ± 2.2</td>
<td>71 ± 16</td>
<td>173 ± 38</td>
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<td>18.7 ± 2.8</td>
<td>9.2 ± 2.0*</td>
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</table>

* Energy efficiency calculated using eqn (1). b Methane efficiency calculated using ultimate methane yield (Rₜ) of 350 mL CH₄/g VS A. maxima. c Energy efficiency calculated using eqn (2). d Note all errors represent standard deviation across all measured or derived dataset.
power to that reported by Jeong et al. (2008). Jeong and colleagues reported that their RMFC produced 0.09 W m\(^{-2}\) when fermenting complex carbohydrates, which was significantly lower than the current study (even though digestion of a more complex solid substrate was investigated).

The optimum conditions for the current study were to operate the digester at an OLR of 1.5 g DW L\(^{-1}\) d\(^{-1}\) as this was the point at which the power and current values began to plateau. With further increased OLR to the AD unit, higher amounts of ammonium ions would be present. Since the RMFC current would not increase substantially, the ammonium ion mitigation (which has been shown to be proportional to the charge transfer) will not increase and so one of the important functionalities of the RMFC will be removed. At that stage, increasing the CE of the RMFC will be the primary objective to improve the charge transfer and so ionic flux through the membrane. Furthermore, the AD : RMFC volume : volume ratio will affect performance.\(^{22}\)

### Methane and energy efficiency of the AD systems

Methane efficiency calculated from the ultimate yield of 350 mL CH\(_4\) per g VS were as high as 50% for the ADMFC system when operated at a low OLR of 0.5 g DW L\(^{-1}\) d\(^{-1}\). With increasing OLR the efficiency decreased for the same reasons as the yields (discussed above in methane production). A major objective of AD systems is to operate at higher loading rates to decrease volumes required. Further improvements need to be made in the system performance to reach OLRs of up to 12 g VS L\(^{-1}\) d\(^{-1}\). The energy recovered from the CSTR, AAR and ADMFC units was normalised with the total energy contained in the influent (\(A.\ maxima\) feed) in order to obtain the total EE (%). At an OLR of 0.5 g DW L\(^{-1}\), the energy efficiency of the CSTR was 17.1 ± 0.8%, whilst the more advanced flow-through AAR was 22.1 ± 2.6% and an even higher efficiency of 29.7 ± 6.8% was obtained in the ADMFC unit. This was expected as both the methane yields and productivity values were higher for the ADMFC system than the other digester orientations. The fuel cell contribution towards energy efficiency was low (average <1%) as the currents obtained were significantly smaller than the energy recovered in the form of methane. Weld and Singh (2010) reported an average contribution of 3% by their microbial fuel cell when the system was operated in hybrid mode. Again a small value compared to the energy recovered in the form of methane. The purpose of the RMFC was for ion mitigation, culture diversity and stability, increased SRT as well as pH buffering via fatty acid removal. It was not expected to be a high-energy contributing unit but rather to improve the methane yields of the digester, which it accomplished successfully. The average energy efficiency (%) of the ADMFC unit was 50% higher than that of the CSTR or AAR systems.

The energy efficiency of the systems analysed in this study were comparable to previous straight AD of \(A.\ maxima\) studies (lit.,\(^{7}\) EE of 6–58%) as well as those obtained from hydrogen fermentation followed by AD of \(A.\ maxima\), which yielded a maximum efficiency of 27.5%.\(^{1}\) This study is significant in testifying the incorporation of microbial fuel cell systems in high nitrogen containing feeds, lowering the energy requirement for ammonium mitigation and achieving high COD removal, thereby creating an energy efficient system.

### Perspectives and opportunities for the integrated ADMFC system

It has been widely reported that one of the major benefits of using algae as a substrate for biofuel production is that it can be cultivated using non-arable land and non-potable water.\(^{9,10,40}\) In addition, productivity and annual biomass yield are suggested to far exceed those obtained for traditional energy crops. If an efficient energy production process can be developed with the biomass as the sole feedstock it bodes well for a successful venture. Microbial fuel cells (MFCs) have become an attractive green energy source to produce electricity from organic fuels by microorganisms while recycling waste to protect the environment.\(^{38}\) The integration of the bioelectrochemical system (RMFC) into the biochemical process (AD) proposed in this study provides a number of new potential opportunities, which are discussed below.

### Digestion of high nitrogen containing wastes

The integration of anaerobic digestion and the two-chamber MFC not only shows great potential for application with \(A.\ maxima\) as a substrate, but for any high nitrogen containing feeds. For instance, poultry waste is known to have very high nitrogen content and could be applied to the current design. Unlike traditional energy intensive techniques such as precipitation/ion exchange columns the microbial fuel cell generates electricity (more so energy saving than production). The modular design will allow implementation into any already operating anaerobic digestion units.

### Ammonia production

Rabaey et al. (2010) emphasise that in order for MFC technology to be economically viable the units must: (1) reach sufficient turnover rates at scale; and (2) generate a product that offsets the investment costs within a reasonable time frame.\(^{42}\) In their work sodium hydroxide (NaOH) production on a litre scale was demonstrated. Sodium ions, originating from a high Na containing wastewater oxidising in the anode chamber, diffused across a MFC membrane and combined with hydroxide in the cathode chamber to from the desired product. It has been shown previously that ammonium ("ammonium" refers to both NH\(_3\) and NH\(_4^+\) whereas the chemical formulae are used to refer to its specific forms) can be removed from the cathode chamber by volatilization of NH\(_3\).\(^{30}\) Using these findings, and following the philosophy of Rabaey and colleagues, concentration and recovery of NH\(_3\) from an ammonium rich cathode effluent becomes a possibility. Ammonia is valuable chemical and can be used for a number of different applications. A sustainable production, even in small amounts, will be beneficial to the current process.

### Polished effluent for liquid fertiliser

Nitrogen, phosphorous and sulphur are key nutrients required for algal growth. The effluent from the ADMFC unit will contain significant concentrations of these elements, therefore could be recycled directly into the growth unit to supply the necessary make-up of nutrients consumed during cell growth.\(^{39}\) Recycling of
the digester effluent has been conducted successfully in two previous studies that investigated closed-loop algal growth and digestion systems.\(^{23,44}\) The compounds in the effluent are also used in the production of industrial fertilisers. It has been widely reported that the effluent of anaerobic digestion can be used directly as a soil fertiliser or an additive compound in fertiliser production.

**Conclusions**

Anaerobic digestibility of *A. maxima* biomass in three different reactor configurations for production of bioenergy was investigated. The close monitoring of liquid phase compounds (COD, VFAs and ammonia) allowed for imbalances in the degradation reactions or inhibition from accumulation of toxic compounds to be monitored. Key parameters that impacted the efficiency of the digestion were also highlighted and varied to aid in optimisation of the reactor systems. The feed concentration and hydraulic retention time influenced the total amount of solid biomass degradation, which in turn controlled the amount of methane produced. Through optimisation of these operating conditions various trends were observed.

Reduced ammonium ion and so free ammonia concentrations in the digester contents of the ADMFC system improved stability of the methanogenic consortia and so higher methane content, productivity and yields were observed for the ADMFC system over the AAR unit for all operating conditions tested. Since the sensitive methanogen cultures do not dominate the MFC culture and the solids retention time (SRT) is improved, further and efficient reduction of COD was seen in the ADMFC.

It is clear that energy production and COD removal were improved by integrating AD and MFC technology. However, substantial improvements in AD and MFC systems should be conducted to achieve higher energy recovery. Optimising the relationship between the two units would help identify the significance of the RMFC unit. Increased OLR would require efficient ammonium ion mitigation and hence improved charge transfer. The ADMFC system presents opportunities for digestion of high nitrogen containing substrates, ammonia production and liquid fertiliser production.

To determine the overall feasibility of such an energy production process, a complete techno-economic analysis should be conducted on the current technology.

**Nomenclature**

<table>
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<tr>
<th>Acronym</th>
<th>Description</th>
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<td>CSTR</td>
<td>Continuously stirred tank reactor</td>
</tr>
<tr>
<td>EE</td>
<td>Energy efficiency</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic retention time</td>
</tr>
<tr>
<td>MFC</td>
<td>Microbial fuel cell</td>
</tr>
<tr>
<td>MPR</td>
<td>Methane productivity</td>
</tr>
<tr>
<td>OLR</td>
<td>Organic loading rate</td>
</tr>
<tr>
<td>PMFC</td>
<td>Polishing microbial fuel cell</td>
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<tr>
<td>RMFC</td>
<td>Recirculation-loop microbial fuel cell</td>
</tr>
<tr>
<td>SCOD</td>
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<tr>
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<td>VFA</td>
<td>Volatile fatty acids</td>
</tr>
<tr>
<td>VS</td>
<td>Volatile solids</td>
</tr>
</tbody>
</table>

**Notes and references**

27. A. Inglesby, MSc Thesis University of Cape Town, 2011.

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