Biochemical Engineering Journal 101 (2015) 248–252

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Regular article

Effects of anode potentials on bioelectrogenic conversion of xylose and microbial community compositions

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ARTICLE INFO

Article history:
Received 23 February 2015
Received in revised form 10 June 2015
Accepted 13 June 2015
Available online 24 June 2015

Keywords:
Microbial fuel cell
Anode potential
Anaerobic processes
Batch processing
Bioconversion
Biocatalysis

ABSTRACT

The results on the effects of different anode potentials on current densities, coulombic efficiencies and microbial communities are contradictory and have not been studied with xylose, an important constituent of lignocellulosic materials. In this study, the effects of different anode potentials (+0.2, 0 and −0.2 V vs. Ag/AgCl) on current generation, xylose degradation and microbial communities were examined with an exoelectrogenic enrichment culture originating from anaerobic sludge. Anode potential of +0.2 V (vs. Ag/AgCl) resulted in the highest current density and coulombic efficiency of 1.5 ± 0.2 A/m^2 and 62 ± 11%, respectively, and there was no accumulation of soluble metabolites. With anode potentials of 0 and −0.2 V the current densities remained low and acetate, butyrate and propionate were detected in the end of batch runs. Different anode potentials resulted in substantial differences in the anodic bacterial species. At more positive anode potentials, *Ochrobactrum intermedium* reported to be capable of direct electron transfer dominated. At more negative anode potentials, a known mediator-producer, *Alcaligenes faecalis*, and *Desulfitobacterium hafnience*, that has been reported to use mediated electron transfer, were detected. This study shows that the anode potential has a substantial effect on microbial communities and on xylose metabolism.

1. Introduction

In microbial fuel cells (MFCs), organic compounds are oxidized at the anode producing current. Anode potential is known to affect the performance of the anode. The anode potential determines the energy available for microbial growth; Theoretical energy gain of anode respiring bacteria (ARB) increases with the difference of redox potentials between substrate and the electrode [1,2]. The energy available for microorganisms is determined by Gibbs free energy ($\Delta G^{\circ\prime}$, Eq. (1)), where n is the number of electrons per reaction mole, F the Faraday's constant, and $E^{0\prime}$ the theoretical oxidation potential of the substrate calculated at standard conditions. Thus, more positive anode potential should increase the growth of bacteria and result in higher biocatalyst density and faster start-up of current production [3,4]. However, *Geobacter* sp., for example, use only a small portion of their net electron flow to ATP production and has been reported to dominate microbial communities at low anode potentials [4]. According to Finkelstein et al. [5], Wei et al. [6] and Wagner et al. [1], the anode potential selects for ARB having redox potentials of the terminal respiratory proteins more negative than the anode potential.

$$\Delta G^{\circ\prime} = -nF\Delta E^{0\prime} \quad (1)$$

The results on the effects of anode potential on current outputs and microbial community compositions are contradictory. For example, higher current densities at more positive anode potentials were reported by Finkelstein et al. [5] and Sun et al. [7], while Torres et al. [8] obtained higher current densities at more negative anode potentials. Similar debate concerns the effects of anode potential on microbial community composition. Zhu et al. [9] concluded that although the biofilm biomass increased with increasing anode potential, the bacterial community composition was unaffected. On the contrary, Torres et al. [8] reported that more positive anode potential resulted in more diverse microbial community, while more negative anode potential enriched *Geobacter sulfurreducens*. Thus, the effects of anode potentials on current production and microbial community compositions remain to be clarified and require further studies (for a review, see Wagner et al. [1]).

In this study, simultaneous fermentation and anaerobic respiration of xylose, an important constituent of lignocellulosic materials, were studied in fed-batch two-chamber MFCs. The aim was to determine for the first time the effects of different anode potentials on current production, xylose conversion and bacterial community

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http://dx.doi.org/10.1016/j.bej.2015.06.007
1369-703X/

composition with an exoelectrogenic culture enriched originally from anaerobic digester.

2. Materials and methods

2.1. MFC experiments

The experiments were conducted in fed-batch two-chamber MFCs [10]. The anode and cathode chambers were separated with anion exchange membrane (AMI-7000, Membrane International, USA) and had working volumes of 75 mL. Plain graphite electrodes (38.5 cm^2, McMaster-Carr, USA) were used. Anode and cathode chambers were kept anaerobic by flushing the chambers with N_2 in the beginning of the experiments and during the feeding. MFCs were grown at 37 °C without mixing. The pH of the anode solution was adjusted to 7.0 ± 0.1 in the beginning and during each MFC feeding. Cathode solution was 50 mM $K_3Fe(CN)_6$ in 100 mM Na_2HPO_4 buffer (pH 7). Synthetic growth medium contained 6.7 mM (1 g/L) xylose and was prepared as described earlier [11].

The MFCs were inoculated with an anaerobic digester culture (10% (v/v)) enriched for electricity production on xylose in similar reactor configurations and with external resistance of 100 Ω [12]. The experiments were done as duplicate. Anode potentials of +0.2 V, 0 V and −0.2 V were adjusted against Ag/AgCl reference electrode (−205 mV vs. SHE; Sentek, UK). If not otherwise mentioned, all the anode potentials are reported against Ag/AgCl. The performance of the MFCs with anode potential control was compared to duplicate MFCs with uncontrolled anode potential run with external resistance of 100 Ω. In addition, control MFCs with external resistance of 100 Ω were run (i) with inoculum and without substrate, and (ii) without inoculum and with substrate. The MFCs were fed regularly by replacing 10 mL of the anodic solution with fresh medium containing xylose (final concentration 6.7 mM). Prior to feeding, samples were taken for the analysis of volatile fatty acids (VFAs), alcohols, and soluble chemical oxygen demand (COD_s) that was used to estimate the remaining xylose concentration. During the feeding, the catholyte solution was also replenished.

2.2. Electrochemical analyses

Data acquisition system (Agilent 34970A, Agilent Technologies, Santa Clara, USA) was used to monitor and record the voltage and anode potential every 2 min. Current and power densities were calculated against the area of the anode electrode. Coulombic efficiency was calculated against the initial xylose concentration (6.7 mM) according to Logan et al. [13]. Potentiostat (μSTAT 8000, DropSens, Asturias, Spain) was used to adjust the anode potential every three minutes. Anode electrode was working electrode, cathode counter electrode, and Ag/AgCl reference electrodes in 3 M KCl solution connected to the anode chambers through glass capillaries (ProSense) were used as references. A complete coulombic analysis was done for each MFC run according to Huang and Logan [14].

2.3. Chemical analyses

VFAs (acetate, butyrate, isobutyrate, propionate, valerate) and alcohols (ethanol, butanol) were analysed with a gas chromatograph (GC-2010 Plus, Shimadzu, Kyoto, Japan) equipped with ZB-WAX plus column (Phenomenex, USA) and flame ionization detector (FID). The injector and detector temperatures were kept at 250 °C. The oven temperature was programmed as follows: 40 °C for 2 min, increasing to 160 °C at 20 °C/min, increasing to 220 °C at 40 °C/min, and then constant at 220 °C for 2 min. Helium was used as carrier gas at a flow rate of 1.0 mL/min. Samples were filtered (0.2 μm) and acidified with oxalic acid. Soluble COD was analysed from filtrated samples (0.2 μm) with dichromate method according to standard SFS5504 [15]. Possible interference of chloride ions from the reference electrode assembly on COD_s analysis was prevented by using $HgSO_4$ during analysis. The concentration of non-degraded xylose was calculated by subtracting the produced VFAs and alcohols (converted to COD equivalents with Eq. (2)) from the remaining CODs.

$$\mathrm{COD} = \left(\frac{8 \times (4x + y - 2z)}{12x + y + 16z}\right) \times \frac{g\mathrm{COD}}{g\mathrm{C}_x\mathrm{H}_y\mathrm{O}_z} \quad (2)$$

2.4. Bacterial community analysis

Duplicate samples from the anode solution and biofilm were taken from the end points of the MFC runs and the samples were stored at −20 °C. DNA extraction, polymerase chain reaction – denaturing gradient gel electrophoresis (PCR-DGGE), sequencing and analysing of partial 16S rRNA genes were done as described earlier by Mäkinen et al. [16].

3. Results and discussion

The exoelectrogenic culture enriched from anaerobic digester [12] produced current densities of 0.37 A/m^2 and 19.1 A/m^3 in similar two-chamber MFCs used in this study with an external resistance of 100 Ω. The coulombic efficiency (CE) with 6.7 mM xylose was 13%. Ethanol was the sole soluble metabolite from xylose and based on the coulombic balance, 40% of the electrons from xylose were directed towards ethanol production while 48% of the electrons were lost to unknown processes. In the end of the enrichment, the culture contained, among others, denitrifying bacteria, *Comamonas denitrificans* and *Paracoccus pantotrophus*, as well as a xylanolytic species, *Ruminobacillus xylanolyticum* [12]. With this culture, the effects of controlled anode potentials on current production, xylose degradation and bacterial community compositions of anode biofilms and solutions were studied.

3.1. Effects of anode potentials on current production

The effects of different anode potentials poised at +0.2, 0 and −0.2 V (vs. Ag/AgCl) on current production were delineated. In control MFCs run either without inoculum or without substrate, no current was produced (results not shown). The maximum current density and CE of 1.47 ± 0.23 A/m^2 and 63 ± 15%, respectively, were obtained with poised anode potential of +0.2 V, while low current densities and CEs were produced at anode potentials of −0.2 and 0 V (Fig. 1). The anode potential of the MFC with uncontrolled anode potential (with 100 Ω external resistance) was at the highest +0.1 V during stable current production. The maximum current density and CE of the MFC with uncontrolled anode potential were 0.40 ± 0.01 A/m^2 and 31 ± 3%, respectively.

Controlling the anode potential at +0.2 V resulted in 2.5 times higher current densities and 2 times higher CEs than obtained in the MFC with 100 Ω external resistance. In the MFC with uncontrolled anode potential, the anode potential was the highest (+0.1 V) after feeding at high xylose concentration and the anode potential decreased (close to −0.2 V) with decreasing xylose concentration. The more positive anode potential is more beneficial for the bacteria due to higher energy available for the bacteria [3,4]. Thus, the maintenance of anode potential at +0.2 V likely increased the growth and activity of exoelectrogenic bacteria resulting in higher current densities. Furthermore, the electron transfer from the microorganisms to the anode electrode depends on the potential of the outer membrane protein involved in the electron transport chain or the potential of the mediator and the anode potential. The anode potential has to be more positive than the protein or mediator