Bioresource Technology 102 (2011) 2758–2766

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Microbial fuel cells meet with external resistance

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ARTICLE INFO

Article history:
Received 13 May 2010
Received in revised form 10 October 2010
Accepted 11 October 2010
Available online 20 November 2010

Keywords:
Microbial fuel cell
External resistance
Biomass yield
Microbial community composition
Anode potentials

ABSTRACT

The influence of external load on the composition of the anodic biofilm microbial community and biomass yield was investigated in a microbial fuel cell fed with glucose and domestic wastewater was used as source of electrogens. Denaturing gradient gel electrophoresis (DGGE) of polymerase chain reaction (PCR) amplified 16S rRNA gene fragments revealed distinct differences in anodic bacterial communities formed at the anode of each MFC operated under a different external load. These results implied that in an MFC, electrogenic bacteria were enriched under higher current densities, i.e., low external load, and were able to sustain better current and effluent quality. The influence of the external resistance applied to the MFCs during formation of the bacterial communities from sewage wastewater was shown to have no significant effect on power performance of the MFCs nor to have a significant influence on their anodic activity with both glucose and brewery wastewater as fuel. As expected, current generation, COD removal and the biomass yield were all directly influenced by the external load. Significantly, when operated under lower external load, the biomass yield in the MFC was less than that in conventional anaerobic digestion (i.e., control).

1. Introduction

Microbial fuel cell (MFC) technology is an emerging research field, in which electrons derived from the metabolism of biodegradable organic matter are converted to electricity (Logan et al., 2006). Barriers to the application of the technology include the use of expensive components (i.e., platinised cathode and proton exchange membrane) and low power densities, caused by poor electron transfer from the bacteria to the anode (Schröder, 2007; Torres et al., 2010). In recent years electricity generation using MFCs has received much attention as a potential source of renewable energy (Logan et al., 2006; Rozendal et al., 2008). In addition to generating electricity, the process can also treat wastewaters. However, in order for this technology to be a viable source of power or wastewater treatment method, further improvements in MFC performance are needed. Most studies have focused on how different MFC reactor configurations, substrates, operating parameters and different types of electrodes affect power generation. A number of potential rate limiting factors have been described and their influence on MFC performance, e.g., rates of substrate oxidation and electron transfer from bacteria to the anode, proton transport through the proton exchange membrane (PEM), oxygen availability and reduction at cathode, have been documented (Jang et al., 2004; Rabaey et al., 2004).

In contrast to a classical fuel cell, MFC electrocatalysis takes place through bacterial metabolism. The effect of external resistance on MFC behaviour has been addressed, in a number of studies primarily focused on the relationship between external resistance, current and Coulombic yield (Gil et al., 2003; Jang et al., 2004; Liu et al., 2006). Liu et al. (2006) observed higher COD removal with the MFC operated under some external resistance when compared to open circuit systems. Menicucci et al. (2006) developed a procedure for selecting an optimal external resistance for maximum sustainable power. In their study, they considered the anode potential produced under different external resistances to determine conditions for the maximum sustainable power. Aelterman et al. (2008) studied the effect of different three-dimensional electrodes on electricity generation, electrochemical and microbial community structure of microbial fuel cells in relation to the applied loading rate and the external resistance. However, the effect of the external resistance on COD removal linked with changes in microbial community composition and influence on biomass growth have not been investigated to date.

Very recently Lyon et al. (2010) have reported the effect of external resistance on the performance of a MFC filled with primary clarifier effluent and fed with acetate as fuel. They found that differences in the external resistance were associated with changes in the bacterial community structure formed on the anode.

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doi:10.1016/j.biortech.2010.10.147

However similar power production was observed regardless of community structure.

Current numerical models of MFCs predict that increased external resistance results in (i) higher biomass growth and (ii) a lowering of current generated at the anode (Picioreanu et al., 2007, 2008). It has also been suggested that increased electrical resistance favours methanogenic microbial growth as opposed to electroactive microbial growth (Picioreanu et al., 2008). In this study we have conducted a series of experiments in batch mode to evaluate the model predictions. We have therefore assessed the influence of external resistance on MFC performance, anodic microbial community composition, especially during the initial phase of anodic biofilm development, and factors of practical significance in wastewater treatment such as COD removal and biomass yield. These data were then compared with conventional anaerobic digestion and MFC operated at open circuit voltage (OCV), equivalent to an infinite external resistance.

2. Experimental

2.1. Feed

The MFC feed solution, which consisted of minimal salts medium with glucose as the electron donor, was prepared by dissolving 500 mg/L glucose and inorganic salts [NH_4–N (NH_4Cl) – 40 mg/L; Mg ($MgCl_2$) – 10 mg/L; Cu ($CuSO_4$) – 0.1 mg/L; Ca ($CaCl_2$) – 5 mg/L; Mn ($MnSO_4$) – 0.1 g/L and Zn ($ZnCl_2$) – 0.1 g/L] in 950 mL of phosphate buffer (0.25 M, pH 7.0). Preceding experimental measurements, 50 mL of biomass inoculum was added, followed by vigorous purging with nitrogen gas for 30 min, at a rate of 40 mL/min, to create anaerobic conditions and uniform microbial distribution. The fuel and inoculum had a combined chemical oxygen demand (COD) of 550 mg/L and a biomass concentration of 56 mg/L as volatile suspended solids (VSS) and 7.9×10^8 cells/mL of bacteria.

Brewery wastewater diluted with domestic wastewater (1:100 by volume) was used as feed in the anodic chamber (anolyte) for some of the experiments in this study. Domestic wastewater was collected from the primary clarifier overflow at a local municipal sewage treatment works (Northumbrian Water, Newcastle upon Tyne, UK) and brewery wastewater was provided by the Federation Brewery (Newcastle upon Tyne, UK). For the MFC tests, brewery wastewater was added to the domestic wastewater followed by vigorous nitrogen gas purging for 15 min, at a rate of 55 mL/min to create anoxic conditions and a uniform microbial distribution. The feed prepared in this way had a soluble chemical oxygen demand (COD) of 700 mg/L.

2.2. Inoculum preparation

Sewage wastewater, collected from the primary clarifier overflow at a local municipal sewage treatment works (Northumbrian Water, Newcastle upon Tyne, UK), was used an inoculum. The biomass was collected by centrifugation (10,000 *g*, 10 min) from sewage wastewater and washed twice with sterile saline solution (0.9% NaCl solution) to remove organic compounds adhered to the microbial cells. The biomass was then re-suspended in 50 mL of sterile phosphate buffer (0.25 M, pH 7.0) and mixed with the anolyte feed medium (inoculum/anolyte ratio, 1:20 [v/v]) to initiate the experiments.

2.3. MFC configuration and operation

Experiments were conducted in a two-chambered fuel cell (150 mL capacity chambers made of borosilicate glass). A 6 cm^2 Nafion 117 proton exchange membrane (PEM) (Sigma, UK), was used to separate the anodic and cathodic chambers. The top of the anode chamber was equipped with sample ports for liquids and gases and for an electrical connection to the anode, which was suspended in the anolyte. The anode consisted of a graphite plate (projected area 12 cm^2), which was sterilised by boiling in 0.1 M H_2SO_4 for 1 h, and washed with distilled water, followed by boiling in distilled water (30 min). A 20 cm^2 (projected area) platinised titanium mesh with 0.30 mg of Pt/cm^2 was used as a cathode. Electrical contacts to the electrodes were made with titanium wire. About 125 mL of feed solution was added to the anodic chamber followed by purging with oxygen free nitrogen gas for 15 min to maintain an anaerobic environment in the reactor. The cathode chamber contained 125 mL of oxygen saturated potassium phosphate buffer (0.25 M, pH 7.0) containing 100 mM potassium ferricyanide.

Duplicate MFCs were operated with different external resistances (0.1 kΩ, 1 kΩ, 10 kΩ, 25 kΩ and 50 kΩ) between the anode and cathode. Additional cells were operated under open circuit conditions, i.e., bioreactors with the same construction as the MFCs except anode and cathode were not connected to an electrical circuit. Conventional anaerobic biofilm reactors (closed bottle with dummy anode) were used as controls to compare organic removal efficiency and microbial community composition with MFCs. All the reactors were monitored for 7 days and samples (3 mL) were withdrawn daily under a stream of oxygen free nitrogen, filtered through 0.2 μm filter membranes (Polyvinylidene fluoride, PVDF, VWR, UK) and analysed.

2.4. Analysis

2.4.1. Electrochemical measurements

The change in fuel cell voltage under different external resistance was recorded hourly using a data acquisition system (ADC 16, Pico Technology Ltd., UK) connected to a personal computer via a BS 232 Pico high resolution analog cable. Energy (mWh) was calculated by integrating power over time. The anode and cathode potentials were monitored using a Ag/AgCl (3 M NaCl, 0.209 V vs. NHE) reference electrode placed in the catholyte solution.

Cell polarizations were obtained by connecting each cell to different external resistances and measuring the voltage. The external resistance was then decreased and voltage measured again after stabilization. From the corresponding voltage values, current densities and power densities were determined using Ohms law. The Coulombic efficiency (%) was calculated according to Logan et al. (2006).

2.4.2. Chemical measurements

At the end of the batch experiment, the total biomass concentration of bulk liquid and the biofilm was estimated using the volatile suspended solids (VSS) method (APHA, 1998) by taking uniformly mixed samples. The attached biofilm around the anode was extracted into a separate container and a sub-sample of 250 μL of the biomass suspension was preserved for DGGE analysis. The remaining extracted anodic biomass was returned to the corresponding reactor anodic solution (i.e., anolyte) to determine the total bacterial biomass.

2.4.3. Microbial measurements

2.4.3.1. Bacterial samples. For microbial community analysis at the end of the experiment, the anodes with attached biofilms were transferred to sterile containers with 5 mL of sterile saline phosphate buffer (3.2 mM Na_2HPO_4, 0.5 mM KH_2PO_4, 1.3 mM KCl, 135 mM NaCl, pH 7.4) and glass beads. The entire biofilm from the anode was suspended in the buffer by shaking followed by sonication (five periods of 30 s separated by 2 min of cooling in order to disturb biofilm structure; 120 W, 40 kHz, Model FS 200b

Deacon, UK). A sub-sample (250 μL) of the biomass suspension was preserved by addition of an equal volume of absolute ethanol (sample/ethanol ratio, 50:50 [v/v]) and stored at −20 °C for microbial community analysis. The remaining biomass suspension from the anode was returned to the corresponding reactor to determine the total bacterial biomass. Samples of the inoculum were also treated with ethanol (1:1 volumetric ratio) for subsequent microbial community analysis.

2.4.3.2. Total cell counts. Bacterial cells (in the inoculum, anolyte and anode biofilms) were fixed with ethanol at a 1:1 volumetric ratio and were stained with SYBR gold (20× diluted) for 30 min at room temperature. Samples were filtered onto 0.2 μm pore-size polycarbonate filters (25 mm diameter; Costar Scientific Corporation, Bucks, UK) by applying a slight vacuum. After filtration, filters were mounted in oil and observed immediately by epifluorescence microscopy using an Olympus BX40F4 microscope (Olympus, Japan). Slides were viewed using a 100× oil immersion lens (UPlanFl 100× lens), under a blue light filter (WB) in the dark. Mean bacterial abundance on each slide was calculated from cell counts in 20 fields of view.

2.4.3.3. Analysis of anodic biofilm bacterial community composition.
2.4.3.3.1. DNA extraction and PCR amplification of 16S rRNA genes. Total DNA was extracted from 250 μL of the ethanol fixed samples, using a Fast DNA Spin Kit (BIO 101, Q-BioGene, UK). The 16S rRNA gene fragments were amplified from the extracted total DNA using primers 1 and 3 (Muyzer et al., 1993), i.e., forward primer (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3′) and reverse primer (5′-ATT ACC GCG GCT GCT GG-3′). Polymerase chain reaction (PCR) amplification was performed in a total reaction volume of 50 μL; 47 μL of PCR mega mix blue, i.e., PCR reaction mix with loading buffer, supplied by BIO 101, Q-BioGene, UK, 1 μL of primer 1, 1 μL of reverse primer 3 and 1 μL of DNA extract. PCR amplification was performed in an automated thermal cycle (Hybaid, Omn-E) with an initial denaturation (95 °C for 3 min) followed, for the first-round amplification, by 24 cycles of denaturation (95 °C for 1 min), annealing for 1 min (starting at 65 °C and decreased by 1 °C every second cycle) and extension (72 °C for 1 min) and a single final extension (72 °C for 10 min).

PCR products were analysed by electrophoresis on a 1% (w/v) agarose gel (NuSieve; FMC Bioproducts) in 1× TAE buffer (40 mM Tris acetate, 1 mM EDTA, pH 8.0, Eppendorf Scientific Inc., New York, USA) for 50 min at 100 V and gel images were recorded using a FluorS gel documentation System (Bio-Rad) after staining with ethidium bromide.

2.4.3.3.2. Denaturing gradient gel electrophoresis (DGGE). Community profiles of the anode biofilms from MFCs operated under different external resistances and at open circuit, and of controls and the inoculum, were obtained by DGGE analysis. Polymerase chain reaction (PCR) amplified 16S rRNA gene fragments (11 μL) were mixed in equal volumes with loading buffer (0.25% (w/v) bromophenol blue, 0.25% (w/v) xylene cyanol FF, 15% (w/v) Ficoll in water) and run on a polyacrylamide gel (acrylamide-*N*,*N*′-methylenebisacrylamide ratio, 37.5:1) in 1× TAE buffer (40 mM Tris acetate, 1 mM EDTA, pH 8.0) containing a chemical gradient of urea and formamide, equivalent to 30–55% denaturant (100% denaturant is 7 M urea and 40% v/v formamide). Electrophoresis was performed using the D-Gene system (Bio-Rad, Hercules, CA, USA) at 200 V constant voltages at 60 °C, for 4.5 h. The separated DNA was stained for 30 min with SYBR green I (Sigma, Poole, UK; diluted 1/20 in 1× TAE). Stained gels were viewed and documented using a Fluor-S MultiImager (Bio-Rad, Hercules, CA, USA).

2.4.3.3.3. Numerical and statistical analysis of DGGE profiles. Scanned DGGE gels were processed using the Bionumerics software (version 3.5, Applied Maths, USA) to analyse the intensity and position of all bands within a single lane in relation to the position and intensity of the bands in all other lanes (Verseveld and Roling, 2008). To correct for variations across the gel, a marker was run on either side of the samples. Further, the Dice index of similarity was used to evaluate the similarity within and between replicate reactors after the similarity values had been checked for normality using an Anderson–Darling test and Box–Cox analysis (Minitab, PA). Dice similarity matrices were converted to a dendrogram using the unweighted pair group method with an arithmetic average (UPGMA) clustering algorithm using the Bionumerics software.

3. Results and discussion

3.1. Current generation

Current generation commenced immediately after start up of the reactors and the MFCs produced different current densities depending on the applied external resistance (Fig. 1). The time taken to attain the peak current density decreased with decreasing external resistance (i.e., increased electron transfer rates) and as expected, higher current densities corresponded to higher anode potentials (Table 1). These observations may be due to different electron transfer rates under different external resistances and/or variations in microbial metabolic activities and kinetic differences in substrate utilization (as reported previously Picioreanu et al., 2008).

Coulombic yield was calculated as a function of substrate concentration and external resistance. A maximum Coulombic yield was observed for MFC operated under 0.1 kΩ (6.15 ± 0.018) and decreased to 3.83% (±0.01), 0.81% (±0.005), 0.47% (±0.003) and 0.44% (±0.008) for external resistances of 1 kΩ, 10 kΩ, 25 kΩ and 50 kΩ, respectively. Even at higher current densities, the Coulombic yield was very low, which demonstrated that the majority of the substrate was not utilized for current generation. This behaviour was perhaps due to competition for electron donor between electrogenic bacteria and fermentative and anaerobically respiring organisms for electron donor during the initial period of anode colonisation. These observations agree qualitatively with model results showing that an increased external resistance favours the anaerobic microorganisms (Picioreanu et al., 2007, 2008). This is explainable by the fact that the high resistance leads to less current and decreased electron transfer rate to anode, leading to less energy generation for the electrogenic microorganisms – hence, their

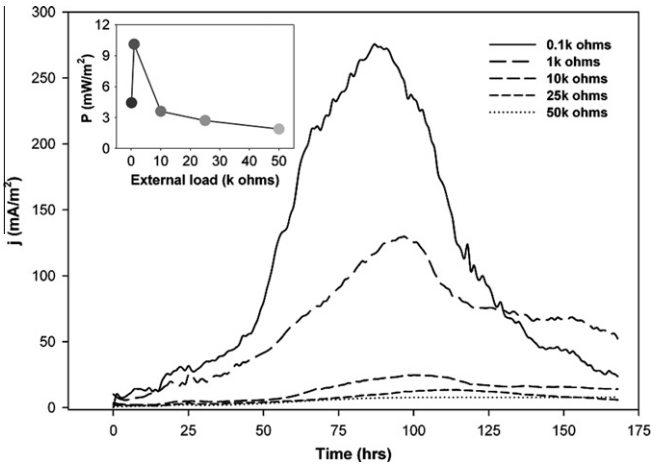

Fig. 1. Differences in current generation with time under different external loads. Inset shows the relationship between peak power generation and external resistance. The graphs show the mean data of duplicate experiments.

Table 1
Maximum current densities achieved for MFCs with different external loads and corresponding time and anode potential. Mean data from duplicate reactors with standard deviation.

External resistance (kΩ)	Peak current density (mA/m^2)	Time to achieve peak current density (h)	Peak anode potential (mV vs. NHE)
0.1	273.6 ± 3.1	87 ± 2	398 ± 5
1	129.7 ± 0.4	94 ± 4	301 ± 15
10	24.5 ± 0.3	101 ± 1	288 ± 7
25	13.4 ± 0.1	113 ± 1	236 ± 2
50	7.8 ± 0.2	167 ± 2	150 ± 13
Open circuit voltage			−142 ± 23

reduced growth rate. A functionally stable anode biofilm harbouring electrogenic microorganisms was required for maximum current/power generation. In other studies (Ren et al., 2011; Sirisha and Katuri, unpublished data), it was observed that functionally stable biofilms develop on anodes when the biofilm was grown for several weeks under constant polarization. The above mentioned studies, found a positive relationship between anodic biomass concentration, Coulombic efficiency, current density and peak power; however, in this study, the experiment was terminated after about 7 days and the cell was fed a single batch of substrate, in order to determine the biomass yields and anodic microbial community developed during the initial phase of anode colonisation under different current generation conditions (i.e., at different external resistances). This probably contributed to the low Coulombic efficiencies obtained in these MFC.

In addition, the ratio of anode area to anolyte volume in the MFC used in this experiment was low for ease of recovery of the anode biofilm for biomass growth and microbial community analysis. Higher currents would be expected with higher anode:anolyte ratios when used in any practical device (Kim et al., 2002; Oh and Logan, 2006). Interestingly, there was lack of correlation between COD removal and CO_2, CH_4 and H_2. This was most probably due to difficulties associated with accurate measurement of the head space gases. Furthermore, COD removal did not correlate with Coulombic yield. This could be a consequence of different microbial communities developing on the anode of the MFCs compared to the control reactor, dominated by non-electrogenic microorganisms.

The inset of Fig. 1 shows the highest power outputs obtained from individual MFCs operated under different external resistance. With an increase in external resistance from 0.1 to 1 kΩ, the power density increased correspondingly, reaching a maximum power density of 10.1 mW/m^2 at 1 kΩ. A fall in power generation was observed at external resistance values from 10 to 50 kΩ. Based on this observation, it was tentatively suggested that the internal resistance of the MFC used in the present study was around 1 kΩ (at that particular operating condition applied). An internal resistance of 1 kΩ is considered to be very high in fuel cell research and this can be an important factor in MFC performance as the higher the internal resistance the lower the current density.

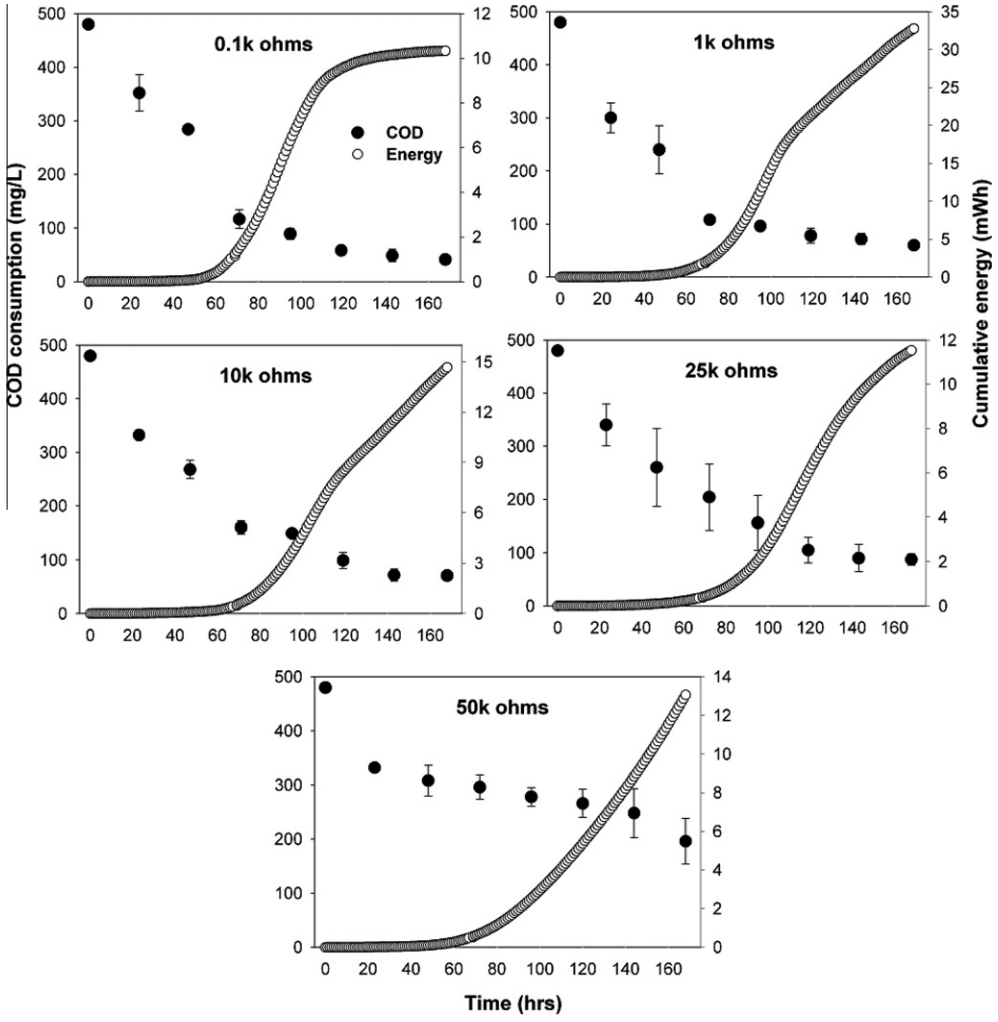

Fig. 2. Cumulative energy generation coupled to COD removal with time under different external resistances. Mean data from duplicate reactors with error bars (±SD).

A relationship between COD consumption and energy generation is evident (Fig. 2). Although COD consumption began immediately, there was a lag of 60–80 h before substantial electrical energy generation occurred (Fig. 2). It seems that in the first 60–80 h a greater portion of reducing equivalents from the oxidation of the glucose was directed towards biosynthesis and growth (mostly growth of anaerobic/fermentative organisms) with less being used for electrical energy generation. Following the lag period, the energy increased exponentially and subsequently entered a plateau as the biodegradable portion of the COD was consumed. The cumulative energy generation with time typically followed a characteristic sigmoid curve with three distinctive phases only for the fuel cell operated at 0.1 kΩ (suggesting that availability of organic substrate was a limiting factor) or linear increase phase for the other resistor values.

Energy generation increased with an increase in external load from 0.1 kΩ to 1 kΩ, and fall in peak energy generation observed by further increase in load as a result of the MFC internal resistance. In the case of 50 kΩ, the energy generation did not follow the sigmoid shape, but increased exponentially, perhaps because of the availability of sufficient residual COD in the feed due to slow rate of substrate utilization by electrogens. This trend was confirmed by the lower observed COD removal using a 50 kΩ external resistance compared to COD removal at lower resistor values <25 kΩ (COD removal of ca. 59.1% vs. ca. 85–92%).

3.2. COD removal

COD removal was observed to be high in the case of MFC operated with 0.1 kΩ (i.e., high current). The extent of COD removal was decreased by only around 6% with external resistance values of 10 k and lower. However, the difference between them (i.e., 0.1–10 kΩ) were found to be statistically insignificant ($P > 0.05$; t-test). MFCs operated with high external resistance (i.e., 50 kΩ) and at open circuit (585 ± 52 mV) had much lower COD removal (Fig. 2 and S1 of supporting information). Nevertheless COD removal in all MFCs was greater than in control reactors which were not operated in MFC configuration. This trend may have two explanations: (i) there was a lower anode potential at higher external resistances, which may alter the metabolic activities of the anodic microbial community, as shown by modeling in Picioreanu et al. (2007), and (ii) the presence of different microbial species (including fermentative species) could have provided different mechanisms for efficient utilization of organic matter. The reality is probably a balance of the both effects. Overall, the data suggest that operating MFCs at higher anode potentials (i.e., low external resistance) may lead to enhanced wastewater treatment since drawing a greater current from the MFC accelerates the COD removal. However, increased treatment efficiency was achieved at the expense of power generation.

The differences in COD removal observed between MFC operated at open circuit, control and for those operated under external resistance were statistically significant ($P < 0.05$; t-test). This suggests that when MFCs were operated under external resistance the effluent quality will increase as the current flows in MFCs and substantial amounts of organic matter are consumed by electro-active bacteria to drive the process. The measured anode potentials for MFCs operated at OCV were low (Table 1). The anode potential at open circuit conditions was −142 ± 23 mV which is modest when compared with a value of −420 mV for glucose oxidation to CO_2, even allowing for slight differences due to lower glucose concentrations and proton concentration. However the COD removal at open circuit was greater than that of the control. In theory, at open circuit, organic carbon removal is not assisted by current generation and it seems likely that the imposition of a potential at the anode and oxidative metabolism due to oxygen penetration through PEM may cause an increase in COD removal rate (Kim et al., 2007). There was no detectable current flowing at open circuit conditions.

Wastewater treatment using an MFC apparently favours organic removal at a higher rate than typically observed in conventional anaerobic systems and this tendency was enhanced when the MFC was operated with a lower external resistance (Fig. S1). Moreover, existing conventional anaerobic treatment technology is considered ineffective for the treatment of low strength wastewaters (COD < 1 g/L). However, based on the results reported, MFC technology may be an effective process for the anaerobic treatment of dilute wastewaters which are usually treated aerobically with large amounts of sludge production and high energy expenditures. This points to a new strategy in the application of MFC, rather than optimizing for power generation, if one were to consider MFC from the perspective of COD removal in wastewater treatment systems, one can envisage the benefits of optimizing an MFC-based anaerobic treatment system optimized to operate at maximum current and low or zero voltage for effective COD removal with reduced biomass generation.

3.3. Biomass growth

The biomass yield from substrate and the total bacterial concentration in the MFC (suspended and attached) were both functions of external resistance (Fig. 3). During MFC operation, the bacterial mass and cell counts increased in all cases compared to the initial inoculum (56 mg/L as VSS and 7.9×10^8 cell/mL); however, the yields were different for the reactors operated at various resistances. This is analogous to the observations of Mclean

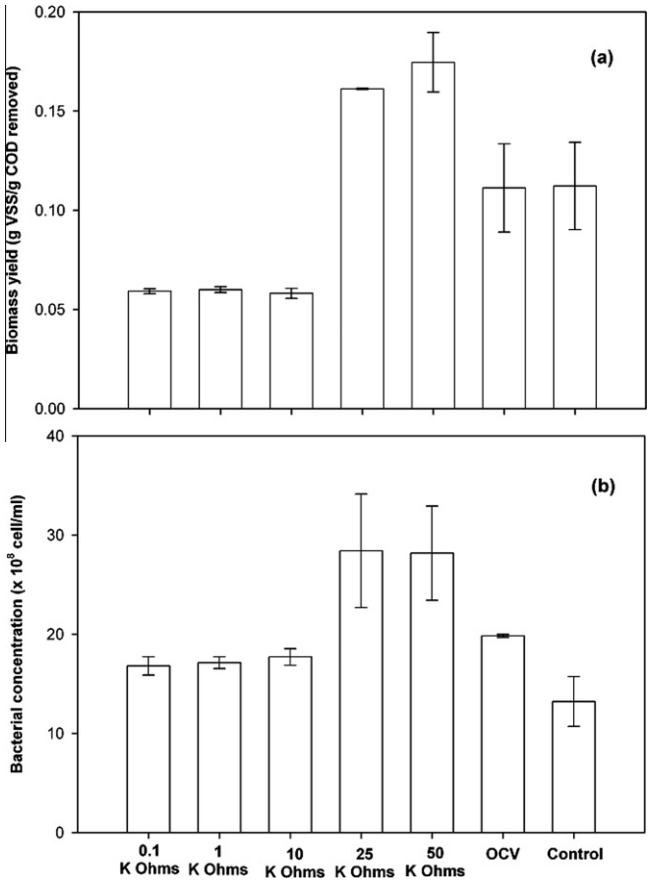

Fig. 3. Total biomass yield under MFC operating with different external resistance, at OCV and in control reactors containing dummy anodes (a) and biomass in terms of cell count (b). Mean data from duplicate reactors with error bars (±SD).

et al. (2010) for growth of *Shewanella oneidensis* MR-1 on graphite plate anodes, where more growth was observed at 1 MΩ external resistance compared to 100 Ω. The biomass yield at low external resistances (<10 kΩ) or higher current densities was lower than in the control reactor (Fig. 3a), while the COD removal was the highest (Fig. S1). In addition, the cell yields were comparable with those expected on the basis of COD consumed in the anaerobic processes from monosaccharides (ranging from 0.05 to 0.15 g VSS biomass/g COD substrate, calculated according to Batstone et al. (2002), at 35–37 °C and using a conversion factor of 1.5 g COD biomass/g VSS biomass). Anaerobic processes generally yield less biomass than aerobic processes (Speece, 2002). Results suggest that a further decrease in biomass yield can be achieved in MFCs operated under an external load. Low sludge yield was reported to be advantageous in the water industry as it accounts for around 25–65% of total plant operating costs (Liu and Tay, 2001).

A possible explanation for lower biomass yield at low external resistance (below 10 kΩ) in MFC compared to the control reactor with a dummy anode (Fig. 3a) is that electrogenic and non-electrogenic processes are competing in mixed culture MFCs and at high current, a greater proportion of the COD is consumed by the electro-active bacteria presumably due to imposition of higher anode potentials. In the case of the MFCs operated at 25 kΩ and 50 kΩ, the biomass yields were higher compared to those bioreactors operated in closed bottles (i.e., controls). The lower biomass yield in control reactors was predictable as this system was essentially anaerobic, but in the case of the MFCs operated at 25 kΩ and 50 kΩ, there may also be some influence of greater oxygen content in the anolyte, as it was operating under less anaerobic conditions due to connection to the oxic cathode chamber.

3.4. Microbial community composition

To understand the relationship between the external resistance applied and the bacterial community composition, and to ascertain consistency in microbial community development DGGE analysis of 16S rRNA gene fragments from the anode community was conducted (Fig. 4). For the purposes of this analysis each DGGE band was considered to represent a single species. The relative intensity of each DGGE band and the sum of all intensities for all bands in a sample were used to estimate relative species abundance. Differences in the DGGE profiles suggested that distinct microbial communities developed at different current densities and that community composition was also affected by the mode of reactor operation (MFC vs. anaerobic digestion; Fig. 4). The bacterial community analysis indicates that the number of detectable bands (i.e., species richness) fell from 27 to 15 as the current density increased from 8 to 272 mA/m^2 at 50–0.1 kΩ respectively. The anodic biofilm developed at 0.1 kΩ showed the simplest DGGE band pattern and was markedly different to that of the feed community. Only a few bands were common between samples (inoculum and 0.1 kΩ). Distinct communities were selected at different external resistance which may represent selection of exoelectrogens at higher current density (i.e., at higher anode potential).

An Anderson–Darling test found that Dice similarities between reactors operated under different conditions were not normally distributed ($P = 0.005$) whilst the similarities between duplicates approximated more closely to a normal distribution ($P = 0.167$). Similarities of all possible pairs of gel tracks were calculated using Dice coefficients and a cluster analysis was performed. Clustering of the profiles revealed large differences among the profiles of the MFC biofilm community developed under different external resistances, at OCV and of the controls (Fig. S2). The profiles of the all samples were separated into four major clusters.

(1) The anodic community developed at 0.1 kΩ belonged to a single cluster.
(2) The anodic community developed at OCV had 62% similarity with the inoculum and formed as a single group.
(3) The anodic communities developed at 1 kΩ and 10 kΩ, had about 72% similarity, and the control biofilm communities were distinct from the MFC biofilm communities. The MFC operated at lower external resistance cluster together (e.g., 1 and 10) and those at higher external resistance cluster together (25 and 50).

Overall, MFCs may exploit bacterial communities to simultaneously degrade organic compounds in wastewater and generate electricity. The assembly of these microorganisms to form communities within reactors is however poorly understood. The communities might arise through selection of specific organisms that best adapt to the prevailing conditions in the reactors, exemplifying deterministic selection. In contrast to conventional wastewater treatment plants, little is known regarding the microbial community composition of microbial fuel cell and the role of various factors, such as external resistance, anodic potentials, on selection of the anodic communities. A hypothesis of this work was that the bacterial community structure would change with external resistance (Fig. 4), and that respective consortia would exhibit different metabolic abilities. We have shown that higher current densities (anode potentials) improved the extent of substrate utilization, and thus wastewater treatment (Fig. S1) and also lead to the formation of distinct anode bacterial communities.

3.5. MFC power performance

In this study we were interested in determining if operating the MFC under different conditions of external resistance would influence the power generation from different bacterial communities formed under different external loads. Fig. 5A shows the steady

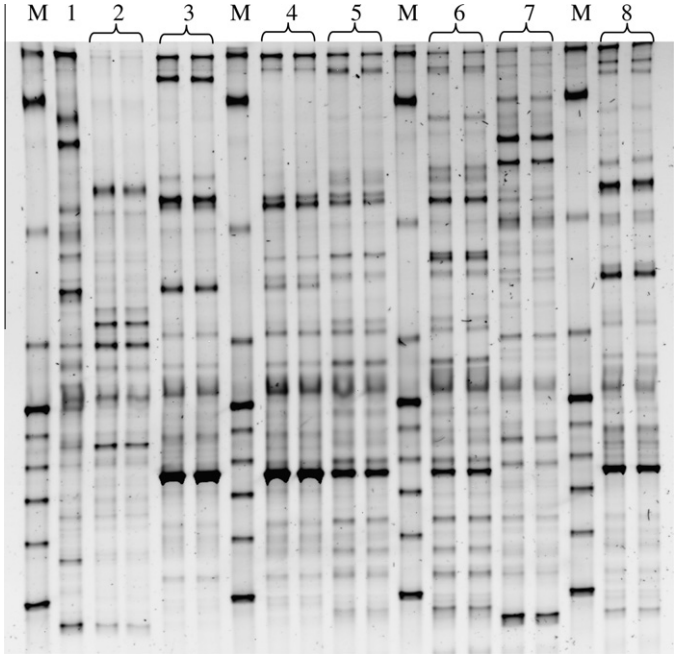

Fig. 4. PCR-DGGE fingerprints for bacterial communities in MFC. Each lane contains PCR-amplified 16S rRNA gene fragments from: (1) feed, anodic community developed at (2) 0.1 kΩ, (3) 1 kΩ, (4) 10 kΩ, (5) 25 kΩ, (6) 50 kΩ, (7) open circuit potential and (8) biofilm formed on the anode in the control reactor. Lanes labeled M contain a reference fingerprint used to correct for differences in fragment migration across the gel.

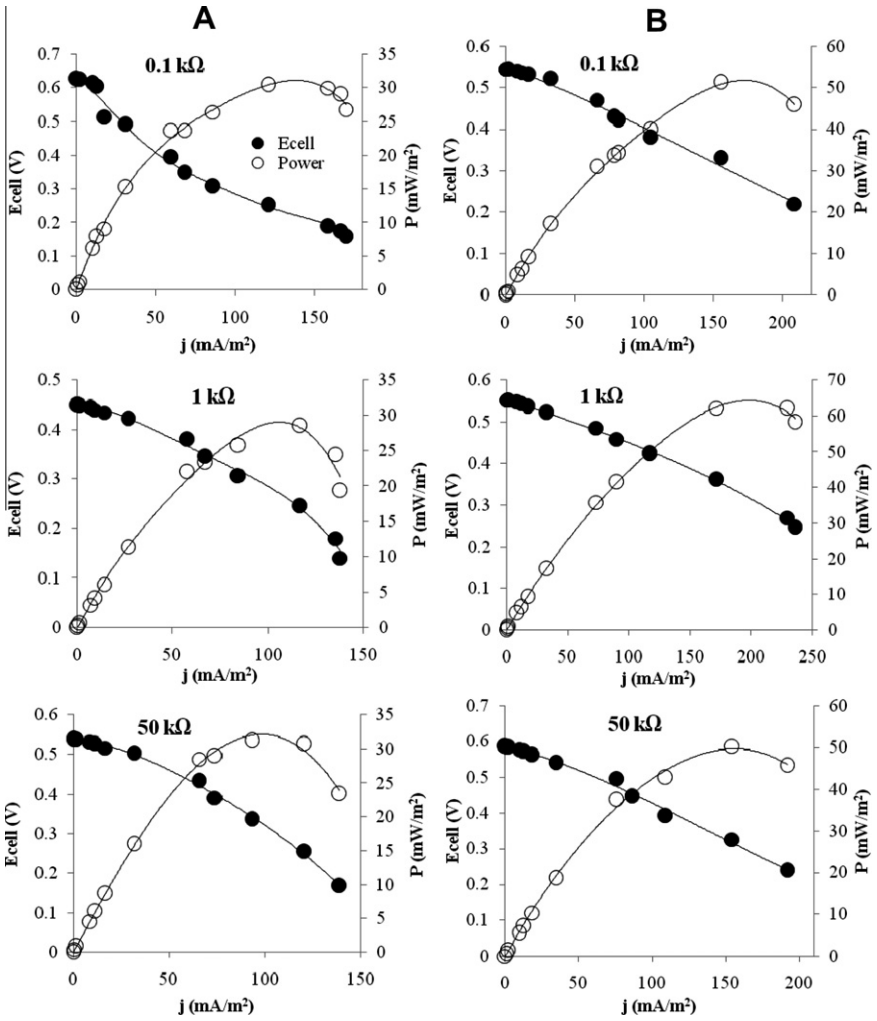

Fig. 5. Cell voltage and power density vs. current density (cell polarization) of MFCs with anode attached biofilms grown under different external resistors. (A) 1st cell polarizations (~100 h age biofilm). (B) 2nd cell polarizations (~180 h age biofilm). These polarizations were performed in separate experiments by forming biofilms under 0.1 kΩ, 1 kΩ and 50 kΩ external resistors at similar operational conditions mentioned above in Sections 2.1–2.3. Prior to polarization, the anodic biofilm was transferred (under nitrogen environment) into new two-chambered MFC replenished with new fuel source with glucose (500 mg/L) but without inoculum, after which the biocatalytic efficacy of the attached biofilms formed over that time period on the anode was assessed by polarization curves. Following this evaluation, anode was removed and placed back in respective original MFC to stimulate further batch growth.

state cell polarization and power density curves obtained from MFCs operated under different external resistances. After initial polarization of the cells, open circuit potentials of ~0.6 V were observed for all cells, eventually yielding maximum current densities of ~150 mA/m^2. There was no significant difference in the peak power produced by the different MFCs (and duplicates), typically peak power densities were 25–30 mW/m^2. Thus, although differences in the microbial communities formed in MFCs operated with different external resistances were identified, it did not appear to impact on the cell power performance.

Subsequently MFCs were left at open circuit conditions followed by further cell polarizations using the same reactors with additional glucose feed added. The cell voltage and power density behaviour obtained from these tests is shown in Fig. 5B. Open circuit potentials were similar to the first set of tests (~0.6 V), although the maximum current densities increased to ~200 mA/m^2 and peak power densities of >50 mW/m^2 were observed Again, no significant difference ($P = 0.36$; t-test) was evident in the behaviour for all MFCs in which bacterial communities were formed under different conditions of external resistance.

Overall it would appear that the formation of different bacterial communities under different external resistances had no significant effect on the power performance of the cells. With the different resistances used, the MFCs were subjected to different ranges of current densities as bacterial communities were developed. With higher resistances the range of current densities was smaller and anode potentials lower, whilst electro-active bacteria activity was lower. This behaviour agrees with the observations of Lyon et al. (2010) in their studies with acetate and carbon cloth anodes. Thus, the indications were that with different fuels and carbon anodes there was no significant influence of the external resistance on the power performance of the MFC inoculated with sewage wastewater. The behaviour reported could indicate that by merely activating the MFC at low anode potentials (and thus current densities) sufficiently active anode reducing communities were produced along with other diverse bacterial communities that can maintain useful power generation regardless of the external resistance. Moreover, because the Coulombic efficiencies are much less than 100% we cannot yet determine which members or the community are electroactive and which are not. We cannot therefore conclude that colonisation of anodes by electro-active bacteria (or communities) was influenced by the external resistance/anode potential, this is the subject of ongoing work.

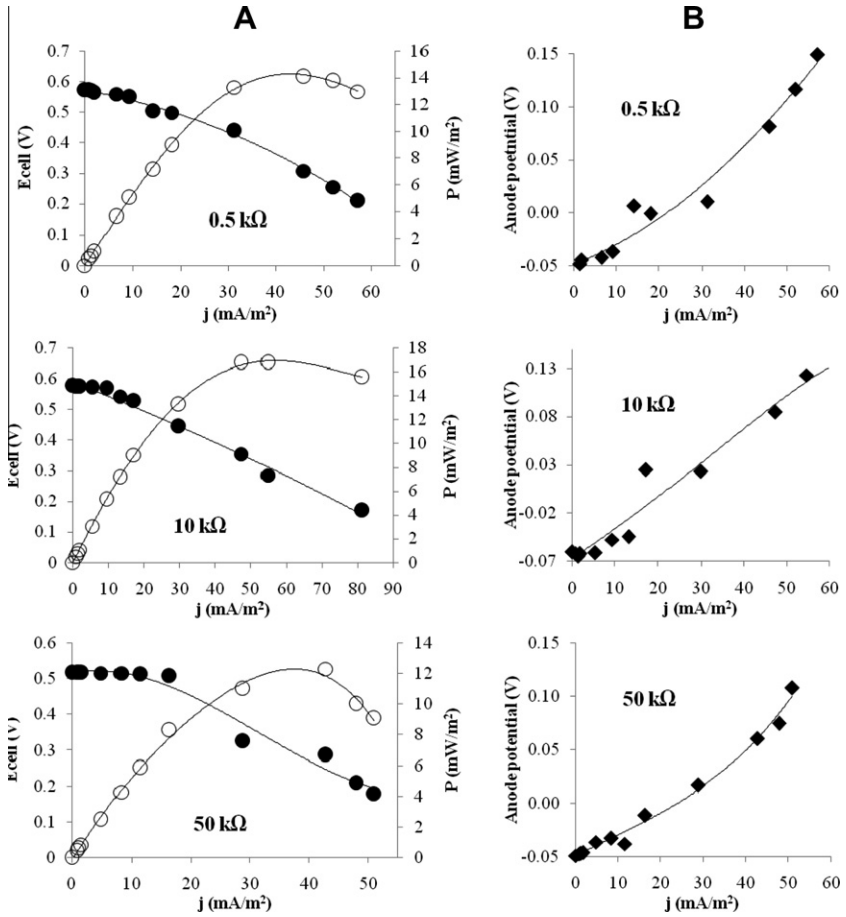

Fig. 6. Cell voltage, power density and anode polarization vs. current density (cell polarization) of MFCs with anode attached biofilms (~100 h age) grown under different external resistors; (A) cell voltage and power density and (B) anode polarizations (vs. NHE). These polarizations were performed in separate experiments by forming biofilms under 0.5 kΩ, 10 kΩ and 50 kΩ external resistors at similar operational conditions mentioned in Section 2.3, except fuel source. Brewery wastewater diluted with sewage wastewater (COD 700 mg/L) was used as feed as well as a source of electro-active bacteria in the anodic chamber (anolyte). Prior to polarization, the MFC was replenished with new fuel (brewery diluted with potassium phosphate buffer (0.25 M, pH 7.0); COD ~ 700 mg/L) but without sewage wastewater, after which the biocatalytic efficacy of the biofilms formed over that time period on the anode was assessed by polarization curves.

In addition to the use of glucose as feed, we have also investigated brewery wastewater in a similar study in which bacterial communities were formed in MFCs operated with different external resistance (DGGE data not shown). Fig. 6A shows the voltage and power density response of the MFCs. The MFCs exhibited similar behaviour starting from OCVs of between 0.55 and 0.6 V and with peak power densities of ~15 mW/m^2. The performance of the MFC was in part limited by the high internal resistance of the two chambers MFC. Using the cell voltage and current data (Fig. 6), and subtracting the anode polarization the combined voltage loss due to the internal resistance and cathode polarization is estimated as 2 kΩ ([280–160 mV]/[50 × 12 × 10^{-4} mA]). Thus conservatively we estimate an internal resistance of around 1 kΩ. The lower power performance was a result of using different feeds, brewery wastewater vs. glucose. Thus again external resistance did not appear to influence the ability of the anode microbial consortia to generate power. Also as shown in Fig. 6B which shows *in situ* measurements of anode potential, the external resistance has no significant influence on the anode community polarization characteristics. This again concurred with the view that exposing MFCs to different anode potentials and current densities did not impact on the ability of the bacterial communities as a whole to generate power, even if as shown different communities were formed. The data generally show that MFCs formed from different mixed communities offer flexibility in operation regarding their ability to provide power.

4. Conclusions

In summary, differences in the external load (resistance) were shown to lead to some significant differences in microbial fuel cell operation. In particular, reduced microbial community composition and lowered biomass yields were observed at greater current densities. The anodic biofilm microbial communities were dissimilar to the feed communities, indicating that there was selection for organisms that colonise anodes under different external loads and at OCV, and of controls. The influence of the external resistance applied to the MFCs during formation of the anodic bacterial communities from sewage wastewater has been shown to have no significant effect on power performance of the MFCs nor to have a significant influence on their anodic activity for both glucose and brewery wastewater.

Acknowledgements

This research was supported by the European Union through Marie Curie Transfer of Knowledge fellowships to Krishna Katuri and Cristian Picioreanu, through Contract No. MTKD-CT-2004-517215.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2010.10.147.

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