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Full Length Research Paper

The impact of electrode reuse on the biofilm community and performance of whey-fuelled H-type microbial fuel cell

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Humankind has always been engaged in a relentless quest for energy to drive daily activities. However, most of the existing conventional strategies of tapping the environment are expensive and polluting. A nascent microbial fuel cell technology, relying on the degradation of wastewater such as cheese whey with the subsequent release of electricity, is thus the focus for sustainable energy. It is therefore important to gain an in-depth knowledge of the biology in these devices and possible avenues of enhancing power generated from them. In an effort to increase power from whey-driven microbial fuel cells (MFCs), graphite sheet anodes were incubated in whey for two months then reused in four cycles of seven days each in MFCs. The concomitant remediation was additionally determined. Highest power density (390 ± 21 W/m^2) was obtained during the third reuse cycle with a coulombic efficiency of 0.25%. The highest tCOD removal (44.6%) was however, noted during the second cycle. The performance of reactors depended on the number of reuse cycles with the third cycle proving to be the best after which there was a decline in power density. In addition, molecular analyses of anodophilic microorganisms showed the presence of three species related to strains from *Lactobacillus helveticus* (85% identity), *Proteus mirabilis* (96%) and *Escherichia coli* (96%). Therefore the necessity of biofilm build up should take into account the limitation of maximum number of reuse cycles where the flora will be performing at its best.

Key words: Cheese whey, green power, microbial fuel cell, biofilm, bioremediation.

INTRODUCTION

With the advent of industrial revolution in the eighteenth century, fossil fuels became the energy currency to drive the world's economy, thus constantly keeping up with the market pressures. However, anthropogenic activities such as intensive extraction by oil drilling rigs with subsequent refining, electricity generation by coal-based power plants, and the petroleum combustion have all resulted in adverse environmental impacts and the depletion of non-renewable energy reserves (Samrot et al., 2010). In order to mitigate the toll on the planet while satisfying the economic objectives, alternative strategies for power generation (solar panels, wind mills, geothermal and biodiesel) are currently in operation and under constant improvement. MFCs, still in their infancy, hold a potential for green electricity generation with a simultaneous decrease of pollutants. Other advantages of MFCs include lower running and maintenance costs since they can be operated at ambient temperatures. Furthermore, the wide microbial substrate base (Min et al., 2005; Huang and Logan, 2008; Masthuriya and Sharma, 2009; Wang et al., 2009; Antonopoulou et al., 2010; Cha et al., 2010; Kassongo and Togo, 2010; Pant et al., 2010; Wen et al., 2010) makes MFCs attractive as an alternative energy source.

A major hurdle in the MFC technology is the lower power yields when compared to chemical fuel cells and other alternatives. Therefore, various approaches have been forged to improve the energy output. These include acclimation of microorganisms (Kim et al., 2007),

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optimisation of process parameters (Yazdi et al., 2008), sedimentation of effluents (Huang and Logan, 2008), co-digestion of wastes (Ward et al., 2008), bio-augmentation of catalysts (Wang et al., 2009), operation in continuous flow systems (Huang et al., 2009), selection of high-performing biocatalysts (Samrot et al., 2010), and addition of salts to enhance conductivity (Wen et al., 2010). Studies in our group revealed that undiluted whey had potential to drive microbial fuel cells with power density directly proportional to the period of biofilm formation in independent vessels. However, elsewhere (Cheng et al., 2008; Ramasamy et al., 2008) external resistors and external circuit parameters have been reported to impact the biofilm build up and nature of microflora on the anode. Therefore it was the aim of this work to investigate this impact on whey-driven microbial fuel cells using the microorganisms inherent to the anolyte (whey). The MFC performance in terms of whey remediation, power density and coulombic efficiency were determined. The final microfloral diversity on the anode biofilm was verified by 16s rDNA sequencing.

MATERIALS AND METHODS

Experimental design

Anodes were stored under an anoxic environment in whey replenished every three days to enrich for anodophilic microorganisms during a period of two months (pre-incubation). Then, electrodes were transferred to whey-fed MFC setups for seven days before being reused in new setups. This electrode recycling was performed four times in reactors and lasted thirty days for a cumulative enrichment period of ninety days, including pre-incubation. Experiments in fuel cells started off with six reactors and one reactor's anode was "sacrificed" to microscopy and molecular identifications at the termination of each round of MFCs. Fresh cheese whey was sourced from Greenways Delli (Kyalami, South Africa) on the day of reactor setups. All the operational parameters, power density calculations and equipments used were as described previously (Kassongo and Togo, 2010).

DNA extraction

Anodes were transferred to 2 mm glass beads and vigorously shaken for 5 min to dislodge cells from surfaces. The medium was centrifuged at 10 000 rpm for 1 min, the supernatant was discarded and the pellet was re-suspended then mixed with Zymo Research ZR Fungal/Bacterial DNA MiniPrep™ kit (Inqaba Biotechnical Industries, South Africa) as per manufacturer's instructions to prepare ultra-pure genomic DNA templates for polymerase chain reaction (PCR).

PCR-amplification

For amplification, one tube contained: 25 µl of 2 X PCR Master mix (Taq DNA polymerase in reaction buffer, $MgCl_2$ and dNTPs - 0.4 mM of each), 1 µl each for reverse and forward primer 16S rDNA (UNIV1392R: 5'-ACG GGC GGT GTG TRC-3', EUB968F AAC GCG AAG AAC CTT AC with GC clamp), 22 µl DNase and RNase-free water (Fermentas, USA) and 1 µl of the extracted DNA aliquot. A second tube was incubated with all the components for PCR, but without the whey-isolated DNA in order to check for any possible contaminations which may have occurred in the course of experiments. Applied Biosytems 2720 Thermal Cycler was used for PCR and set at the following parameters: initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation (30 s at 94°C), annealing (45 s at 60°C and extension – 90 min at 72°C), and a final extension at 72°C for 7 min before storage at -4°C. The products were electrophoresed on an agarose gel to confirm their presence, quality and purity (Jong et al., 2006).

Table 1. Profile of the whey before reactor setups.

Parameter	Values
tCOD (g/l)	76 ± 19
Solids (g/l)	3.4 ± 0.12
PO^{3-}_4 (mg/l)	1410 ± 14
NO^{-}_3 (mg/l)	90 ± 8.5
Glucose (mg/l)	261.1 ± 33

Denaturing gradient gel electrophoresis (DGGE)

The goal with DGGE was to examine the microbial diversity/composition on the anode biofilm by running a mix of amplified 10 µl PCR products and 5 µl of the DGGE loading dye in 6% PAG at 130 V, 60°C for 4 h. After that, the gel was stained in 250 ml 0.5 X TAE buffer (10 $mg.ml^{-1}$ ethidium bromide) for 15 min followed by a distaining for 15 min in 0.5 X TAE buffer without ethidium bromide (Muyzer et al., 1993). A Gel Doc System was used to view the gel under UV light and help in the cutting out of dominant bands which were soon immersed in 50 µl TE buffer at 4°C overnight to remove the DNA template from the DGGE gel (Lee et al., 2003). A second re-amplification as specified above (without GC clamp on the forward primer) followed by another agarose gel electrophoresis were performed to confirm presence of DNA before sequencing at Inqaba Biotechnical Industries (South Africa) (Azbar et al., 2009).

Sequencing and identification

Sequences received from Inqaba Biotechnical Industries (South Africa) were edited with FinchTV and a nucleotide BLAST search for highly similar sequences was performed in the NCBI database. The strongest matches obtained were used together with the sequence of the isolate to construct the phylogenetic tree (Figure 6) in the DNAMAN sequence analysis software.

RESULTS

Whey composition

Phosphates, nitrates and glucose were present in the whey used for reactor setups (Table 1). These parameters remained relatively stable between repetitive effluent samplings from the factory. Both phosphates and nitrates had similar patterns of concentration changes across batch cycles with anodes reuse. The lowest concentrations were found in the first batch cycles whereas the fourth cycles experienced an increase of

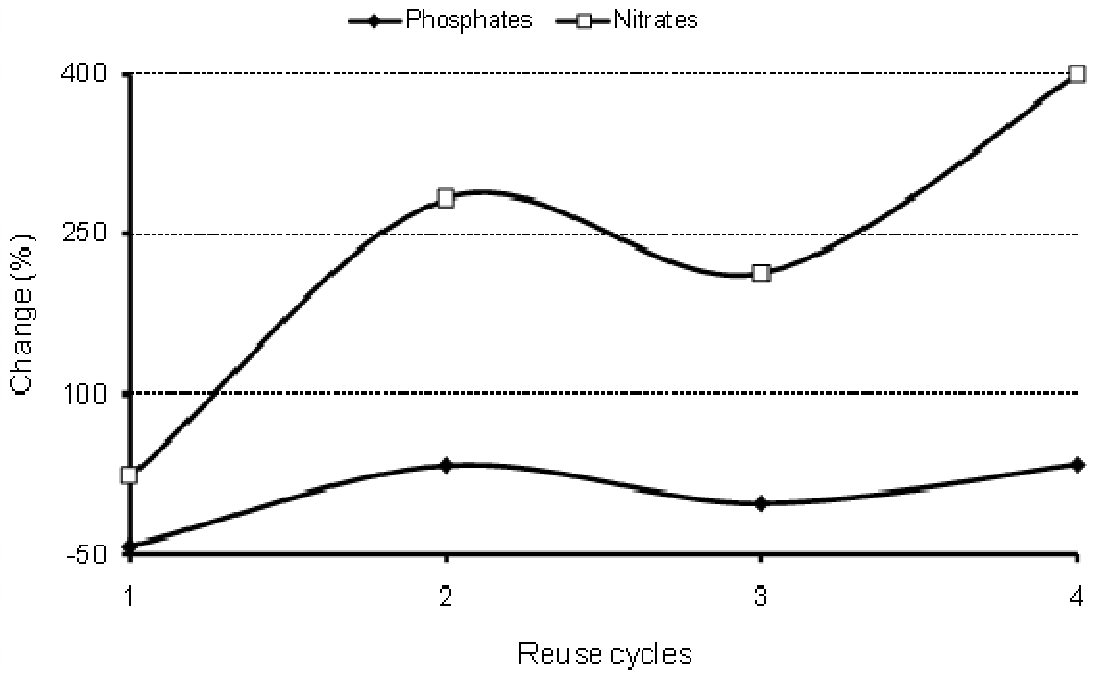

Figure 1. Relative changes (%) in nitrates, phosphates and coulombic efficiency for during each cycle of anode reuse.

concentrations in the reactors (Figure 1).

Power generation

Significant increase ($P < 0.05$) in power density was observed during the third cycle while the first and second had low power densities (Figure 2a). A significant ($P < 0.05$) decrease in maximum power density was observed in the fourth cycle. Similarly, the coulombic efficiency exhibited the same trend as that of power density during these cycles (Figure 2b). However, the trend for the time it took to produce maximum power density per reactor was neither similar to that of maximum power nor coulombic efficiency (Figure 2a). There was no significant difference between the times required to produce maximum power density from the first to third cycles, while the time was significantly short in the fourth cycle (Figure 2a).

Bioremediation

The solids removal efficiencies were not significantly different ($P > 0.05$) between the first and second cycles, 66.5 and 66.2%, respectively (Figure 3). There was variable tCOD removal through successive anodes use (Figure 3) with significant differences observed between the first and fourth cycles. Glucose removal pattern was similar to that of the tCOD.

Scanning electron microscopy (SEM)

After the two-month period of anode acclimation ahead of reactor setups, electrodes had dense biofilms comprising of rod-shaped cells. Microbial density on the electrode surfaces increased with prolonged anode reuse and cell aggregates were observed on the biofilms (Figure 4).

Molecular analysis and phylogeny

In the case of the DGGE, there was an increasing brightness characterised by a progressive refinement and resolution of bands with anode reuse across reactors (Figure 5). As illustrated, the DNA extracted from anodophiles on the first MFC cycle appeared as a single and faint band (lane 1; Figure 5) which became well defined in the third (lane 3; Figures 5 and 6) and fourth batch cycles (lane 4; Figure 5). Band 1 had 85% identity to strains of *Lactobacillus helveticus* while bands 2 and 3 had 96% identity to *Proteus mirabilis* and *Escherichia coli*, respectively.

DISCUSSION

The pre-incubation period of two months allowed anodophiles to attach, colonise electrodes, consequently increasing the concentration of the biocatalyst as confirmed by microscopy. Occurrence of the different

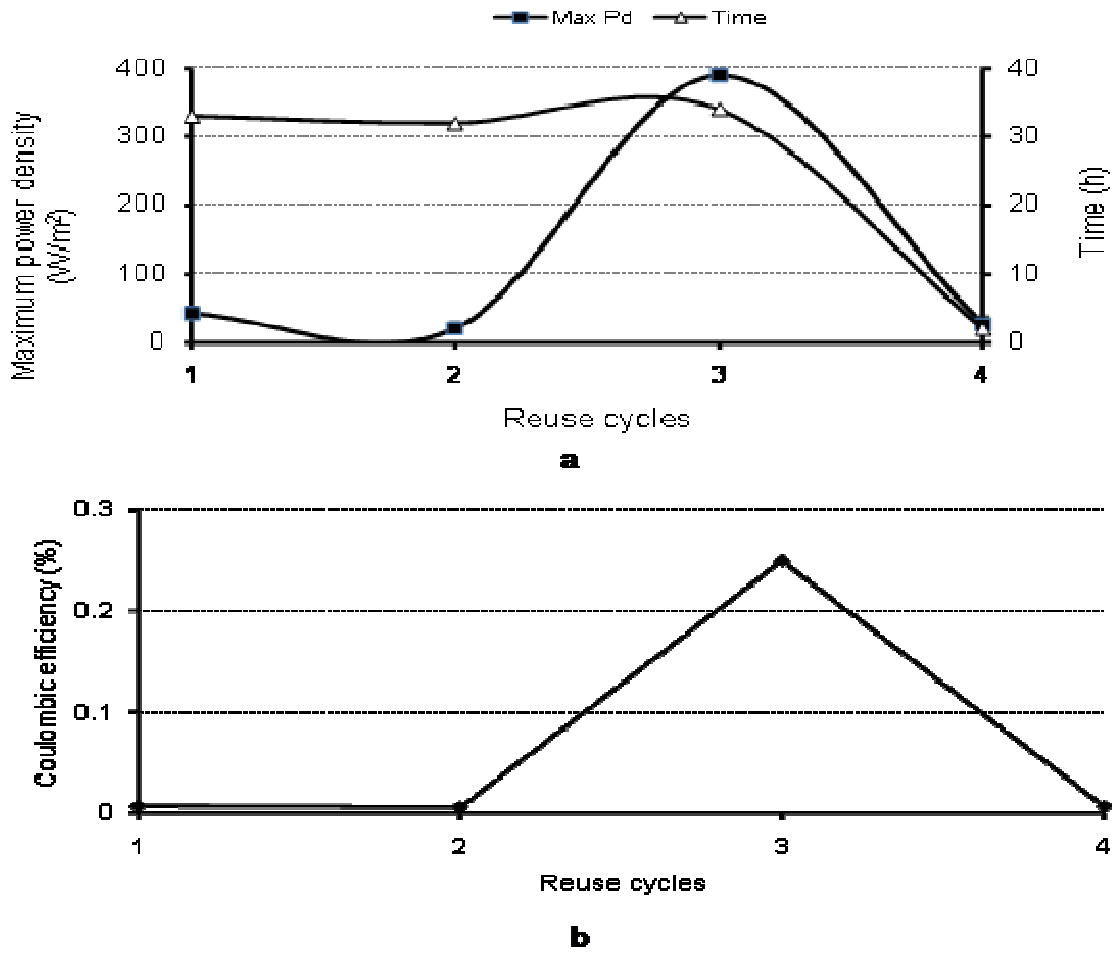

Figure 2. Maximum power density and time taken to reach each peak density in different stages of anode reuse.

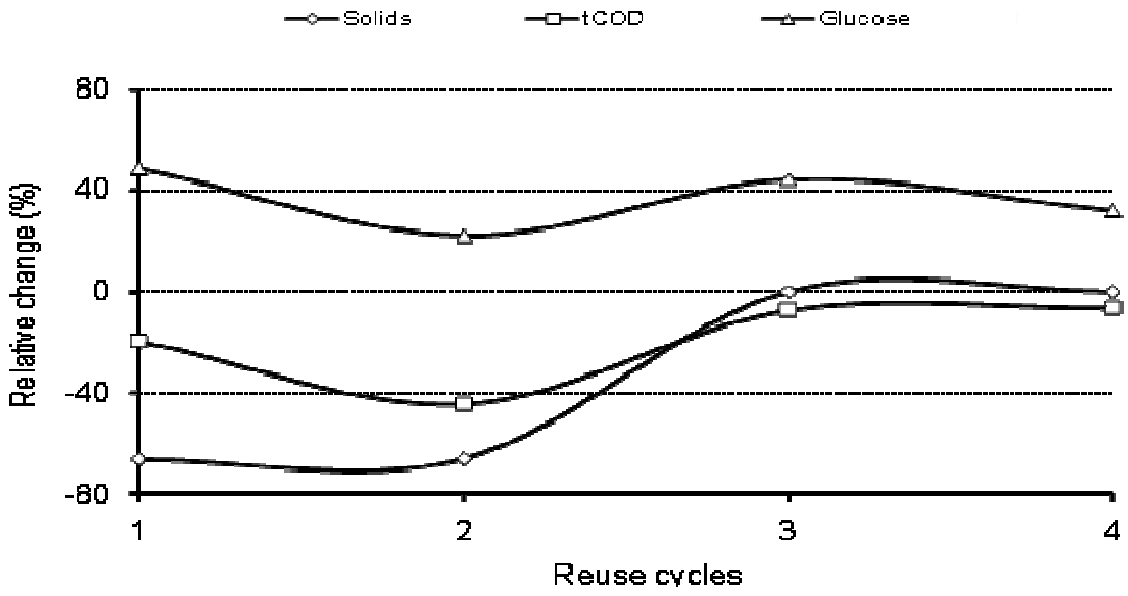

Figure 3. Changes (%) in whey parameters (a) and the substrate degradation rate (b) for each anode reuse setup. The 100 % concentrations for nitrates and phosphates were the initial concentrations given in Table 1.

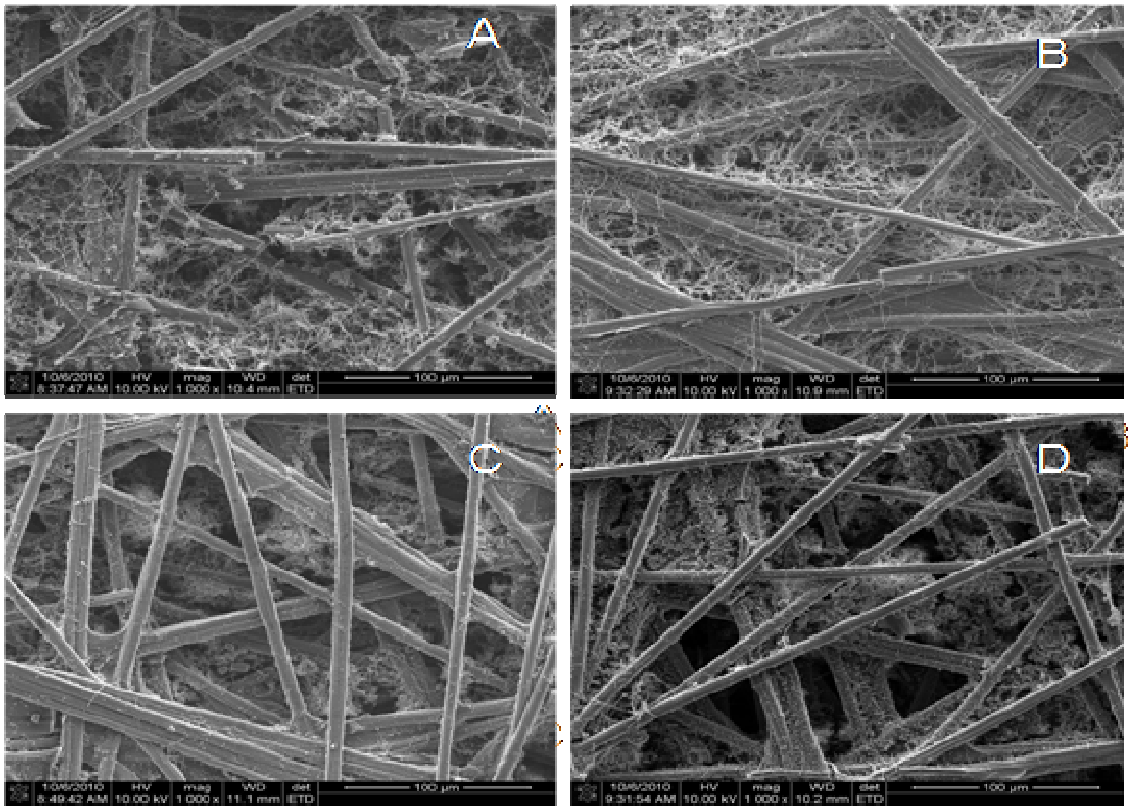

Figure 4. Scanning electron micrographs of anodes at the termination of the first (A), second (B), third (C) and fourth (D) MFCs reactor sets.

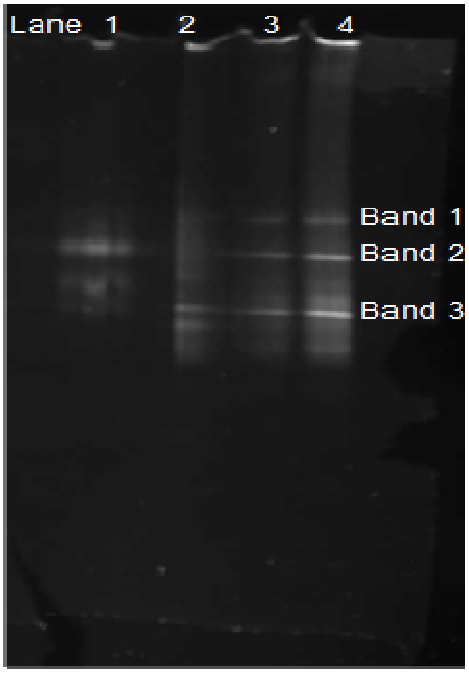

Figure 5. Profiles of DGGE analyses. Lane numbers represent the DNA samples extracted from the anodes in their respective MFC batch cycles.

species on the biofilm reinforces the requirement for a consortium to improve performance of microbial fuel cells. *L. helveticus* is common to cheese whey (Gatti et al., 2004) while *E. coli* and *P. mirabilis* could have been present at undetectable levels or were fortuitous contaminants. The latter is unlikely considering that MFCs select for electricigenic microorganisms (Rabaey et al., 2004; Kiely et al., 2010) and the repeated use of the anodes proved a selective advantage of the electrogenic, *E. coli* and *P. mirabilis* (Du et al., 2007).

The relative changes in tCOD illustrated that glucose could have been mainly utilised in he first and second cycles. Glucose is not the best substrate for electricity generation when compared to acetate (Ahn and Logan, 2010; Pant et al., 2010). Acetate can be generated in lactic acid metabolism (Caplice and Fitzgerald, 1999). Hence maximum power can be attributed to non utilisation of or less dependency on glucose. The trends for power density and pollutant parameters highlight the need for a fine balance between maximum power density generation and removal of pollutant indicators.

Increase in power density with electrode reuse up to the third cycle was expected partly because of increase in the population of electricigenic microorganisms and improved efficiency that comes with acclimation (Rodrigo et al., 2009). The decrease in power density during the

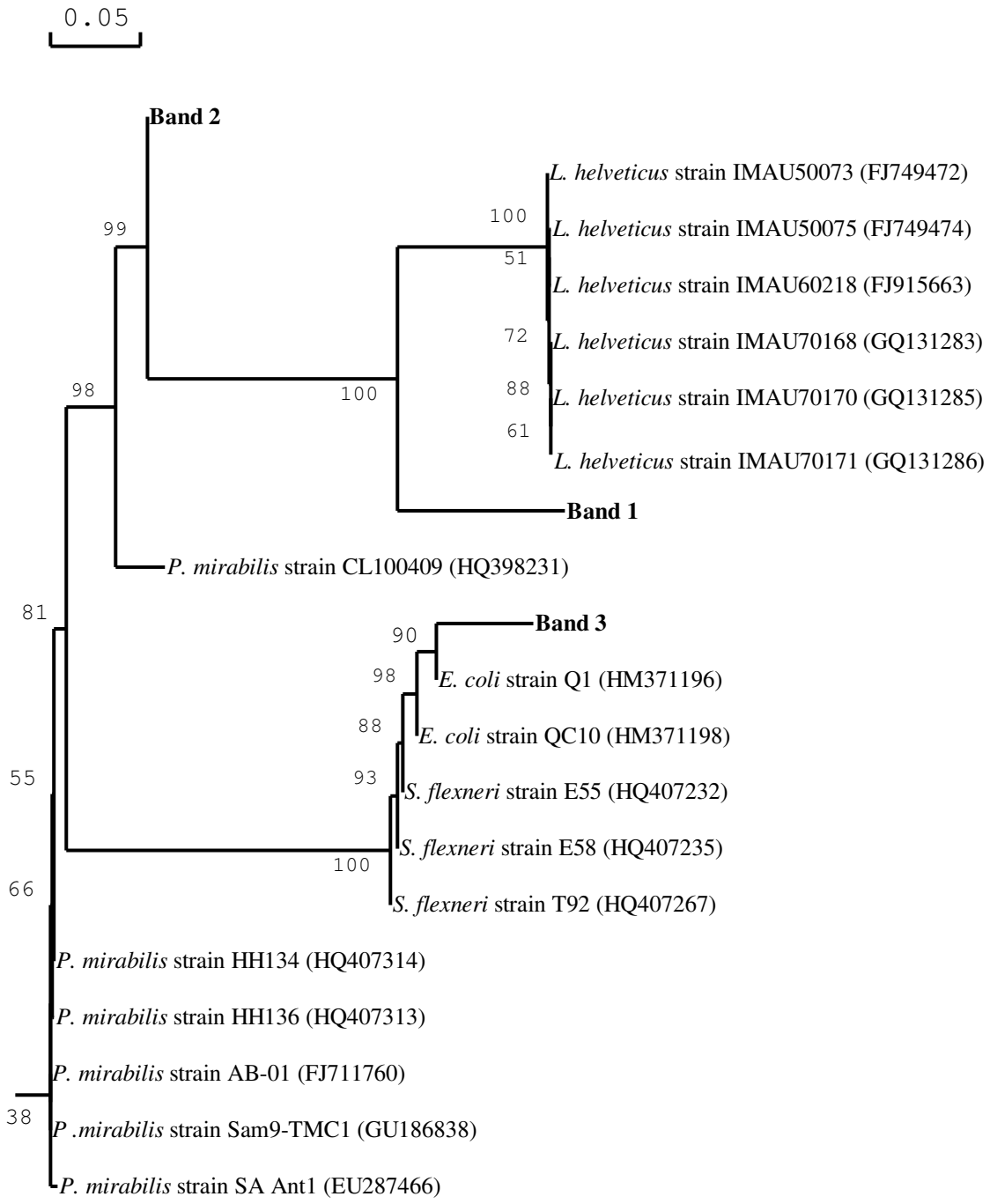

Figure 6. A phylogenetic tree based on the 16S rDNA from the anode of MFC in the third cycle. Numbers at internodes indicate bootstrap values (percentages of 1000 replications).

fourth cycle can be attributed to microbial exhaustion (Venkataraman et al., 2010). Microbial exhaustion can be supported by the decrease in the time the maximum peak was observed. One would have expected the times to remain constant but the decrease in the time could be due to other factors (summed as exhaustion) that saw failure of the microbial community to sustain their systems for the same period as that observed in previous cycles. Confirmation of this would need independent real-time viability studies. Decrease in coulombic efficiency can be partly attributed to the existence of alternative pathways that do not generate electricity. For example, *L. helveticus* is known for its lactic acid producing abilities and production of LA alone does not guarantee improved electricity generation.

While the role of biofilm build up and acclimation of the

microorganisms is important in the enhanced performance of microbial fuel cells, the trends observed call for the need to consider the maximum number of cycles and life span of these biofilms in order to sustain the desirable power generation. This may however vary with microorganism species and type of waste. Therefore, anode reuse promotes development of electricigenic consortium and enhanced power in whey driven microbial fuel cells. Further studies on the viability of the biofilm species and contribution of the lactic acid producing bacteria would need to be performed. It can be postulated that the biofilm maybe providing an efficient conduit for electron flow, but this requires to be verified by further studies. Hence, future studies should entail construction of polarisation curves and cyclic voltammograms for more insight on the internal resistance, optimising external resistors and investigation of possible mediators contributed by the microbial species in the biofilm.

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