Full Length Research Paper

The potential of whey in driving microbial fuel cells: A dual prospect of energy recovery and remediation

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Renewable and green energy resources are paramount to environmental sustainability. Microbial fuel cells (MFCs) are potential candidates for these alternatives but there is need to search for cheaper fuels to drive the MFCs for realistic large scale applications. A high strength effluent such as whey, which poses a serious environmental threat, is a good candidate for fueling the MFCs with an added advantage of bioremediation. Thus, cheese whey was evaluated for its ability to drive MFCs and the extent of whey remediation was also investigated during the operation of MFCs in this study. Three experimental anodic setups: Raw (unamended) whey alone, heat treated (sterile) whey inoculated with Enterobacter cloacae subspecies dissolvens, and raw whey inoculated with E. cloacae where employed. Native whey microbes achieved 44.7 ± 0.2% total chemical oxygen demand (tCOD) removal efficiency and 0.04% coulombic efficiency ($\varepsilon_{cb}$). The maximum power density generated was 0.4 W/m$^2$ (normalized to the anode surface area). Upon introduction of an exogenous electricigenic E. cloacae culture in the whey, the tCOD removal efficiency dropped to 5% while $\varepsilon_{cb}$ was the highest (3.7%) with maximum power density of 16.7 ± 1.8 W/m$^2$. However, a combination of E. cloacae and unsterilized/unamended whey gave 1.1 W/m$^2$, $\varepsilon_{cb}$ of 0.5% and 22.1% tCOD removal. The results confirmed the ability of whey to be used as a fuel in the anodic chamber to drive electricity generation in an MFC system with its partial remediation, but absence of synergism between E. cloacae and the electricigen is inherent to whey.

Key words: Microbial fuel cell, cheese whey, coulombic efficiency, bioremediation, sustainable energy, electricigen.

INTRODUCTION

The need for alternative energy sources to fulfil the environmental friendliness goals in energy production and substitute the depleting fossil fuel reserves has seen active research and a variety of potential alternatives. Microbial fuel cells (MFCs) are amongst the potential alternative solutions to the energy dilemma. MFCs take advantage of the oxidation of organic compounds to produce electricity. Their advantages include combining bioremediation and electricity production, their environmental friendliness and ability to be used in remote areas.

However, the wide scale application and realistic scaling up of MFC to significantly substitute current fossil fuel energy generation techniques remain elusive. This is because of the requirement of large area of operation for meaningful power production, low power output due to internal resistances, poor reproducibility of the setups and expensive artificial/defined media for the anode chamber (Logan and Regan, 2006 a, b; Liu and Li, 2007). Furthermore, the biology of microbial consortia involved is yet to be fully understood (Bullen et al., 2006).

Despite the highlighted shortcomings, considerable progress has been made in electrode modifications to increase power output. Such efforts entail incorporation of nanoparticles and catalysts on the electrodes (Scott et al., 2007; Sharmaa et al., 2008; Nambiar et al., 2009; Tsai et al., 2009). While these efforts have yielded notable increases in power, the power remains insufficient to fill in the required credibility gap (Ieropoulos et al.,...
2005; Pant et al., 2010). Hence, searching for cheaper substrates and high performing microorganisms can augment the current electrode modification effort. Research into cheaper substrates and alternative microbial sources is ongoing (Asad et al., 2007; Pant et al., 2010) but the searching is not exhaustive. The prospect of simultaneous bioremediation and electricity generation is attractive. This study was aimed at contributing towards current efforts of alternative cost effective substrates for driving microbial fuel cells. The work focused on investigating the performance of microbial fuel cells with cheese whey as a substrate in the anode chamber. In addition, presence of native electricigens as a substrate in the anode chamber. In addition, presence of native electricigens and high performing microorganisms in the effluent in question and their behaviour upon introduction of an exogenous electricigenic Enterobacter cloacae culture were investigated.

MATERIALS AND METHODS

MFC setup and analyses

An H-type MFC was setup and used as described by Nambiar et al. (2009) with a 470 - Ω resistor. Both anolyte and catholyte were of equal volumes (200 ml). Each MFC setup was run for 10 – 14 days. Voltage was measured using a TopTronic T830 digital multimeter (Apelectronics, South Africa) at two-hour intervals, while pollution indicators such as chemical oxygen demand (COD) and total solids where measured at the beginning and end of every MFC cycle. Ohm’s law (I = V/R) was applied to calculate the current and subsequent power density (P_d) calculated using

\[ P_d = \frac{VI}{A} \]

Where, V is voltage, I is current and A is surface area (Rabaey and Verstraete, 2005). The coulombic efficiency (ε_d) which is the percentage of total charge transferred to the anode surface over the maximum charge extractable upon complete oxidation of the substrate to electricity was calculated as described by Huang and Angelidakis (2008) and Antonopoulos et al. (2010).

Whey sampling

Cheese whey was obtained from Greenways Deli (Kyalami, South Africa). Following collection, samples were stored at 4°C to retard possible changes in biochemical composition and prevent further acidification (Najafpour et al., 2009). Parameters measured from the raw whey (effluent) included pH, total (t) COD and glucose concentration.

Experimental setups

Three sets of MFCs were used: (i) Raw (untreated) whey, (to investigate the presence of electricigens in the whey and remediation ability of inherent microorganisms), (ii) raw whey with E. cloacae to investigate synergism between the native microorganisms and the E. cloacae, and (iii) sterile effluent inoculated with E. cloacae only to determine the amount of power generated by the pure culture alone as well as the suitability of whey as a media for other microorganisms. E. cloacae has been shown to produce electricity in an MFC setup (Nambiar et al., 2009). Sterility of the medium was achieved by autoclaving whey at 121°C for 15 min.

The E. cloacae culture was obtained from Rhodes University, (South Africa). Identity of this culture was confirmed by 16S rRNA sequencing at Inqaba Biotechnology Laboratories (South Africa). Each setup consisted of 200 ml of effluent supplemented with 30 mg of cysteine in order to maintain anoxic conditions. When E. cloacae culture was inoculated into the anode chamber, a 10% (v/v) of 16 - 18 h old culture was used. The operation of the MFCs was performed at ambient temperature (22 ± 1°C). Three independent runs for each experimental set up were performed and data reported mean ± standard deviation.

Determination of COD

tCOD was determined by a colorimetric method as outlined in the Hanna instruments kits (South Africa). Briefly, two millilitres of each, mixed and appropriately diluted, effluent were mixed with 1.5 ml digestion and 3.5 ml catalyst solutions, incubated at 150°C for two hours and then cooled at room temperature; absorbance was measured at 600 nm. The COD concentration was calculated using a potassium hydrogen phthalate standard curve from solutions processed in the same way as the whey samples.

Total solids

Total solids were determined using the drying method (SFS, 1990) both before and after the experiment. Briefly, the effluent was mixed thoroughly and 50 ml of which was pipetted into a pre-weighed glass Petri dish, heated in an oven (100°C) with periodic cooling (in a dessicator) and weighing until a constant weight was recorded between the heating and cooling intervals.

Scanning electron microscopy (SEM) and species identification

Visualisation of the electrode surfaces under SEM was performed to determine the degree of microbial colonisation. Following each MFC run, anodes (from the reactors) were prepared for SEM. Microorganisms on the electrodes were fixed by immersing the electrodes in 2.5% (v/v) gluteraldehyde and dehydrated by successive immersion for 10 min in ethanol at the following concentrations: 30% (v/v), 50, 70, 80, 90 and 100%. This was then followed by critical point drying, gold palladium coating (Brunk et al., 1981) and visualisation using a JEOL 840 SEM.

RESULTS

Raw whey characteristics

The raw whey parameters are given in Table 1. It is important to note the high strength of the effluent as typified by the high tCOD (96.5 g/l; Table 1). There was a degree of conductivity (6.3 mS/cm) in the whey and high amount of glucose (59.5 g/l; Table 1).

Power generation

Generally, the maximum power was obtained between
Table 1. A profile of the whey investigated before treatment in MFCs.

| Parameter          | Value     |
|--------------------|-----------|
| pH                 | 7.85 ± 0.1|
| tCOD (g/l)         | 96.5 ± 4.2|
| Total solids (g/l) | 3.6 ± 0.3 |
| Glucose (g/l)      | 59.5 ± 1.4|
| Conductivity (mS/cm)| 6.3 ± 0.1|

Figure 1. A typical MFC performance when fed with heat-treated whey and using *E. cloacae* inoculum. Maximum power density: 16.7 ± 1.75 (W/m²); the positive control (RCM inoculated with *E. cloacae*) is also shown.

Remediation and coulombic efficiencies

There was a decrease in glucose content, tCOD and total solids in all three setups (Table 2). An inverse relationship between the maximum power density and the remediation efficiency with respect to tCOD and total solids removal (Table 2), and glucose utilisation (not shown in Table 2) was observed. The heat treated whey with *E. cloacae* had the least COD and solid removal while the whey with its native microorganisms had the highest remediation efficiency (Table 2). Interestingly, while the pH values in unsterilized whey reactors decreased, there was a significant (P < 0.05) increase in the pH at the end of the experiment in the heated whey inoculated with *E. cloacae* only anodic chamber. In all three setups glucose was utilised, with the native whey setup showing the highest decrease (51.3%) in the sugar concentration at the end of the experiment, followed by native whey with *E. cloacae* (40.6%) and then sterilised whey with *E. cloacae* (29.0%).

The coulombic efficiency was directly proportional to the power density reported amongst the three setups. The *E. cloacae* and heat treated whey had the highest (3.7%) coulombic efficiency while the setup with the native whey microorganisms had the lowest (0.1%; Table 2).

Electrode colonisation

There was evidence of microbial colonisation on the electrodes as illustrated by the micrograph in Figure 3. The
Figure 2. Maximum power densities produced from the three MFC setups.

Table 2. Changes in total solids and other parameters in MFC setups using whey and different microbial combinations in the anode chamber.

| MFC setup Pre-treatment | Microbial composition | Final pH* | COD removal (%) | Solids removal (%)* | Coulombic efficiency (%)* |
|-------------------------|-----------------------|-----------|-----------------|---------------------|-------------------------|
| Autoclaved              | E. cloacae only       | 6.1       | 5.0 ± 0.7       | 3.4                 | 3.7                     |
| None                    | Native and E. cloacae | 4.2       | 22.1 ± 2.8      | 11.7                | 0.5                     |
| None                    | Native only           | 4.8       | 44.7 ± 0.2      | 19.7                | 0.1                     |

*Standard deviations are less than 0.1 were correct to 1 decimal place.

Figure 3. Scanning electron micrograph illustrating the anode colonisation by bacteria (biofilm) after termination of the MFC with heat treated whey and E. cloacae.
density of microorganisms on the electrode surface was directly proportional to the power generated (micrograph not shown).

**DISCUSSION**

Results from this study established that, whey can drive power generation in MFCs with variations in power density based on the microbial composition in the anode chamber. The power generation was coupled to partial remediation of the effluent. However, similar to power amount of power produced, the extent of whey remediation in the MFC setup depended on the microbial communities in the reactors. Our findings of the trends in power peaking and dropping (Figure 1) are similar to observations reported by Nambiar et al. (2009) when they used reinforced clostridial media (RCM) in the anode chamber. However, in their case, maximum power was attained after two hours. Such differences in the time for peak power production are mainly attributed to the media complexity because whey is more complex than RCM. When a medium is complex, the lag period for maximum power generation is prolonged. Rapid decrease in the power density from the maximum is not desirable because one of the objectives in MFC technology is to maintain maximum power production over a longer period. Under such circumstances the MFC has to be modified to feed batch system and maintain a steady state when maximum power is generated. The maximum power density from sterilised whey with *E. cloacae* is considerably higher than the one that was reported (18.4 mW/m²) from MFC driven by diluted whey and inherent microorganisms by Antonopoulou et al. (2010). This high power density (16.7 W/m²) can be partly attributed to the strength of the raw whey typified by the elevated tCOD value (96.5 ± 4.2 g/l) and partly to the initial conductivity of whey (Table 1). Whey COD has been reported to be between 61 and 80 g/l (Kalyuzhnyi et al., 1997; Re et al., 1998; Cali et al., 2002; Pant et al., 2010), that is lower than the value reported in this study. The advantage of high COD at start-up would be that the substrate ceases to be the limiting factor in electricity generation. Higher conductivity reduces the resistance of the medium, thus, generally increasing the power output and shortening the onset of the maximum power density (Ramasamy et al., 2008; Mohan and Das, 2009).

The tCOD removal in MFCs with native whey only and that with native whey and *E. cloacae* shows insignificant synergy in remediation power production. One would have expected to have both tCOD removal and power density to be higher than that of sterilised whey and *E. cloacae* if there was significant synergy. This low power production could be attributed to competition for the nutrients and synthesis of intermediates that accepted electrons within the chamber. The latter is supported by the low coulombic efficiencies observed in this study. Coulombic efficiency gives an indication of the proportion of electrons extracted from organic matter that go towards electricity production (Huang et al., 2009; Antonopoulou et al., 2010).

The indirect relationship between power density and COD removal can be expected since COD removal is used as a component in the denominator when calculating the coulombic efficiency. Thus, there is some degree of antagonism between maximum remediation and power generation (electron transfer to the anode). This calls for cautious designing of MFCs and consideration of the microbial composition in the anodic chambers. While mixed cultures are favourable for MFCs that utilise complex substrate (Ren et al., 2007; Chae et al., 2009), their ecology has to be understood in order to achieve desirable augmentation in waste degradation and power generation. Therefore a high coulombic efficiency (3.7%) in sterile whey and *E. cloacae* implies that in the absence of other microflora, there was electron interception before reaching the anode. This could be a result of metabolic intermediates from the competing cells.

Electron interception maybe useful if the culture can be used as a biocathode. Further investigations to this effect are under way in our group to explain this and evaluate potential of using the live whey *E. cloacae* as a biocathode. In addition, heat treatment could have hydrolysed proteins, making them readily available to *E. cloacae* consequently producing more power than the rest of the reactors.

The direct relationship between power generation and microbial density on the electrode suggests a direct transfer of electrons to the anode. In addition, there is a benefit on the kinetics due to reduced activation loss (Ramasamy et al., 2008). Thus, increased microbial competition could also have limited process or biofilm formation in the mixed culture and consequently, less power was produced.

Generally, the pH of whey was within the range of pH values in fermented milk (Mufandaedza et al., 2006). The continual decrease in pH in unheated setups points out the dominance of lactic acid bacteria that fermented the milk. This continuation in fermentation also leads to a decrease in the solids of the milk hence, the significant (P < 0.05) drop in the solids of the whey with native microflora only. Such low pH in the two setups (Table 2) could partly account for low power generation by the reactor with *E. cloacae* and whey microflora. It would be interesting to perform a study to determine the survival of the *E. cloacae* under these conditions. This will shed more light on the observed power trends. The increases in pH of the heat treated reactor is partly accounted for by absence of competition from the acid producing, lactic acid bacteria and partly the denatured proteins giving the amino acids that have a buffering capacity. The latter was partially confirmed, in a separate study by our group. The
observed decrease in glucose concentration after the experiment suggests that the microorganisms could have used it as a carbon source even if it is not the best substrate in MFCs (Chae et al., 2009).

This study illustrated that whey can drive microbial fuel cells and opened a possibility of using it in the cathode chamber. However, more work requires to be performed to gain more understanding and maximum benefit from using such effluents. The work entails the understanding of microbial dynamics, metabolite tracking and mixing whey with other effluents rich in specific constituents such as starch. The latter will be important for a balanced carbon and nitrogen sources for enhanced microbial performance since whey is rich in proteins.

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REFERENCES

Antonopoulou G, Stamatelatou K, Bebelis S, Lyberatos G (2010). Electricity generation from synthetic substrates and cheese whey using a two chamber microbial fuel cell. Biochem. Eng. J. doi:10.1016/j.bej.2010.02.008.

Asad S, Amoozegar MA, Pourbabae AA, Sarbolouki MN, Dastgheib SMM (2007). Decolorization of textile azo dyes by newly isolated halophilic and halotolerant bacteria. Bioresour. Technol. 98: 2082-2088.

Brunk U, Collins VP, Arro E (1981). The fixation, dehydration, drying and coating of cultured cells of SEM. J. Microsc. 123: 121-31.

Bullen RA, Arnot TC, Lakeman JB, Walsh FC (2006). Biofuel cells and their development. Biosens. Bioelectron. 21: 2015-2045.

Huang L, Angelidaki I (2008). Effect of humic acids on electricity generation integrated with xylese degradation in microbial fuel cells. Biotechnol. Bioeng. 100: 413-422.

Huang L, Cheng S, Rezaei F, Logan B (2009). Reducing organic loads in wastewater effluents from paper recycling plants using microbial fuel cells. Environ. Technol. 30(5): 499-504.

Ieropoulos IA, Greenmana J, Melhuish C, Hart J (2005). Comparative study of three types of microbial fuel cell. Enzyme Microb. Technol. 37: 238-245.

Kalyuzhnyi SV, Martinez EP, Martinez JR (1997). Anaerobic treatment of high-strength cheese-whey wastewaters in laboratory and pilot UASB-reactors. Bioreosur. Technol. 60: 59-67.

Liu ZD, Li HR (2007). Effects of bio- and abio-factors on electricity production in a mediatorless microbial fuel cell. Biochem. Eng. J. 36: 209-214.

Logan BE, Regan JM (2006a). Electricity-producing bacterial communities in microbial fuel cells. Trends Microbiol. 14: 512-518.

Logan BE, Regan JM (2006b). Microbial fuel cells-challenges and applications. Environ. Sci. Technol. 40(17): 5172-5180.

Mohor D, Das D (2009). Effect of ionic strength, cation exchanger and inoculum age on the performance of microbial fuel cells. Int. J. Hydrogen Energ. 34: 7542-7546.

Mufandaeza J, Viljoen BC, Feresu SB, Gadaga TH (2006). Antimicrobial properties of lactic acid bacteria and yeast-LAB cultures isolated from traditional fermented milk against pathogenic Escherichia coli and Salmonella enteriditis strains. Int. J. Food Microbial. 108: 147-152.

Najafpour GD, Tajallipour M, Komeili M, Mohammadi M (2009). Kinetic model for an up-flow anaerobic packed bed bioreactor: dairy wastewater treatment. Afr. J. Biotechnol. 8(15): 3590-3596.

Nambari S, Togo CA, Limson, JL (2009). Application of multi-walled carbon nanotubes to enhance anodic performance of an Enterobacter cloacae-based fuel cell. Afr. J. Biotechnol. 8(24): 6927-6932.

Pant D, Bogaert GV, Diels L, Vanbroekhoven K (2010). A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. Bioresour. Technol. 101: 1533-1543.

Rabaey K, Verstraete W (2005). Microbial fuel cells: Novel biotechnology for energy generation. Trends Biotechnol. 23: 291-298.

Ramasamy RP, Ren Z, Mench MM, Regan JM (2008). Impact of initial biofilm growth on the anode impedance of microbial fuel cells. Biotechnol. Bioeng. 101: 101-108.

Re GD, Giacomo GD, Aloisio L, Terreiri L (1998). RO treatment of wastewater from dairy industry. Desalination, 119: 205-206.

Ren Z, Ward TE, Regan JM (2007). Electricity production from cellulose in a microbial fuel cell using a defined binary culture. Environ. Sci. Technol. 41: 4781-4786.

Sharma T, Reddy ALM, Chandraa TS, Ramaprabhub S (2008). Development of carbon nanotubes and nanofluids based microbial fuel cell. Int. J. Hydrogen Energy, 33: 6749-6754.

Scott K, Rimbau GA, Katuri KP, Prasad KK, Head IM (2007). Application of modified carbon anodes in microbial fuel cells. Process Saf. Environ. 85: 481-488.

SFS (1990). SFS 3008: Determination of total residue and total fixed nitrogen in water, sludge and sediment. Finnish Standards Association (SFS). p. 3.

Tsai H-Y, Wub C-C, Lee C-Y, Shiha EP (2009). Microbial fuel cell performance of multiwall carbon nanotubes on carbon cloth as electrodes. J. Power Sources, 194: 199-205.