Enrichment of Exoelectrogens on Xylose from Anaerobic Digester Sample

M. E. Nissilä**, M. L. K. Sulonen* and J. A. Puhakka*

* Department of Chemistry and Bioengineering, Tampere University of Technology, P.O.Box 541, 33101 Tampere, Finland (E-mail: marika.nissila@tut.fi; mira.sulonen@tut.fi; jaakko.puhakka@tut.fi)

Abstract
Exoelectrogenic communities were enriched in two-chamber microbial fuel cells from anaerobic digester sample on xylose. During the enrichment, the current density and Coulombic efficiency (CE) increased from 0.16 to 0.35 A/m^2 and from 4.7 to 11.8 %, respectively. In the last enrichment step, 40 % of the electrons were recovered as ethanol. Xylose concentration (from 0.5 to 4.0 g/L) affected electricity production and the highest current density and CE were obtained at 2.5 and 1.0 g/L, respectively. The enrichment cultures were characterized and consisted, e.g.,of a xylanolytic species, *Ruminobacillus xylanolyticum*, and a denitrifying bacteria, *Comamonas denitrificans*.

Keywords
Microbial fuel cell; bioelectricity; xylose; ethanol; butanol

INTRODUCTION

Lignocellulosic materials consist of lignin, cellulose and hemicellulose that can be hydrolysed into hexoses (e.g. glucose and mannose) and pentoses (e.g. xylose and arabinose) (Hendricks and Zeeman 2009). These sugars are amenable to electricity production in microbial fuel cells (MFCs) (Catal et al. 2008). In MFCs, sugars are first fermented into soluble metabolites that are further converted into electricity by exoelectrogens (Huang and Logan 2008). Pure exoelectrogenic cultures, such as *Shewanella putrefaciens* and *Geobacter sulfurreducens*, have been widely studied. Mixed cultures, however, have generally higher electricity yields, wider substrate versatility, and facultative anaerobes consume the trace amounts of oxygen (Liu and Logan 2004, Logan and Regan 2006).

In this study, exoelectrogenic cultures were enriched from anaerobic digester (AD) sample on xylose, an important constituent of lignocellulosic materials, in fed-batch two-chamber MFCs. The developments of bacterial communities in anode solutions and biofilms were characterized.

MATERIALS AND METHODS

Reactor experiments and enrichment procedure

Sludge was obtained from an anaerobic digester integrated to a sewage treatment plant (Viinikanlahti, Tampere, Finland). The anaerobic digester sludge was inoculated into fed-batch two-chamber MFCs to enrich electricity producers. Anode and cathode (working volumes: 75 mL) were separated with cation exchange membrane (CMI-7000S), and plain graphite electrodes (38.5 cm^2) and 100 Ω external resistance were used. Cultures were enriched at 37°C, pH was maintained at 7.0±0.1 at both chambers, and 50 mM $K_3Fe(CN)_6$ in 100 mM Na_2HPO_4 was used as catholyte. Synthetic growth medium was as described by Mäkinen et al. (2012) except for following alterations: 1.0 g/L xylose was used as substrate if not otherwise mentioned, yeast extract concentration was 0.81 g/L and no Na_2SeO_3, $NaWO_4$, resazurin or Na_2S were added. In the beginning, 10 % (v/v) of AD sample was added to the anode chamber. In the following enrichment steps, 10 % (v/v) liquid enrichment culture was transferred to a new MFC containing fresh medium. Every two or three days, samples were taken and MFCs were fed by replacing 10 mL of the anodic solution with fresh medium. A control MFC without inoculum was also run.

Chemical and microbial analysis

Polarization curves were obtained by changing the external resistance from 1 MΩ to 5 Ω every 5 min. Coulombic analysis was done for each MFC according to Huang and Logan (2008). Gas production in anode was monitored with gas-bags. Only negligible amount of gas was detected and the gas content was not further analysed. Volatile fatty acids (VFAs) and alcohols were analysed with a gas chromatograph equipped with HP-5MS column and flame ionization detector. The injector and detector were at 250 and 280°C, respectively, and helium (1.0 mL/min) was used as carrier gas. The oven temperature was the following: 50°C for 3 min, increasing to 100°C at 20°C/min, increasing to 150°C at 35°C/min, and then constant for 2 min. Xylose was analysed with phenol-sulphuric acid method modified from Dubois et al. (1956), where the sample, 5 % phenol and sulphuric acid volumes were 1, 0.5 and 2.5mL, respectively. Bacterial communities were characterized using DNA extraction and polymerase chain reaction – denaturing gradient gel electrophoresis (PCR-DGGE) of partial 16S rRNA genes followed by their sequencing. Duplicate bacterial community samples were taken in the end of the enrichment phases from the anode solution and biofilm and stored at -20°C. The analyses were done as described by Nissilä et al. (2011).

RESULTS AND DISCUSSION

Production of electricity and alcohols

The control MFC without inoculum produced a voltage of 5 mV, which was extracted from other results. Electricity production and CEs increased considerably during the enrichment of AD sample from 8.3 to 18.0 A/m^3 and from 4.7 to 11.8 %, respectively (Table 1). Ethanol was the only soluble metabolite in the end of each enrichment run and the electron recovery as ethanol increased considerably from 0 % to 40 % during the three enrichment phases. VFAs were produced in the beginning of the experiments but were successfully utilized for electricity production by the end of the runs. Electricity production was hindered by the high internal resistance of 227 Ω (calculated from the polarization curve), which resulted in the loss of 48 % of electrons in the end of the enrichments. These results are in the range of current densities and CEs reported in other studies producing electricity from xylose in two-chamber MFCs. For example, Huang and Angelidaki (2008) reported CE of 18 % and power density of 69 mW/m^2 from 1.5 g/L xylose. Higher power densities have been reported in air-cathode MFCs (Catal et al. 2008, Huang and Logan 2008) that have lower internal resistance and thus, better performance than two-chamber MFCs.

Table 1. Voltage (V), current density (ID), Coulombic efficiency (CE) and electrons recovered as ethanol (EtOH) or lost (Losses) during the different enrichment (enr.) steps.

Enr. step	V (mV)	ID (A/m^2) / PD (mW/m^2)	ID (A/m^3) / PD (W/m^2)	CE (%)	EtOH (%)	Losses (%)
1	62	0.16 / 10.0	8.3 / 0.51	4.7	0	95.8
2	47	0.12 / 5.7	6.3 / 0.29	5.9	2.5	90.6
3	135	0.35 / 47.2	18.0 / 2.43	11.8	39.9	47.6

After the three enrichment phases, the electrical connections were optimized resulting in voltage, current (power) density and CE of 490 mV, 65.3 A/m^3 (32.0 W/m^3) and 35 %, respectively. Internal resistance dropped to 112 Ω and no soluble metabolites were produced. These results were in the range of the power densities and CEs of 17 W/m^3 (Huang and Logan 2008) and 31 % (Catal et al. 2008), respectively, obtained in air-cathode MFCs. However, the internal resistance remained relatively high and could be further decreased by, e.g., increasing the electrode and membrane areas (Oh and Logan 2006) or shortening the distance between the electrodes (Venkata Mohan and Chandrasekhar 2011).

The effect of xylose concentration on electricity production was studied with the AD enrichment

culture. Xylose concentrations of 0.5 and 4.0 g/L resulted in low CEs, while the current density was relatively high with 4.0 g/L xylose (Figure 1). The highest current density of 8.7 A/m^3 and the highest CE of 3.4 % were obtained with 2.5 and 1.0 g/L xylose, respectively. In this study, the CE increased from 0.5 to 1.0 g/L xylose and decreased at higher xylose concentrations. The decrease in CE with 2.5 and 4.0 g/L xylose was associated with the production of acetate, propionate, isobutyrate and/or butyrate. In other studies, the highest CEs have been obtained with the lowest xylose concentrations of 0.08-0.23 g/L (Huang and Angelidaki 2008, Huang et al. 2008). During the concentration experiment, the AD enrichment culture produced mainly butanol with electron recoveries of 28 and 35 % as butanol with 0.5 and 1.0 g/L xylose, respectively. Changes in bacterial community composition likely resulted in decreased CEs and high butanol production in the concentration experiment. The high butanol yield reduced the CEs by re-directing electrons and may also have inhibited exoelectrogens or fermentative bacteria.

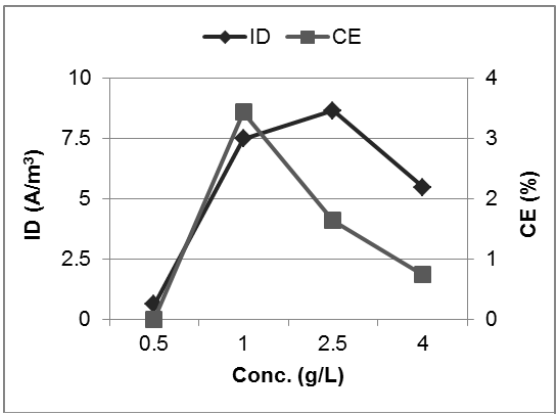

Figure 1. Current densities and Coulombic efficiencies obtained with AD enrichment culture at different xylose concentrations.

Use of fermentable substrate, such as xylose, for electricity production in MFCs often results in the accumulation of soluble metabolites in the anode chamber requiring a further polishing step of the discharged solution. In this study, ethanol was the sole soluble metabolite during the enrichment and could be easily separated by distillation. Furthermore, butanol was mainly produced during the concentration experiment. Ethanol and butanol are high-energy compounds that can be used as direct replacement or as additives for transportation fuels. Simultaneous production of electricity (170 mW/m^2) and ethanol (0.25-0.33 g/L) has been earlier reported from food waste in a solid phase MFC (Venkata Mohan and Chandrasekhar 2011). Lakaniemi et al. (2012) reported simultaneous production of electricity and butanol from microalgal biomass in a two-chamber MFC.

Bacterial community analysis

Bacterial communities were characterized at the end of each enrichment step and after concentration experiment with 1.0 g/L xylose (4th enr. step). Samples from the anode solutions and biofilms were analysed with PCR-DGGE followed by band sequencing (Figure 2). The number of different bacterial strains decreased in anode solutions from 13 to 9 (from 2nd enr. step to the end of the experiment) and in biofilms from 12 to 7 (from 2nd to 3rd enr. step). Mainly *Proteobacteria* and *Bacteroidetes* were present in the enrichments. Bacteria closely related to *Ruminobacillus xylanolyticum* (Band G, 97.2% similarity), *Comamonas denitrificans* (Band K, 98.7%) and *Paracoccus pantotrophus* (Band R, 98.7%) were present in all the samples. Electricity production with *C. denitrificans* from acetate has been reported by Xing et al. (2010). It has been suggested that denitrifying

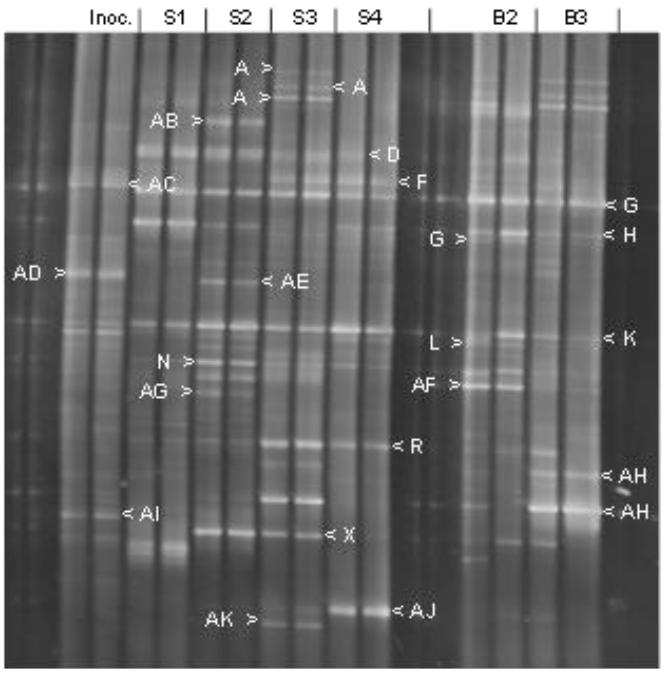

Figure 2. Bacterial DGGE profile of AD enrichments. Inoc.:inoculum, S1-S4: bacteria in solutions of the 1st to 4th enr. steps, B2-B3: bacteria in biofilms of the 2nd and 3rd enr. steps

bacteria may play an important role in electron transfer in MFCs where *Geobacter* and *Shewanella* sp. are not present (Rezaei et al. 2009).

Direct electricity production from xylose has not been reported with bacteria detected in this study. Thus, oxidation of xylose into soluble metabolites must precede electricity production. Ethanol, acetate, propionate, isobutyrate and butyrate were detected in the middle of the experiments. However, ethanol was the only soluble metabolite in the end of the experiment suggesting that other metabolites were efficiently utilized for electricity production. A xylanolytic species, *R. xylanolyticum*, was present in all samples. Furthermore, two *Bacteroidetes* sp. (Bands H and N, 97.6 and 94.7%) were detected. Bacteroidetes are obligately anaerobic and saccharolytic bacteria (Shah 1992). Many bacteria present in the enrichment cultures may produce ethanol as soluble metabolite. Butanol production during the concentration experiment was associated with the appearance of two bacteria, *P. pantotrophus* and *Spirochaeta coccoides* (Band AJ, 90.2%), containing alcohol dehydrogenase.

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REFERENCES

Catal, T., Li, K., Bermek, H., Liu, H. 2008. Electricity production from twelve monosaccharides using microbial fuel cells. *Journal of Power Sources* **175**, 196-200.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28**, 350-356.

Hendricks, A.T.W.M., Zeeman, G. 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology* **100**, 10-18.

Huang, L, Angelidaki, I. 2008. Effect of humic acids on electricity generation integrated with xylose degradation in microbial fuel cells. *Biotechnology and Bioengineering* **100**, 413-422.

Huang, L., Logan, B.E. 2008. Electricity production from xylose in fed-batch and continuous-flow microbial fuel cell. *Applied Microbiology and Biotechnology* **80**, 655-664.

Huang, L., Zeng, R.J., Angelidaki, I. 2008. Electricity production from xylose using a mediator-less microbial fuel cell. *Bioresource Technology* **99**, 4178-4184.

Lakaniemi, A.M., Tuovinen, O.H., Puhakka, J.A. 2012. Production of electricity and butanol from microalgal biomass in microbial fuel cells. *BioEnergy Research* **5**, 481-491.

Liu, H., Logan B.E. 2004. Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. *Environmental Science and Technology* **38**, 4040-4046.

Logan, B.E., Regan J.M. 2006. Electricity-producing bacterial communities in microbial fuel cells. *Trends in Microbiology* **14**, 512-518.

Mäkinen, A.E, Nissilä, M.E., Puhakka, J.A. 2012. Dark fermentative hydrogen production from xylose by a hot spring enrichment culture. *International Journal of Hydrogen Energy* **37**, 12234-12240.

Nissilä, M.E., Tähti, H.P., Rintala, J.A., Puhakka, J.A. 2011. Effects of heat treatment on hydrogen production potential and microbial community of thermophilic compost enrichment cultures. *Bioresource Technology* **102**, 4501-4506.

Oh, S.E., Logan, B.E. 2006. Proton exchange membrane and electrode surface areas as factors that affect power generation in microbial fuel cells. *Applied Microbiology and Biotechnology* **70**, 162-169.

Rezaei, F., Xing, D., Wagner, R., Regan, J.M., Richard, T.L., Logan, B.E. 2009. Simultaneous cellulose degradation and electricity production by *Enterobacter cloacae* in a microbial fuel cell. *Applied and Environmental Microbiology* **75**, 3673-3678.

Shah, H.N. 1992. The genus Bacteroidetes and related taxa. In Balows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K.H. (eds). The Prokaryotes. Springer-Verlag, New York, 3593-3697.

Venkata Mohan, S., Chandrasekhar, K. 2011. Solid phase microbial fuel cell (SMFC) for harnessing bioelectricity from composite food waste fermentation: Influence of electrode assembly and buffering capacity. *Bioresource Technology* **102**, 7077-7805.

Xing, D., Cheng, S., Logan, B.E., Regan, J.M. 2010. Isolation of the exoelectrogenic denitrifying bacterium *Comamonas denitrificans* based on dilution to extinction. *Applied Microbiology and Biotechnology* **85**, 1575-1587.