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Tunable production of ethanol and acetate from synthesis gas by mesophilic mixed culture fermentation in a hollow fiber membrane biofilm reactor

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ABSTRACT

Researches on environmentally friendly pathways in mixed culture fermentation (MCF) to convert synthesis gas (syngas), including carbon monoxide (CO) and hydrogen (H_2), to biofuels and chemicals are attracting worldwide attention. The hollow-fiber membrane biofilm reactor (HFMBR) offers a potential technology for in-situ utilization of syngas. In this work, the performance of syngas MCF in the HFMBR at acidic pH 4.5 was studied for the first time. Sole ethanol was produced using an HFMBR in batch mode and the maximum concentration reached 16.9 g/L. The results also showed that the partial pressure of H_2 (P_{H2}) and CO (P_{CO}) could tune the acetate and ethanol production in HFMBR, in a manner whereby high P_{H2} and P_{CO} favored ethanol production, while low P_{H2} and P_{CO} benefited acetate production. Microbial analysis revealed that the dominant genus in the HFMBR biofilm was *Clostridium* (86.3%), which is consistent with the experimental results. Overall, the adjustable production of acetate and ethanol from syngas in an HFMBR would be useful for the utilization of syngas MCF in the future.

1. Introduction

Mixed culture fermentation (MCF) is an environmentally friendly process to convert the degradable organic wastes to biofuels and chemicals, which are gaining worldwide attention (Henstra et al., 2007; Latif et al., 2014; Lin et al., 2018; Miltner et al., 2010). Gasification, a kind of thermochemical process, can convert mineral fuels or biomass to synthesis gas (syngas) as a mixture of carbon monoxide (CO), hydrogen (H_2) and carbon dioxide (CO_2) (Latif et al., 2014). Syngas as a derivative of various organic sources, including biomass and hydrocarbon feedstock, can then be further utilized for production of biochemicals and fuels by syngas fermentation (Krishnamoorthy and Pisupati, 2015; Wang et al., 2015). To date, CO utilization is mainly reported in pure culture fermentation of *Clostridium* spp., such as *C. ljungdahlii*, *C. drakei* and *C. autoethanogenum* (Datar et al., 2004; Liou et al., 2005; Tanner et al., 1993). For example, in pure culture fermentation of *C. autoethanogenum*, Abubackar et al. reported that, at pH 4.75, no acetic acid was produced and the ethanol concentration reached a maximum of 0.87 g/L, while at a higher pH of 6.0, almost equal amounts of ethanol and acetic acid were formed from CO, reaching 0.91 g/L (Abubackar et al., 2015). Liu et al. proposed the co-culture of syngas fermenter of the *Alkalibaculum bacchi* strain CP15 and the producer of propionic acid *Clostridium propionicum* to enhance the production of ethanol, propanol and n-butanol by 50% (Liu et al., 2014).

Since MCF consumes less power and is more stable to environmental changes (Kleerebezem and van Loosdrecht, 2007; Massaro et al., 2015), syngas MCF is a potential approach to utilize syngas for production of biofuels (such as ethanol) and volatile fatty acids (VFAs), including acetate and propionate, and medium-chain fatty acids (MCFAs), such as caproate and caprylate (Latif et al.,

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2014; Zhang et al., 2013b). However, the low solubility of H_2 and CO in the water phase limits the utilization of syngas (Henstra et al., 2007). The use of the hollow-fiber membrane biofilm reactor (HFMBR) successfully solves this problem and H_2 in the reactor headspace is even undetected (Zhang et al., 2013b). In the HFMBR, syngas permeates from inside of the membrane lumen and is directly consumed by biofilms naturally attached on the outer surface of the hollow-fiber membrane (Zhang et al., 2013a). Furthermore, the biofilm formed on the outer surface of the hollow fiber membrane may also enhance the bacterial resistance to CO toxicity. However, researchers have mainly focused on H_2 utilization in the HFMBR (Rittmann, 2018; Wang et al., 2018), and research on the use of MCF for the production of biochemicals and alcohol from syngas in the HFMBR is rarely reported.

Several researchers demonstrated that factors such as the partial pressure of H_2 (P_{H2}), partial pressure of CO (P_{CO}) and pH can change the dominant bacteria and alter the metabolite distribution in syngas MCF (Peintner et al., 2010; Steinbusch et al., 2008; Temudo et al., 2008; Zhang et al., 2013a, 2013b). For example, Steinbusch et al. found that VFAs, such as acetate, propionate and butyrate were reduced by MCF with a P_{H2} of 1.5 bar, and the final alcohol concentrations were 0.17 g/L, 0.48 g/L and 0.27 g/L for ethanol, propanol and n-butanol, respectively (Steinbusch et al., 2008). In HFMBR feeding with H_2 and CO_2, under mesophilic condition, the dominant bacteria at pH 6.0 were *Clostridium* spp., such as *C. ljungdahlii* and *C. kluyveri*, and the product was a mixture of acetate, butyrate, caproate and caprylate (Zhang et al., 2013b). As the pH decreased to 4.5, the enriched bacteria shifted to *C. ljungdahlii* (>70%), and the percentage of acetate reached 100% (Zhang et al., 2013a). Singla et al. enriched several mixed cultures and optimized their growth conditions for ethanol production, achieving a maximum concentration of 2.3 g/L of ethanol (Singla et al., 2014). Recently, Ganigué et al. found that the control of the fermentation pH to a final value of around 4.8 in batch mode could produce alcohols of ethanol (1.7 g/L), butanol (1.1 g/L), and hexanol (0.6 g/L) from syngas (32% H_2, 32% CO, 8% CO_2, and 28% N_2) (Ganigué et al., 2016). Accordingly, a low pH and a high P_{H2} may regulate the acetate and ethanol production in syngas MCF.

However, Bertsch et al. reported that the H_2-dependent CO_2 reductase of *Acetobacterium woodii* was very sensitive to CO, consequently, *A. woodii* was not able to grow on CO as sole carbon and energy source (Bertsch and Müller, 2015). Until now, mesophilic syngas fermentation at acidic pH and high CO and P_{H2} for ethanol production has seldom been reported, especially in the HFMBR. In our earlier work, we found sole acetate production from H_2 and CO_2 in the HFMBR at pH 4.5, which was favorable for acetate recovery and utilization (Zhang et al., 2013a). Accordingly, the main objective of this work was to determine the effect of acidic pH on the production of metabolites from syngas (CO and H_2) MCF in an HFMBR at 35 °C, with a setup that was the same as that of a previous work (Zhang et al., 2013a). The effect of high P_{CO} and P_{H2} in the HFMBR at pH 4.5 was also elucidated. In addition, the dominant bacteria in the HFMBR biofilm were identified by sequencing analysis on the Illumina MiSeq high-throughput sequencing platform. Therefore, it is expected that the results obtained will support the application of syngas MCF in future.

2. Materials and methods

2.1. The setup and operation of the HFMBR

The HFMBR was configured and the fiber used were the same as that used by Wang et al. (2017). The hollow-fiber membrane was purchased from Haotian Membrane Technology Development Co., Ltd (Hangzhou, China). The mean pore size of the ultrafiltration membrane was 0.01 μm and the total membrane area was 0.023 m^2. The inoculum was obtained from a local mesophilic digester treating starch wastewater (Shandong Province, China), in which the total solid and volatile suspended solid of the sludge were 30 and 11.7 g/L, respectively.

The inoculum (30 mL) and anaerobic medium (285 mL) were added into the reactor with a working volume of 320 mL. Temperature was maintained at 35 °C using a water bath. Syngas (60% CO and 40% H_2) was fed into the system through the hollow-fiber membrane. The inlet pressure was manually controlled to maintain the P_{CO} and P_{H2} in the headspace of the HFMBR. The pH was automatically maintained at 4.5 ± 0.1 with a 1-M NaOH solution. Bromoethane sulfonate (BES) was used to inhibit methanogens activity. The HFMBR was initially run in batch mode and the inlet pressure was kept at 0.4 atm. To demonstrate the effect of the P_{CO} and P_{H2} on the metabolite distribution, the reactor was changed to continuous operation and the hydraulic retention time (HRT) was 9 days, and the inlet pressure was manually controlled between 0.15 and 0.4 atm. The details of the medium composition was previously described by Zhang et al. (2013b).

2.2. Chemical analysis and scanning electron microscopy (SEM) imaging of the biofilm

The VFAs, MCFAs, ethanol and butanol were measured using a Gas Chromatograph (Agilent 7890 GC, Agilent Technologies, Santa Clara, CA, USA). The H_2, CO and methane contents in the headspace were analyzed with a Gas Chromatograph (Lunan model SP7890, CN; Shandong, China). Details of the method were previously described by Wang et al. (2017). The biofilm on the outer surface of the HFMBR was analyzed by SEM (SIRION200; FEI, Hillsboro, OR, USA) and the details of the method were previously described by Zhang et al. (2013b).

2.3. Illumina Miseq high-throughput sequencing analysis

The biofilm attached on the fiber was pushed down and used for Illumina Miseq high-throughput sequencing analysis. DNA samples were then extracted using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA). DNA was amplified using the universal 16S rRNA primers which covered the V3-V4 regions of the 16S rRNA gene: modified 341F and modified 806R (Wang et al., 2017). The whole sequencing process was conducted at the Novogene Institute (Beijing, China). Operational taxonomical units (OTUs) were picked at 97% sequence identity to phylogenetically analyze the bacterial community diversity. The sequencing data of the inoculum and the collected biofilm in this work were archived in NCBI Sequence Read Archive with the accession number of SRR5998768 and SRR5998767, respectively.

3. Results and discussion

3.1. The metabolite production in batch mode

The production of ethanol from syngas at acidic pH 4.5 in the HFMBR is shown in Fig. 1. The inlet pressure of the HFMBR was maintained at 0.4 atm. Since the initial biofilm was not stable, the P_{H2} and P_{CO} in the headspace were all above 0.3 atm. A total of 20 days were needed to enrich the syngas fermentation bacteria and ethanol was initially detected at day 20. As shown in Fig. 1, ethanol was the sole metabolite in the liquid solution, while other metabolites, such as acetate and butyrate, were undetected. The ethanol concentration increased gradually and reached 8.7 g/L at day 51, but it did not increase much, due in part to the ethanol toxicity to bacteria. As it is widely known, the accumulation of ethanol causes