

Hydrogen production profiles using furans in microbial electrolysis cells

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Abstract Microbial electrochemical cells including microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) are novel biotechnological tools that can convert organic substances in wastewater or biomass into electricity or hydrogen. Electroactive microbial biofilms used in this technology have ability to transfer electrons from organic compounds to anodes. Evaluation of biofilm formation on anode is crucial for enhancing our understanding of hydrogen generation in terms of substrate utilization by microorganisms. In this study, furfural and hydroxymethylfurfural (HMF) were analyzed for hydrogen generation using single chamber membrane-free MECs (17 mL), and anode biofilms were also examined. MECs were inoculated with mixed bacterial culture enriched using chloroethane sulphate. Hydrogen was successfully produced in the presence of HMF, but not furfural. MECs generated similar current densities (5.9 and 6 mA/cm² furfural and HMF, respectively). Biofilm samples obtained on the 24th and 40th day of cultivation using aromatic compounds were evaluated by using epi-fluorescent microscope. Our results show a correlation between biofilm density and hydrogen generation in single chamber MECs.

Keywords Aromatic compounds · Biofilm · Electricity · Hydrogen

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Introduction

Microbial electrochemical cells including microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) have great potential in wastewater treatment and as power sources for autonomous sensors. Significant increase in energy generation has been achieved in recent studies either using mixed bacterial culture enriched from wastewater, activated sludge, anaerobic sludge (Wilson and Kim 2016) or using pure bacterial species, such as *Geobacter* spp. (Reguera et al. 2007), *Shewanella* spp. (White et al. 2016) and *Pseudomonas* spp. (Cary et al. 2000). Mixed culture can be easily adapted to utilize complex organic materials for practical applications, such as waste treatment, and energy generation from lignocellulosic biomass (Fan et al. 2015). Studies on MFC using mixed bacterial culture mainly focused on cell configuration, electrode materials, and solution chemistry (Sreelatha et al. 2016). Community analysis of the mixed bacterial culture on the anode and in the solution has also been made by several groups (Bermek et al. 2013). However, few studies have been conducted to characterize the anode biofilm structure of the mixed bacterial culture (Yamashita et al. 2016). Previous work on anode biofilms with pure culture has focused primarily on cell viability (Reguera et al. 2007) and little attempt has been made to assess the anode biofilm density and hydrogen generation. Aromatic compounds are suitable to analyze the formation of biofilms over time because of their interesting effects on nature, and their effects on hydrogen production in MECs have not been published yet.

Sugars, starches and vegetable oils are first generation biofuels mainly produced from terrestrial plants, and hydrolyzation of them leads to the formation of aromatic compounds. As reported in a previous study, a more promising feedstock lignocellulose emerges as it is more

abundant, cheaper and potentially more sustainable (Wang et al. 2016). Lignocellulose requires expensive, complex processes for producing biofuels. Hence, some processes such as pyrolysis or gasification convert the whole lignocellulose to biofuels, and others focus on using its valuable sugars by converting them to ethanol (Wang et al. 2016). Recently, there has been an increased interest to convert sugars to different types of fuels rather than ethanol. One of the most common attempts is converting glucose to hydroxymethylfurfural (HMF) and subsequently upgraded to dimethylfuran (Roman-Leshkov et al. 2007). 2,5-Dimethylfuran, a potentially good gasoline component, is produced by C–O hydrogenolysis of HMF and it has heating value of 35 MJ/kg and boiling point at 93 °C higher than those of ethanol (22.6 MJ/kg and 78 °C, respectively). Furfural is also another important option as a potential source for biofuels. Hydrolysis and dehydration of xylan in lignocellulose produce furfural which offers a rich source of derivatives of potential biofuel components (Hayes et al. 2008). Hydrodeoxygenation form of a trimer obtained from the aldol-condensation of two molecules of furfural and one of acetone produces linear paraffins which could be used as jet or diesel fuel components (Xing et al. 2010). Our preliminary results show that the acid hydrolysis of hazelnut leaves produce hydrogen gas in single MECs (data not shown). Lignocellulosic hydrolysates contain high amount of phenolic compounds such as furfural and HMF. Most of these aromatic compounds show inhibitory effects on microorganisms. The effects of furfural and 5-HMF on anode biofilm formation for hydrogen production have not been researched in MECs.

In this study, the effects of furfural and 5-HMF on the performance of hydrogen production were investigated in single chamber membrane-free MECs. The features of the anode biofilm formation were compared microscopically.

Materials and methods

Construction of MECs

Single chamber membrane-free MECs were constructed according to a previous study (Catal et al. 2015). Glass serum vials (17 mL) were used to investigate hydrogen production by mixed microbial culture enriched from a local domestic wastewater treatment plant (Pasakoy Advanced Biological Wastewater Treatment Plant, Istanbul, Turkey) using 20 mM chloroethane sulphonate (CES) as reported previously (Catal 2016). The area of both the anode and cathode used in this system was 4 cm². The anodes (LOT: 14032102, FuelCells, College Station, TX, USA) and the cathodes (LOT: CTO32414, FuelCells) containing 0.5 mg/cm² Pt catalyst were made of carbon cloth. The cathode

was prepared according a previous report (Catal et al. 2015; Hu et al. 2008). Titanium wire was used to connect the electrodes. MECs were sealed with rubber stoppers and aluminium seals, and purged with N₂/CO₂ gas (80:20 mixtures) to remove oxygen. MECs were operated at an applied voltage of 0.7 V (n=2). The MEC without inoculum was used as control (n=2). After achieving stable gas production in MECs, the medium solutions were refreshed with 0.1 mM furfural (n=2) or HMF (n=2) in the presence of 20 mM sodium acetate, respectively.

Operations

For MEC operations, sodium acetate (10 mM) was initially used as the carbon source in each MEC with a medium solution containing: NH₄Cl (0.31 g/L); NaH₂PO₄·H₂O (5.84 g/L); Na₂HPO₄·7H₂O (15.47 g/L); KCl (0.13 g/L); a mineral solution (12.5 mL) and a vitamin solution (12.5 mL) as reported previously (Lovley and Phillips 1988). After purging the MECs with the mixture of N₂/CO₂ gas for 10 min, 0.7 V was applied to the MECs by connecting the positive pole of a power supply (GPS-3030DD, GEN851658, Suzhou New District Jiangsu, China) to the anodes. A multimeter (input ranges, ±39 to ±2500 mV) (ADC24, Picolog; Cambridgeshire, UK) connected to a computer was used to record voltage measurements across a 2.2 Ω external resistor placed in series between the positive terminal of the power supply and anode of each reactor. Four MECs were run simultaneously.

All operations were done in batch modes; the whole solution was replaced with fresh medium using a sterile syringe when the produced current started to decrease. All experiments were conducted at 30 ± 2 °C in a temperature controlled chamber.

Gas analysis

The observed biogas production and the cumulative gas volumes for each gas fraction was calculated as previously described (Hu et al. 2008). The expected biogas production was calculated as $V_{E,t} = C_t V_M / 2F$, where, $V_{E,t}$ (mL) is the expected specific biogas production at sample time t based on integrated current over time in coulombs (C), F Faraday's number (96.485 C/mol), C_t the total Coulombs by integrating the current over time, and V_M the molar gas volume (25.200 mL/mol at 30 °C) (Hu et al. 2008).

The gases produced in serum vial MECs were measured and released using an air tight glass syringe (1 mL). All gases were sampled regularly and the composition was analyzed using a gas chromatograph (Agilent, 7820 A GC System, CN14042004, Shanghai, China) equipped with a thermal conductivity detector (AGT-64332A) and a column (113-3133 GS-CARBONPLOT, 30 m, 0.32 mm, 3 mm,

USC703614H, J&W Scientific, Folsom, CA, USA) with argon as the carrier gas (Hu et al. 2008). The following conditions were used in the gas analysis; column temperature at 185 °C, column carrier gas flow rate of 2.8 mL/min, and pressure at 12.734 psi (Catal et al. 2015).

Biogas production was analyzed in the presence of 5-hydroxymethyl-2-furaldehyde (TCI, Tokyo, Japan) and furfural (Alfa Aesar, Karlsruhe, Germany) together with 10 mM sodium acetate as substrate. Furfural and 5-HMF were examined at the concentration of 0.1 mM together with 10 mM sodium acetate. All experiments were duplicated.

Fluorescent microscopic analysis

Biofilm samples on anode surfaces were gently washed in phosphate buffer (100 mM, pH 7.0) to remove planktonic cells, fixed in 3.5% formaldehyde for 15 min and stored in 70% ethanol for microscopic analysis (Catal et al. 2010). Anodic biofilms were stained with propidium iodide (1.5 μ M staining solution) by diluting a 1 mg/mL (1.5 mM) stock solution in PBS and examined using an upright fluorescent microscope (Olympus BX60, Olympus, Tokyo, Japan). The DP72 camera was attached to Olympus BX60 upright microscope was used. Propidium Iodide stained samples were visualized by using U-MWIG3 (green excitation) filter set with excitation wavelength, 530 nm and emission wavelength, 615 nm. Ten randomly selected fields of vision were observed at 100 \times magnification.

Results

Hydrogen production using the mixed culture

Gas production was observed in the presence of furfural and 5-HMF together with 10 mM sodium acetate. Methane generation was not observed in any of the experimental operation because enrichment of microorganisms using CES eliminated methanogenic microorganisms (Catal et al. 2015). Furfural generated up to 7 mL of carbon dioxide on 14th day of operation while a decrease in carbon dioxide level was detected in the consecutive batch (Fig. 1a). There was no hydrogen production during operations although 0.7 V applied voltage. This result indicates that furfural inhibited hydrogen production by CES treated microbial culture at concentration level of 0.1 mM in single chamber MECs.

Figure 1b shows gas production using 0.1 mM 5-HMF and 10 mM acetate in MECs. Up to 10 mL of accumulated hydrogen was observed on the 14th day of operation, and hydrogen generation was not inhibited in the second batch. Carbon dioxide production was detected in all operations (Fig. 1b). This result indicates that 5-HMF did not inhibit hydrogen production in single chamber MECs at concentration level of 0.1 mM. Our results suggest that these two aromatic compounds have different effects on hydrogen production in MECs at the same concentration level. MECs generated similar current densities (5.9 and 6 mA/cm² furfural and HMF, respectively) (Fig. 2).

Fig. 1 Gas production by mixed culture in single chamber membrane-free MECs in the presence of furfural ($C_5H_4O_2$) (a) and 5-HMF ($C_6H_6O_3$) (b)

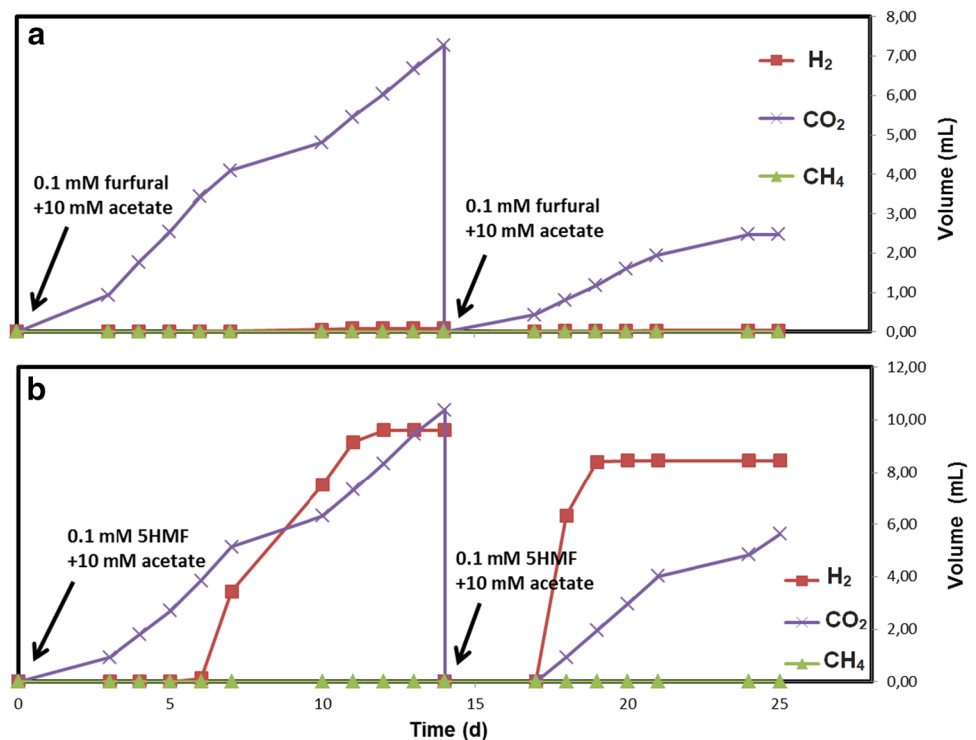
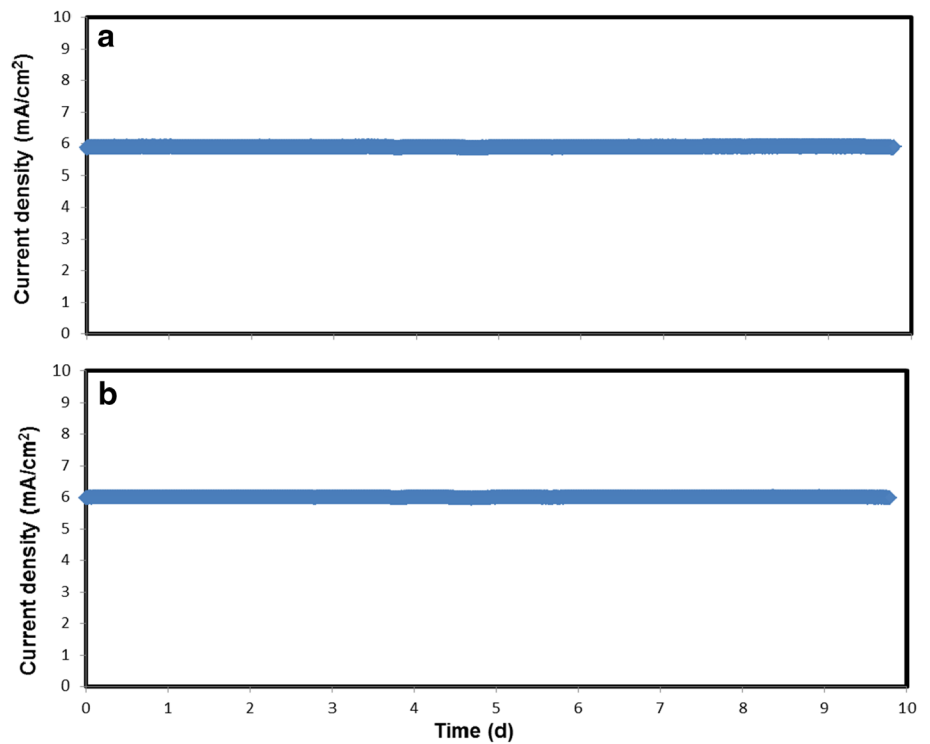


Fig. 2 Current densities at applied voltage of 0.7 (pH 7.0) by mixed culture in single chamber membrane-less MEC using 10 mM sodium acetate (external resistance of 2.2 Ω) in the presence of furfuraldehyde (a), and 5-HMF (b) over time



In our current study, a novel approach was also examined to enrich furfural-adapted microorganisms from mixed bacterial culture anaerobically grown in the presence of acetate as carbon source without CES. Furfural was used in the cultivation of microorganisms from original activated sludge with different concentrations (0.1 and 0.5 mM) in the presence of 1.2 g/L glucose as a carbon source. Microorganisms were grown anaerobically at 37 °C in an incubator for 1 month until clearly sighting the growth of the microbial culture. The growth rate was slow in microorganisms treated with furfural. Although microbial growth was observed under anaerobic conditions, there was no hydrogen production with low level of carbon dioxide and methane production referring to fermentative and methanogenic activity of microorganisms in MECs (data not shown).

Biofilm results

Figure 3 shows fluorescent images of enriched biofilms in the presence of furfural. Biofilm formation was observed on 24th cultivation day on anode surface. On the 40th day of cultivation, increase in biofilm density seems not significant which is unexpected since there was no hydrogen production occurred during operations in the presence of furfural.

Figure 3 demonstrates fluorescent images of biofilms fed with 5-HMF at 100 \times magnification. Biofilm density in operations, especially in samples collected on 24th

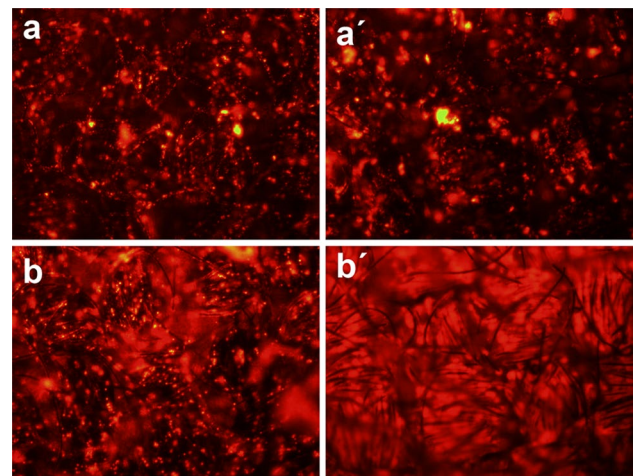


Fig. 3 Fluorescent images of enriched biofilms in the presence of furfural at 100 \times magnification on the 24th day (a) and 40th day (a') of cultivation. Fluorescent images of enriched biofilms in the presence of 5-HMF at 100 \times magnification on the 24th day (b) and 40th day (b') of cultivation

day and 40th day of cultivation was compared. Results are also in parallel manner with hydrogen production results since hydrogen generation was observed in conditions using 5-HMF. These results indicate that anode biofilm may be also important in the formation of hydrogen production in single chamber membrane-less MECs.

Discussion

Most challenging part of biofuel production from cellulosic compounds is production of toxic sub-metabolites during biochemical reactions. Pretreatment processes result in the formation of toxic components including acetic acid along with furfural, HMF and phenolic components (Hayes et al. 2008). Environmental factors such as acid concentration and pretreatment temperature highly influence the formation of degradation products including HMF, furfural, and numerous phenolic lignins, which show severe inhibitory effects on downstream processes (enzymatic hydrolysis and fermentation) (Cruz et al. 2013). The most abundant sugar degradation products during acid hydrolysis are 4-HMF, 5-HMF, 2-furoic acid and furfural. 5-HMF is an organic compound produced from lignocellulose-derived sugars which is a potential feedstock for fuels and chemicals (Hayes et al. 2008). The presence of 5-HMF has some effects on microorganism's growth rate, fermentation rate and cell composition. Hydroxymethyl furfural which is formed from dehydration of hexose sugar has inhibitory effects on microorganisms (Larsson et al. 1999). The compound shows its inhibitory effect on reducing enzymatic and biological activities, breaking down DNA, inhibiting protein and RNA synthesis, and is considered among the most potent inhibitors (Khan and Hadi 1994). Similarly, furfural is the most common simple furan in the environment. It is also known as one of the toxic fermentation inhibitors. The inhibition for the enzymolysis of xylan and glucan hydrolyzation can be done by furfural. During fermentation, the growth rate and the ethanol production rate after pulse additions in both anaerobic and aerobic batch cultures are decreased by furfural (Zheng et al. 2015). Furfural has been linked to NADPH starvation resulting from the activity of NADPH-dependent furfural reductases, for which furfural reduction has been observed in *E. coli* as well as other microorganisms related to its toxicity (Glebes et al. 2014). Furfural damages DNA and inhibits glycolysis. Thus, it affects the metabolism of sugar in microorganisms. Furfural is reduced by aldehyde oxidoreductases into furfuryl alcohol which is less toxic compound than furfural. Nevertheless, this process also decreases cell growth (Seo et al. 2016). Furfural was also found to be easily degraded using sewage bacterial starter culture under aerobic conditions, however anaerobic conditions are also known to block degradation of furfural by some microorganisms. Various microorganisms were reported by Wang et al. (1994) for their ability to degrade furaldehyde. Some strains of the aerobic bacteria such as *E. coli*, *Pseudomonas putida*, and *Rhodococcus erythropolis* and the yeast *Hyphozyma roseniger* were able to degrade the compound (Cary et al. 2000).

Lignocellulosic materials can be used in MFCs and MECs for electricity and hydrogen production, respectively (Catal 2016). Pretreatment of lignocellulosic biomass is necessary in most cases in MFCs (Bellucci et al. 2016). Aromatic compounds such as furfural and HMF are formed during the breaking down process showing inhibitor features. Furfural was reported as inhibitor on yeast during bioethanol production, fungi and bacteria (Wang et al. 2016; van der Pol et al. 2016). While biodegradation of furfural can be achieved by special strains of microorganisms such as *Bacillus subtilis* (Zheng et al. 2015), these aromatics are known with their inhibitor characteristics in most cases. Fermentation activity of hydrogen producer microorganisms is negatively inhibited by HMF, and several bacteria such as *Clostridium* spp. was reported as possible candidate to decrease the toxicity of HMF (Bellucci et al. 2016).

Furfural was reported as inhibitor on electricity generation, while HMF was tolerable by electrogenic microorganisms in MFCs (Catal et al. 2008). Phenolic compounds such as furfural were reported as strong inhibitor on dark hydrogen fermentation (Lin et al. 2015). Hence, in order to operate MEC process, furfural needs to be carefully considered as a challenging part for efficient hydrogen production. Various other phenolic compounds such as propyl galate were reported as inhibitor on electricity generation in MFCs (Bermek et al. 2013). Although up to 75% of total phenolics could be removed during MFC operations, only specific parts of total phenolics are removed, efficiently (Catal et al. 2011). So, it seems furfural would be the most possible phenolic component in lignocellulose-based materials depending on the type of microbial inoculum. It was reported that some phenolic compounds such as phenolic dyes significantly affect microbial diversity causing an enriched phenol-degrading microorganisms in MFCs (Kumru et al. 2012). It is known that electroactive biofilm gets more dense over time in MFCs (Catal et al. 2010), and that microbial consortium can also be significantly affected by phenolic-containing substrates in MFCs (Bermek et al. 2013). For example, it was reported that *Brevundimonas*-, *Sphingomonas*- and *Novosphingobium*-related phylotypes are predominantly found in mixed culture suggesting a possible role of these microorganisms in degradation process of phenolic compounds (Bermek et al. 2013). Yang et al. (2013) suggested that *Geobacter* spp., *Pseudomonas* spp. and *Shewanella* spp., are also all capable of degrading aromatic compounds in MFCs. Therefore, microbial dynamics should be analyzed in the presence of HMF and furfural to find the possible dominant species in order to use in MEC applications to enhance hydrogen production levels, and to develop possible inoculation strategies to use lignocellulosic materials in hydrogen production process in MECs.

Conclusions

In conclusion, our results suggest a relationship between biofilm density and hydrogen generation in single chamber MECs. Microorganisms can tolerate HMF during hydrogen production in MECs, while furfural remains a challenge in lignocellulosic biomass utilization depending on microbial dynamics which should be investigated in advance.

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