Bioresource Technology 241 (2017) 821–829

Contents lists available at ScienceDirect

Bioresource Technology

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

Acetate production and electron utilization facilitated by sulfate-reducing bacteria in a microbial electrosynthesis system

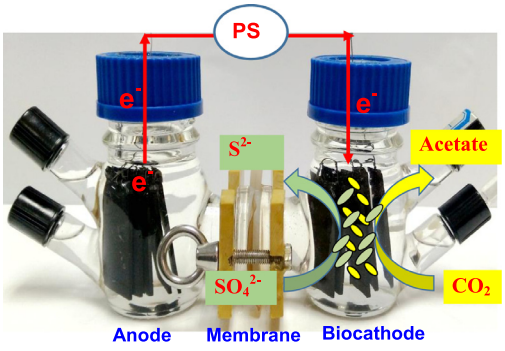
Yinbo Xiang, Guangli Liu, Renduo Zhang, Yaobin Lu, Haiping Luo *

Guangdong Provincial Key Laboratory of Environmental Pollution Control and Remediation Technology, School of Environmental Science and Engineering, Sun Yat-sen University, Guangzhou 510275, China

HIGHLIGHTS

- MES with sulfate achieved higher acetate production at −0.7 and −0.8 V.
- MES with sulfate achieved maximum 240% improvement in electrons output.
- Biomass and microbial activity of biofilm increased with the effect of sulfate.
- MES with sulfate had higher abundance of *Desulfovibrionaceae* at −0.7 V biocathode.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 18 April 2017
Received in revised form 2 June 2017
Accepted 3 June 2017

Keywords:
Microbial electrosynthesis system
Acetate production
Sulfate
Cathode biofilm
Sulfate reducing bacteria

ABSTRACT

The aim of this study is to investigate the effect of sulfate-reducing bacteria on performance of a mixed culture microbial electrosynthesis system (MES). The two-chamber MESs were operated under different cathode potentials (−0.5, −0.6, −0.7, and −0.8 V) with or without addition of 6 mM sulfate. At −0.7 V, acetate production and electrons harvesting in the MES with the sulfate addition were 31.81 mM and 5152 C, respectively, which improved by 2.7 and 2.4 times compared to that without sulfate. With sulfate, the biomass, proportion of live cells, and electrochemical activity of cathode biofilm were enhanced at all the potentials. At −0.7 V, the relative abundance of *Acetobacterium* and *Desulfovibrionaceae* was 14.2% and 36.7% with sulfate, respectively, compared to 17.4% and 7.3% without sulfate. At −0.7 and −0.8 V, the sulfate-reducing bacteria can promote the electron transfer of cathode biofilm and enhance the acetate production.

1. Introduction

Microbial electrosynthesis system (MES) has been developed recently to supply electrons to homoacetogenic bacteria (HB) for CO_2 fixation in commodity chemicals (Bajracharya et al., 2015; Blanchet et al., 2015; Nevin et al., 2011; Su et al., 2013). In the MES, the HB can accept electrons directly or/and indirectly (via H_2) from the cathode surfaces (Nevin et al., 2010). Compared with physical-chemical CO_2 fixation pathways, the MES has many advantages, such as high energy efficiency, low cost, and high environmental sustainability of final products (Lovley and Nevin, 2013). Moreover, since the driven energy can be provided by solar, wind and even wastewater, the MES also provides new method to store electrical energy from renewable energy in chemical bonds (Xiang et al., 2017).

Recently, much attention has been paid to the MES using mixed culture, which can achieve higher biomass and acetate production,

* Corresponding author.
E-mail address: luohp5@mail.sysu.edu.cn (H. Luo).

http://dx.doi.org/10.1016/j.biortech.2017.06.017
0960-8524/

and be more tolerant and convenient to future applications than the pure-culture MES (Batlle-Vilanova et al., 2016; Jourdin et al., 2014). For example, using *Sporomusa ovata* and a mixed culture as the cathode catalyst, the acetate production rates in the MES were 0.17 and 1.125 mM d^{-1}, respectively (Mohanakrishna et al., 2015; Nevin et al., 2010). However, it is still challenging to utilize the mixed culture MES for real applications, such as the low energy recovery efficiency. With mixed culture, the competition of electrons between electrosynthesis and non-electrosynthesis bacteria causes electron loss on the cathode. Except the HB, some species of sulfate reducing bacteria (SRB) and methanogen (MA) can use the cathode as sole electron donor (Cheng et al., 2009; Su et al., 2012). Sulfate is one of the most abundant anions found in the environment (Liamleam and Annachhatre, 2007), indicating possibly ubiquitous existence of SRB in a mixed culture system. Phylogenetic analysis of the active electrosynthetic microbiome revealed that HB (e.g. *Acetobacterium* spp.) and SRB (e.g. *Desulfovibrio* spp. and *Sulfurospirillum* spp.) dominated the active microbial population on the cathode and supernatant in the MES (LaBelle et al., 2014; Marshall et al., 2012; Patil et al., 2015a; Xafenias and Mapelli, 2014). Marshall et al. (Marshall et al., 2013) found abundance of *Sulfurospirillum* genus in electrode biofilm (18.9–26.9%) and in supernatant (82.8–89.3%). Therefore, the competition between HB and SRB is a crucial issue in the MES studies.

Theoretically, the SRB can outcompete HB for electron harvesting from the cathode according to the standard electrode potential E_0'(V) of the cathodic reaction as follows: SRB (E_0' = −0.22 V) > HB (E_0' = −0.28 V) (Kato et al., 2015). The addition of acetate can accelerate the startup of a SRB dominated biocathode for hydrogen producing (Jeremiasse et al., 2012). Therefore, the SRB plays a negative role on the MES performance by the competition of electrons with HB or consumption of acetate. On the other hand, some SRBs show good electrochemical activity and the ability of electron transfer interspecies. For example, *Desulfovibrio* spp. possess outer membrane cytochromes, soluble cytochromes, and hydrogenases in the periplasm, which may readily facilitate the electron transport from electrode to hydrogenase and produce H_2 (Croese et al., 2011; da Silva et al., 2013; Venceslau et al., 2010). As the metabolism product, H_2 can transfer interspecies and play a positive role in the metabolism of the electrosynthetic microbiome. The H_2 route is considered as one of the most important pathways in up-scaling electrosynthesis for microbial CO_2 reduction (Blanchet et al., 2015). The competition between HB and SRB in the cathode of MES can be affected by many aspects and needs further investigation.

The cathode potential is one of the critical factors that affect the performance of the biocathode in bioelectrochemical system. The metabolic pathways and end-products of mixed culture MES are highly dependent on the set cathode potentials (Jiang et al., 2013; Marshall et al., 2013). Cathode potential and current also affect the desulfuration performance in autotrophic biocathode (Luo et al., 2014). Huang et al. (Huang et al., 2011) found that set potentials of −150 and −300 mV improved the performance of biocathode for Cr (VI) reduction because of the higher utilization of metabolic energy gained, as compared to the set potentials of +200 and −450 mV. Therefore, the cathode potential can impact the competition of species in the biofilm.

The objective of this study was to investigate the effect of SRB on the mixed culture biocathode performance in the MES. To better stimulate the growth of SRB, 6 mM sulfate was added in the cathodic medium. The effect was investigated under cathode potentials of −0.5, −0.6, −0.7, and −0.8 V, respectively, in terms of acetate production, electron recovery efficiency, biomass, and microbial population of the biocathode.

2. Materials and methods

2.1. Dual chamber MES reactor

A two-chamber MES was constructed using a previously described design (Xiang et al., 2017). Two custom glass chambers were clamped together with a 6.15 cm^2 Nafion 117 proton exchange membrane (DuPont, USA) as separator. Anode and cathode was made of five pieces of polished graphite plate (2 cm × 5 cm × 0.2 cm) that connected by titanium wires (0.5 mm of diameter). All graphite electrodes were pretreated by washing in acetone and drying, followed by immersion in 1 M NaOH, and 1 M HCl for 24 h. The treated electrodes were rinsed and stored in deionized water before use (LaBelle et al., 2014). A saturated calomel electrode (+0.241 V *vs* standard hydrogen electrode (SHE)) was used as reference electrode in the cathode chamber to fix the electrode potential. Total and working volumes of the cathode chamber (and anode chamber) were 150 and 130 mL, respectively.

2.2. Inocula and operation

The biocatalysts were from raw granular sludge taken from the Zhujiang beer brewery (Guangzhou, China). It was acclimatized under H_2/CO_2 (80/20, v/v) more than a year and already had about 5.16 mM/d average acetate production rate. The liquid cultures centrifuged (6000*g*) for 5 min to collect bacterial cells as inoculum (10% (v/v)). The cathode chamber was filled with 130 mL of mineral synthetic medium that was prepared based on DSMZ_Medium311 growth medium (Xiang et al., 2017). To inhibit MA, 10 mM sodium 2-bromoethanesulfonate was added to the medium (Marshall et al., 2013) during the whole experimental period. To make the conductivity of anolyte be equal to that of the catholyte, DSMZ_Medium311 growth medium with additional 1.5 g/L KCl and 2 g/L NaCl was filled in the anode chamber with 130 mL.

Our previous work showed that an initial 6 mM sulfate significant improved acetate production with H_2/CO_2 as the substrate in the same mixed cultures (data not show). To investigate the effect of sulfate-reducing bacteria, 6 mM sulfate was added into the substrate in the MES. Four sets of MESs were operated at fixed cathode potentials of −0.5, −0.6, −0.7, and −0.8 V (*vs*. SHE) (duplicate reactors) with the sulfate addition in the catholyte. For the MES without sulfate, 6 mM NaCl was added to balance the conductivity (Struchtemeyer et al., 2011). Other four MESs were operated under the same potential conditions but without sulfate in the catholyte. The cathode chamber was flushed with ultrapure CO_2 (purity > 99.999%) for 10 min to remove headspace air and dissolved oxygen after inoculation, then was sealed with rubber stoppers. To avoid the dramatic changes of pH (Marshall et al., 2012; Xiang et al., 2017), the cathode chamber was intermittently sparged with ultrapure CO_2 every 2 d (10 min per time). The abiotic MESs were filled with sterile medium under the same conditions. For each cycle, the anolyte and catholyte in the MES were refreshed at the beginning and the MES was operated for 12 d, during which samples were periodically taken for analysis. All MESs were operated for at least 3 cycles (i.e., 36 d) at 30 °C. The presented results below are values of the mean value and standard deviation of data collected during 3 cycles.

2.3. Analysis methods and calculations

The cathode, anode and reference electrode were connected to the three-electrode system of a potentiostat (CHI1000C, Shanghai Chenhua Instruments Co., P.R. China). Concentration of acetate and sulfate were determined in every 2 d before flushing ultrapure