African Journal of Biotechnology Vol. 10(69), pp. 15564-15570, 7 November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.146
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Evaluation of full-strength paper mill effluent for electricity generation in mediator-less microbial fuel cells

J. Kassongo and C. A. Togo*

School of Molecular and Cell Biology. University of the Witwatersrand, P. Bag 3, Wits 2050, Johannesburg, South Africa.

Accepted 26 April, 2011

In the search for renewable, sustainable and affordable energy sources, microbial fuel cells (MFCs) offer the advantage of a biological oxidation of pollutants to the direct generation of electricity by microorganisms. We thus examined the biodegradability and suitability of unamended paper mill effluent for power production in MFCs. In addition, an investigation of the response from indigenous waste microbes upon introduction of an exogenous *Enterobacter cloacae* culture was performed. Unamended effluent alone reached a substrate degradation rate (SDR) of 0.112 kg COD/m^{3}day and 29.4 ± 2.4% total chemical oxygen demand (tCOD) removal, the power density peaked at 24 ± 3 mW/m^2 and 47.7% glucose increase at the termination of the reactor cycle after 12 ± 3 days. The introduction of *E. cloacae* in separate setups lowered electricity generation, but benefited remediation, power density decreased to 13 ± 2 mW/m^2 whereas the SDR increased to 0.257 kg COD/m^{3}day. Also, there was 44.1% glucose removal in the presence of *E. cloacae*. It was concluded that unamended paper mill effluent can fuel electricity generation in MFCs with its concomitant remediation. The addition of *E. cloacae* to live paper mill effluent may be antagonistic in terms of electricity generation.

Key words: Microbial fuel cells, paper mill effluent, bioremediation, substrate degradation rate, wastewater, renewable energy, electricity.

INTRODUCTION

Over the years, various methods have been designed and tested in the treatment and recovery of water from paper mill effluent. The paper manufacturing industry was ranked amongst the largest consumers of freshwater and polluters globally (Ali and Sreekrishnan, 2001). In addition, a value as high as 11 g/l tCOD has been reported (Thompson et al., 2001; Basu et al., 2009) which is detrimental to aquatic life. As a result, chemical, physical (clarification and filters), aerobic biological treatments and/or anaerobic incubation in appropriate operation tanks have been put in place (Thompson et al., 2001).

Despite the considerable feats achieved such as water recirculation and reduction of pollutants prior to disposal, conventional treatments are limited by fouling of membranes and filters, poor dissolution of oxygen in wastewater and tremendous input of electricity in aeration tanks (Thompson et al., 2001; Ahn and Logan, 2010). On the other hand, the hallmark of anaerobic treatment of effluents has been the substantial reduction of sludge accompanied by a chemical reduction otherwise recalcitrant to aerobic treatment (Bogaert et al., 2010).

An increasing number of studies, within the field of renewable energy, have considered anaerobic treatment for the generation of volatile fatty acids, biogases (Kaksonen et al., 2003; Najafpour et al., 2009) and direct generation of electricity in microbial fuel cells — MFCs (Nimje et al., 2009; Cha et al., 2010; Kassongo and Togo, 2010). In the latter, effluent is degraded by microorganisms in an anoxic chamber with the release of electrons that are captured by an electrode and channelled externally to a final electron acceptor in an aerated compartment. Protons are also released and migrate through a selectively permeable membrane to

*Corresponding author. E-mail: catogo@gmail.com. Tel: +27 11 717 6312. Fax: +27 11 717 6351.

reduce oxygen to water (Mohan et al., 2008; Mathuriya and Sharma, 2009). Recent works involving paper mill effluent have been focused on single-chambered MFC designs at ambient temperatures. For example, upon improving conductivity of the effluent supernatant with addition of 100 mM PBS in a 500 h fed-batch configuration, up to 672 ± 27 mW/m^2 maximum power density (P_d) and 76 ± 4% tCOD removal were attained (Huang and Logan, 2008). However, the maximum P_d was reduced to 210 ± 7 mW/m^2 and tCOD removal was 26 ± 2% when effluent of the same type was fed to MFCs operated in a continuous flow system (Huang et al., 2009). Mathuriya and Sharma (2009) also attempted to generate bioelectricity while achieving bioremediation of different wastes, including paper industry wastewater. They found that an increase of operational parameters namely temperature, agitation, and effluent concentration directly increased the P_d within a specific range.

However, little has been documented on the feasibility of electricity generation from a full-strength paper mill effluent while achieving significant remediation in H-type MFCs. In this study, attention was devoted to keep, strictly at minimum, any input of nutrient (s) and/or energy to allow for practical projections in future upper-scale studies. In addition, molecular analyses of electrodes were performed to identify the anodophilic microbial species.

MATERIALS AND METHODS

Experimental setups and analyses

The H-type MFCs, as described by Nambiar et al. (2009), were used. Briefly, each 250 ml reactor (anode and cathode chambers) consisted of 200 ml of respective solutions separated by a proton exchange membrane (Nafion™ 117). Paper mill effluent from Mondi Paper (Springs, South Africa) was used as the anolyte while the catholyte comprised of 0.02 M potassium ferricyanide in 0.1 M sodium phosphate buffer (pH 6.8). The external circuit was linked by a 470 Ω resistor. Following collections, samples not used in setups were stored at 4°C to delay possible changes in bio-chemical composition (Najafpour et al., 2009). Three sets of MFCs were set up as previously explained (Kassongo and Togo, 2010). The operation of the MFCs was performed at ambient temperature (22 ± 3°C). Each MFC setup was run for 10 to 14 days and voltage was measured at 2 h intervals using a TopTronic T830 digital multimeter (APelectronics, South Africa), while pollution indicators such as tCOD and solids were measured at the beginning and at the 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: $P_d = (VI)/A$, where, V is the voltage; I is the current and A is the surface area (Rabaey and Verstraete, 2005). In addition, the power yield, the substrate degradation rate (SDR) and the coulombic efficiency (ε_{cb}), were calculated according to Huang and Angelidaki (2008), Mohan et al. (2008) and Antonopoulou et al. (2010) respectively.

Scanning electron microscopy (SEM)

The preparation of anodes (fixing, dehydration and coating) was performed to assess the density of microbial population on carbon electrode surface (Kassongo and Togo, 2010).

Anode biofilm diversity studies

To gain insight on microfloral community diversity playing participating in electricity generation and bioremediation, diversity of microorganisms on the anode was investigated using molecular techniques. The latter entailed DNA extraction, PCR, DGGE and sequencing.

DNA extraction

At the termination of reactors, anodes were transferred to 20 g of 2 mm glass beads in 50 ml sterile reinforced clostridial medium and shaken by hand for 5 min to dislodge cells from surfaces. The medium was centrifuged at 10,000 rpm for 1 min at 4°C, 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 PCR.

PCR-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 isolated DNA in order to check for any possible contaminations which may have occurred in the course of the 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. An agarose gel electrophoresis was later conducted to confirm for the presence, quality and purity of PCR products (Jong et al., 2006).

Denaturing gradient gel electrophoresis (DGGE)

The goal with DGGE was to examine the microbial diversity of samples 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). Dominant bands were cut and template DNA removed from the DGGE gel by immersing in 50 µl TE buffer at 4°C overnight (Lee et al., 2003). A second re-amplification as specified previously (without GC clamp on the forward primer) followed by another agarose gel electrophoresis were performed to confirm the presence of DNA before sequencing (Azbar et al., 2009) at Inqaba Biotechnical Industries (South Africa).

Sequencing and phylogenetic tree

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.

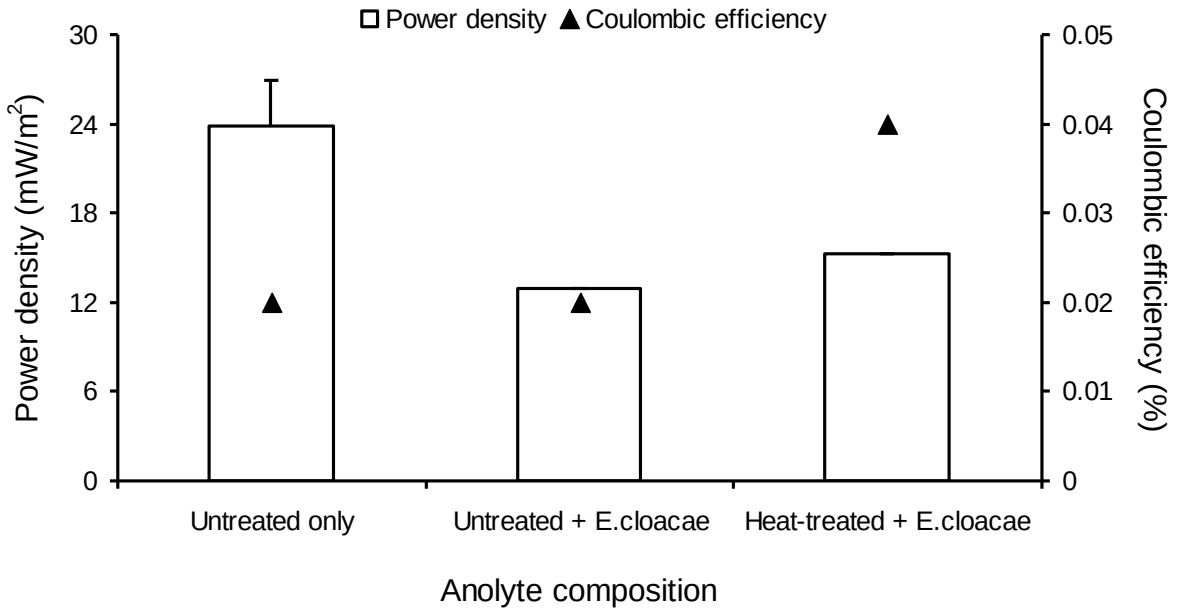

Figure 1. Maximum power densities and respective coulombic efficiencies from different MFC setups fed with paper mill effluent.

The strongest matches obtained were used together with the sequence of the isolate to construct the phylogenetic tree in the DNAMAN sequence analysis software.

Statiscal analysis

Experiments were performed in triplicates and reported as mean ± standard deviation.

RESULTS

Effluent profile

The pH of the effluent was 6.61 ± 0.1 with a conductivity of 4.65 mS/cm at sampling. Initially, tCOD and total solids were 43.7 ± 0.3 g/l and 584 ± 0.2 mg/l, respectively. The glucose concentration was 1.4 ± 0.02 g/l. These parameters remained relatively stable during storage.

Electricity generation

Without *Enterobacter cloacae*, setups did not have stable potential differences. However, introduction of *E. cloacae* in the medium generally reduced both the magnitude and the variations of power densities, stabilising voltage readings close to 0.3 mV. The maximum power density from inherent waste microbes in the presence of *E. cloacae* was 13 ± 2 mW/m^2. The highest power density, 24 ± 3 mW/m^2, was observed on setups with wastewater only (Figure 1).

Although, setups using paper mill effluent only generated the highest maximum power density, its coulombic efficiency (ε_{cb} = 0.02%; P_Y = 0.1 µW/kg COD_R) was lower than that for sterile effluent acted upon by *E. cloacae* (ε_{cb} = 0.04%; P_Y = 0.3 µW/kg COD_R). However, the maximum power density for untreated waste incubated with *E. cloacae* (P_d = 13 ± 2 mW/m^2 and ε_{cb} = 0.02%) was lower than that for sterile wastewater using *E. cloacae* (P_d = 15.3 mW/m^2) (Figure 1).

Bioremediation

Pollution parameters such as tCOD and total solids were reduced in all set ups. However, glucose concentration was increased in reactors containing the unamended effluent together with *E. cloacae* (Figure 2). There was a direct relationship between the final pH of setups and the solids removal efficiencies. Up to 98.8% settable solids removal efficiency and a final pH of 7.42 when *E. cloacae* was used together with microorganisms inherent to paper mill effluent. In the absence of *E .cloacae*, unamended waste achieved a settable solids removal efficiency of 20.1% and a final pH of 6.17. The presence of *E. cloacae* most likely contributed to the final pH values higher than those initially (6.61 ± 0.1); the pH dropped in the absence of *E. cloacae* compared to setups where it was introduced.

Organic matter degradation was inversely proportional to the maximum electricity generated across different MFC configurations. Two setup types; sterile effluent with *E. cloacae* alone and unamended effluent only, had

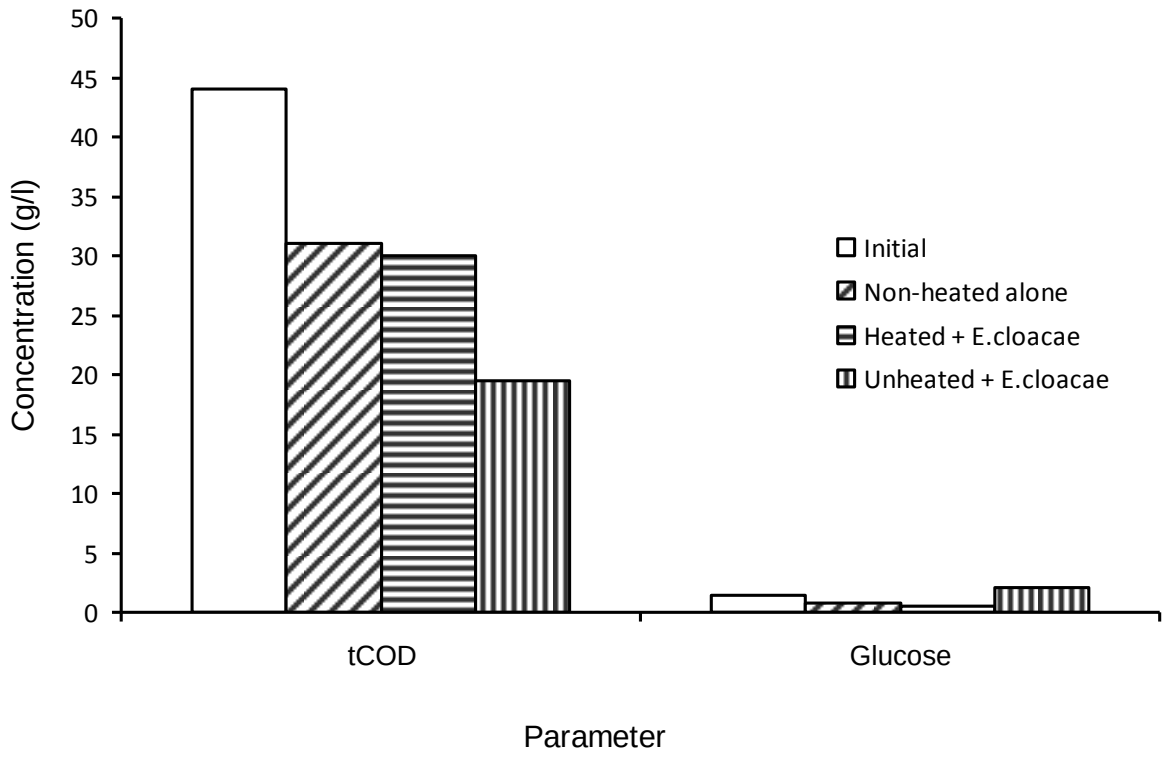

Figure 2. Bioremediation profile of paper mill effluent in different setups after MFC cycles in relation to initial concentrations.

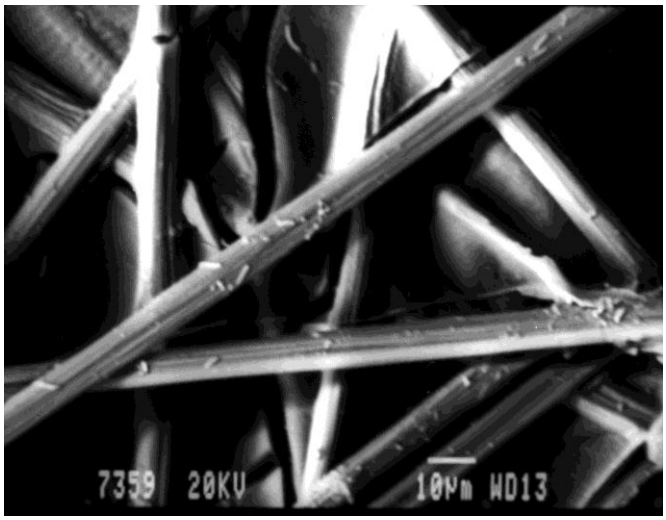

Figure 3. A scanning electron micrograph of a sparsely colonised anode electrode.

relatively close SDRs, 0.125 kg COD/m^3 day and 0.112 kg COD/m^3 day, respectively. Addition of *E. cloacae* to native effluent increased the SDR to 0.257 kg COD/m^3day (55.4 ± 0.7% COD removal efficiency and ε_{cb} = 0.02%) resulting in 13 ± 2 mW/m^2, the lowest maximum power per anode surface area.

Microscopy

There was a sparse microbial colonisation of electrodes by rod-shaped bacteria and isolated micro-colonies of round-shaped bacteria as illustrated in Figure 3. However, similar electrodes showed a dense microbial population when incubated in reactors fed with reinforced clostridial medium (RCM) used as a control (micrograph not shown). Thus, the density of microorganisms on electrode surfaces was directly proportional to the power generated.

Molecular analyses

PCR amplicons from the anodophilic microorganisms (after reactor setups) showed a smear on the denaturing gradient gel electrophoresis (DGGE) without a distinctive separation of bands. An arbitrary cut on the DGGE was performed, sequenced and subsequently analysed (BLAST search) for the 16S ribosomal partial sequence, revealing 99% identity to strains of the *Leuconostoc mesenteroides* subsp. *mesenteroides* (Figure 4). The complete GenBank search revealed a total of 102

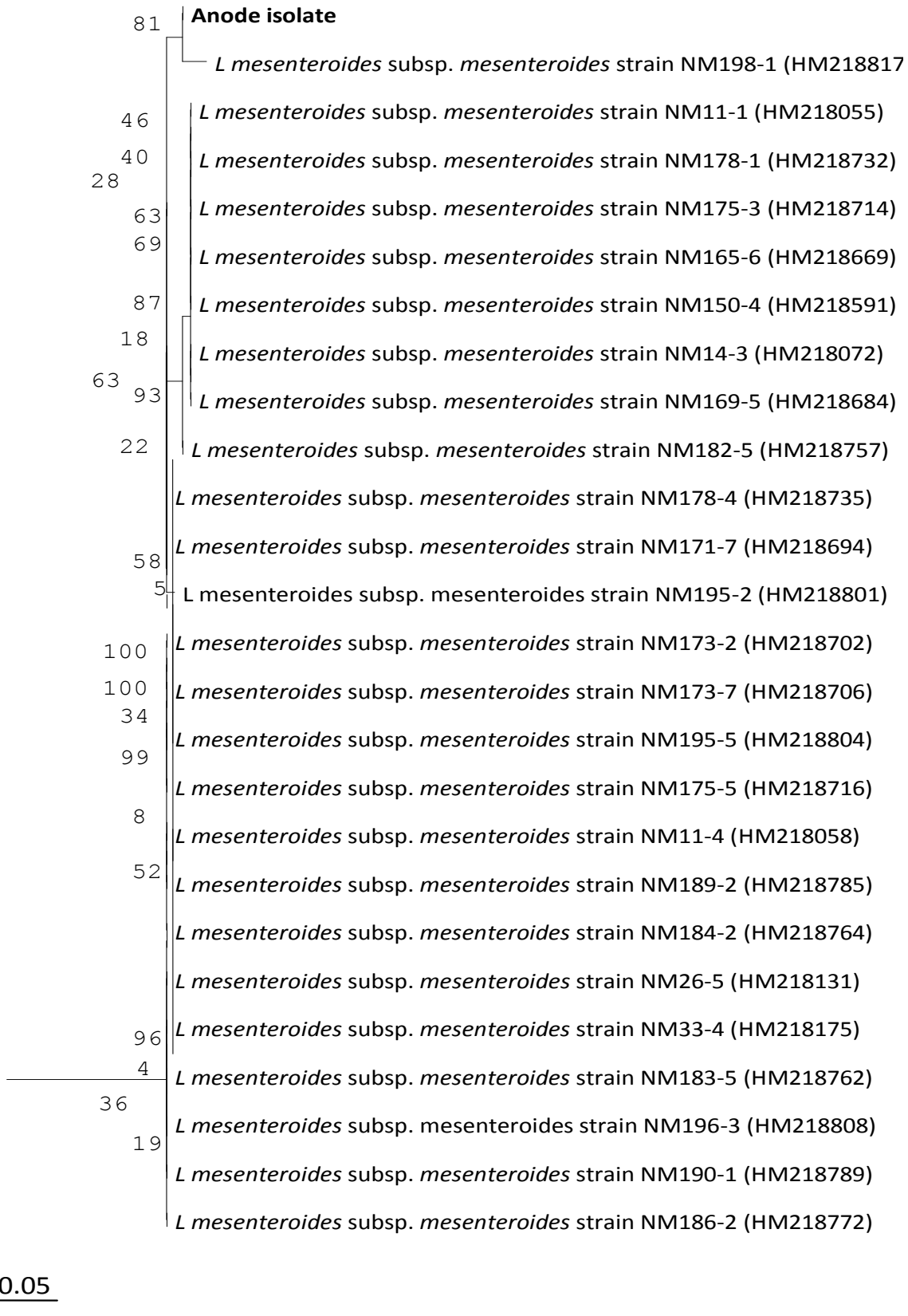

Figure 4. An unrooted phylogenetic tree of the 16S ribosomal RNA, partial sequence. Species matching (all 99% identity) the paper mill effluent anodophilic isolate and their respective GenBank accession numbers are shown in brackets. Bootstrap values (1000 replications) are indicated at the nodes.

different strains with the same percentage of identity to the paper mill effluent isolate.

DISCUSSION

This study used a concentrated paper mill effluent which contained alkalis used throughout a wood processing plant. It could be expected that such a non-pre-treated wastewater containing strong oxidizing chemicals would increase the survival pressure for microbial life and not necessarily favourable to electron generation and transport (Huang et al., 2009; Hakeem and Bhatnagar, 2010). Therefore, the lack of a steady state in power generation and a sparse microbial growth on electrodes were noted (Mathuriya and Sharma, 2009). The fluctuation in power densities may also be attributed to the breakdown of cellulose which is a very slow process and may even get intermittent in stagnant conditions or in absence of ideal mixing of bacteria within the wastewater (Huang and Logan, 2008). Moreover, a lack of microbial acclimation may have resulted in lack of stability of power densities (Wang et al., 2009). Nonetheless, addition of exogenous *E. cloacae* in setups reduced both the magnitude and frequency of fluctuations of electricity with a bimodal distribution of power densities in most cases. Presumably, *E. cloacae* may have contributed with a supplementary metabolic pathway noted by an increase in pH as well as an accelerated removal of certain substrates such as glucose, thus shifting the dynamics in MFCs. Further investigations will look at the nature of such metabolites and their possible involvement as additional electron shuttles in the medium. At present, *E. cloacae* is capable of degrading cellulose-rich materials with a direct transfer of electrons to a carbon electrode without a requirement for exogenous mediators (Rezaei et al., 2009). This is in line with the power generated exclusively with *E. cloacae* observed on setups fed with sterile cellulose-rich paper mill effluent in this study.

There was a break down of pollutants and reduced sludge when *E. cloacae* degraded paper mill effluent together with inherent waste microbes (up to 98.8% settable solids removal efficiency); however, the drop in glucose removal (44.1%) may be an indication of an absence of synergism with the exogenous culture. Two possible mechanisms may explain this lack of microbial cooperation. First, microorganisms may have decreased the degradation of glucose and resorted to using excess electrons which are ordinarily captured by the anode. Alternatively, *E. cloacae* may be consuming or inhibiting metabolites which can be viewed as precursors toward power generation by mixed microbial consortia in the effluent (Trzcinski et al., 2010).

It could have been expected to have the setup of sterile effluent acted on by *E. cloacae* to yield the highest maximum power density due to its highest coulombic efficiency (0.0004%) coupled to a low SDR and a relatively high power yield. Surprisingly, setups with unamended effluent generated the highest power possibly due to the lowest SDR and the increase of glucose (up to 47.7%) in the medium; whereas it was being decreased in all other setups. The slow degradation of organics together with the glucose build-up may have amounted to a long-term energy store for microorganisms toward the high electricity generated. However, the highest power yield in sterile waste metabolised by *E. cloacae* holds an indication on the adaptation of the microbial culture and the continuation of electricity generation above power densities of both untreated waste alone and those of untreated waste with *E. cloacae* (Mohan et al., 2008).

The coulombic efficiency has long been an important gauge for comparison amongst power generation studies. However, additional factors may need to be considered such as waste composition, including both biotic and abiotic factors. For example, initially high tCOD content can induce bacteriostatic and bacteriocidal effects on certain microbial populations, resulting in reduced coulombic efficiencies (Min et al., 2005; Jong et al., 2006), hence the SDR was also considered. Interestingly, the coulombic efficiencies and power yield may be low in this study, but electricity generated can be significant when taking into consideration the projection of the total effluent discharge volume produced daily by a processing mill over a year, assuming that the scale-up generates electricity within the same proportion as in a pilot study (Wen et al., 2010). The lack of defined bands in the DGGE may be an indication of consortia of closely-related species which could not be properly separated on polyacrylamide (Trzcinski et al., 2010). This observation was also supported by a phylogenetic analysis revealing a large number of strains (102) matching the paper mill effluent anodophilic isolate at the same level of identity (based on 16S rRNA partial sequence) which belonged to facultative and gram-positive lactic acid bacteria from *Leuconostoc mesenteroides* subsp. *mesenteroides.* The very little DNA to be amplified owing to little microbial colonization of electrodes resulted in a low intensity of bands in the DGGE (Riemann et al., 1999). In addition, *Leuconostoc* strains are commonly found and extensively used in dairy products and plant materials (Hemme and Foucaud-Scheunemann, 2004; McSweeney and Sousa, 2000). Therefore, the predominance of sparse microbial growth on electrodes in a medium is not of preference such as paper mill effluent. This exploratory study has established that full-strength paper mill effluent can be metabolised and serve as both carbon and energy sources to endogenous microbes with a detectable electricity generation in fuel cells.

ACKNOWLEDGMENTS

The authors thank the University of the Witwatersrand

(South Africa) and Carnegie Foundation for the funding and Prof. J. Limson (Rhodes University) for the donation of research consumables to commence the project.

REFERENCES

Ahn Y, Logan BE (2010). Effectiveness of domestic wastewater treatment using microbial fuel cells at ambient and mesophilic temperatures. Bioresour. Technol 101: 469-475.

Ali M, Sreekrishnan TR (2001). Aquatic toxicity from pulp and paper mill effluents: A review. Adv. Environ. Res. 5: 175-196.

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. 50: 10-15.

Azbar N, Dokgoz FTC, Keskin T, Korkmaz KS, Syed HM (2009). Continuous fermentative hydrogen production from cheese whey wastewater under thermophilic anaerobic conditions. Int. J. Hydrogen Energ. 34: 7441-7447.

Basu N, Ta CA, Waye A, Mao J, Herwitt M, Arnason JT, Trudeau VL (2009). Pulp and paper mill effluents contain neuroactive substances that potentially disrupt neuroendocrine control of fish reproduction. Environ. Sci. Technol. 43: 1635-1641.

Cha J, Choi S, Yu H, Kim H, Kim C (2010). Directly applicable microbial fuel cells in aeration tank for wastewater treatment. Bioelectrochemistry, 78: 72-79.

Hakeem AS, Bhatnagar S (2010). Heavy metal reduction of pulp and paper mill effluent by indigenous microbes. Asian J. Exp. Biol. Sci. 1: 201-203.

Hemme D, Foucaud-Scheunemann C (2004). *Leuconostoc*, characteristics, use in dairy technology and prospects in functional foods. Int. Dairy J. 14: 467-494.

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

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

Huang L, Logan BE (2008). Electricity generation and treatment of paper recycling wastewater using a microbial fuel cell. Appl. Microbiol. Biotechnol. 80: 349-355.

Jong BC, Kim BH, Chang IS, Liew PWY, Choo YF, Kang AGS (2006). Enrichment, performance, and microbial diversity of a thermophilic mediatorless microbial fuel cell. Environ. Sci. Technol. 40: 6449-6454.

Kaksonen AH, Vanhanen MLR, Puhakka JA (2003). Optimization of metal sulphide precipitation in fluidized-bed treatment of acidic wastewater. Water Res. 37: 255-266

Kassongo J, Togo CA (2010). The potential of whey in driving microbial fuel cells: a dual prospect of energy recovery and remediation. Afr. J. Biotechnol. 9: 7885-7890.

Lee J, Phung NT, Chang IS, Kim BH, Sung HC (2003). Use of acetate for enrichment of electrochemically active microorganisms and their 16S rDNA analyses. FEMS Microbiol. Lett. 223: 185-191.

Mathuriya AS, Sharma VN (2009). Bioelectricity production from various wastewaters through microbial fuel cell technology. J. Biochem. Tech. 2: 133-137.

McSweeney PLH, Sousa MJ (2000). Biochemical pathways for the production of flavour compounds in cheeses during ripening: a review. Lait. 80: 293-324.

Min B, Kim JR, Oh SE, Regan JM, Logan BE (2005). Electricity generation from swine wastewater using microbial fuel cell. Water Res. 39: 4961-4968.

Mohan SV, Saravanan R, Raghavulu SV, Mohanakrishna G, Sarma PN (2008). Bioelectricity production from wastewater treatment in dual chambered microbial fuel cell (MFC) using selectively enriched mixed microflora: effect of catholyte. Bioresour. Technol. 99: 596-603.

Muyzer G, Waal ECD, Uitterlinden AG (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59: 695-700.

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: 3590-3596.

Nimje VR, Chen CY, Chen CC, Jean JS (2009). Stable and high energy generation by a strain of *Bacillus subtilis* in a microbial fuel cell. J. Power Sources, 190: 258-263.

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

Rezaei F, Xing D, Wagner R, Regan JM, Richard TL, Logan BE (2009). Simultaneous cellulose degradation and electricity production by *Enterobacter cloacae* in a microbial fuel cell. Appl. Environ. Microbiol. 75: 3673-3678.

Riemann L, Steward GF, Fandino LB, Campbell L, Landry MR, Azam F (1999). Bacterial community composition during two consecutive NE Monsoon periods in the Arabian Sea studied by denaturing gradient gel electrophoresis (DGGE) of rRNA genes. Deep-Sea Res. Pt. II 46: 1791-1811.

Thompson G, Swain J, Kay M, Forster CF (2001). The treatment of pulp and paper mill effluent: a review. Bioresour. Technol. 77: 275-286.

Trzcinski AP, Ray MJ, Stuckey DC (2010). Performance of a three-stage membrane bioprocess treating the organic fraction of municipal solid waste and evolution of its archaeal and bacterial ecology. Bioresour. Technol. 101: 1652-1661.

Wang X, Feng Y, Wang H, Qu Y, Yu Y, Ren N, Li N, Wang L, Lee H, Logan BE (2009). Bioaugmentation for electricity generation from corn stover biomass using microbial fuel cells. Environ. Sci. Technol. 43: 6088–6093.

Wen Q, Wu Y, Sun Q (2010). Production of electricity from the treatment of continous brewery wastewater using a microbial fuel cell. Fuel, 89: 1381-1385.