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Conversion of syngas (CO and H_2) to biochemicals by mixed culture fermentation in mesophilic and thermophilic hollow-fiber membrane biofilm reactors

Nan Shen [a, b, 1], Kun Dai [a, 1], Xiu-Yang Xia [a], Raymond Jianxiong Zeng [a, **], Fang Zhang [a, *]

[a] Centre of Wastewater Treatment and Resource Recovery, College of Resources and Environment, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, China
[b] School of Environment, Nanjing Normal University, Nanjing, Jiangsu 210023, China

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ABSTRACT

Syngas (CO/H_2) fermentation provides an alternative method for organics utilization. The hollow-fiber membrane biofilm reactor (HfMBR) offers a potential way to use CO and H_2 effectively. However, studies on syngas utilization by mixed culture fermentation (MCF) in HfMBR were seldom reported. This study is the first to demonstrate and compare the in-situ production of volatile fatty acids via syngas by mesophilic and thermophilic MCF, in which CO and H_2 utilization exceeded 95% and even reached 100%. Acetate (4.22 g/L), butyrate (1.35 g/L), caproate (0.88 g/L), and caprylate (0.52 g/L) were detected at 35 °C, whereas acetate was the main metabolite at 55 °C. The proportion of acetate ranged from 47.5% to 62.7% at 35 °C and exceeded 90% at 55 °C in the batch mode. Notably, the acetate concentration and the production rate could reach 24.6 g/L and 16.4 g/(L·d), respectively, at pH 6.5 in the continuous mode at 55 °C. Thus, thermophilic conditions are preferable for high-purity and high-concentration acetate production from syngas. As revealed by Illumina high-throughput sequencing, *Clostridium* (41.6%) *and Thermoanaerobacterium* (92.8%) were the dominant bacteria under mesophilic and thermophilic conditions, respectively. This study demonstrates a potential technique for syngas (CO/H_2) utilization and biochemicals production by MCF in HfMBR.

1. Introduction

Syngas consists of the main components CO and H_2 and the minor components CO_2, CH_4, H_2S, and NO_X. Syngas utilization by biotechnological methods, such as syngas fermentation, provides a potential pathway to utilize the hardly degradable organic wastes such as lignocellulose and sludge (Jing et al., 2017; Liew et al., 2016). Thus far, syngas fermentation mainly focuses on pure culture and co-culture under mesophilic conditions and is proposed to convert syngas to volatile fatty acids (VFAs, such as acetate and butyrate), ethanol, butanol, and/or caproate via microbes, such as *Clostridium carboxidivorans* and *Clostridium ljungdahlii* (Martin et al., 2016; Ramió-Pujol et al., 2015; Wang et al., 2018). For example, syngas (60% CO, 35% H_2, and 5% CO_2) was purged into a 2-stage system with *C. ljungdahlii* inoculated; the maximum concentrations of acetate and ethanol were 13.1 and 10.5 g/L, respectively (Martin et al., 2016). Syngas of 20% CO, 15% CO_2, 5% H_2, and 60% N_2 was also supplied for co-culture fermentation (*Alkalibaculum bacchi* and *Clostridium propionicum*) in a continuous mode; the maximum concentrations of ethanol, n-propanol, and n-butanol were 8, 6, and 1 g/L, respectively (Liu et al., 2014).

Pure culture or co-culture fermentation is typically challenged by high operating costs, strain degeneration, and contamination, thus, mixed culture fermentation (MCF) is considered as a potential approach to produce biochemicals including acetate, ethanol, and caproate (Esquivel-Elizondo et al., 2017; Lay et al., 2012; Reddy et al., 2018). Moreover, CO toxicity to some bacteria is a main obstacle for CO conversion (Esquivel-Elizondo et al., 2017; Jing et al., 2017). Bertsch and Müller (2015) demonstrated that the hydrogen-dependent CO_2 reductase of *Acetobacterium woodii* was highly sensitive to CO, consequently impeding the growth of *A. woodii* on CO as a sole carbon and energy source. Thus, mixed culture syngas fermentation may facilitate the simultaneous

* Corresponding author.
** Corresponding author.
E-mail addresses: rzeng@ustc.edu.cn (R.J. Zeng), zhfang@ysu.edu.cn (F. Zhang).
[1] These authors contributed equally to this work.

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conversion of H_2 and CO by different enriched bacteria.

The poor aqueous solubility of H_2 and CO is another major limiting factor in syngas fermentation (Esquivel-Elizondo et al., 2017). High impeller speeds can provide high gas/liquid mass transfer coefficients but can also lead to high-power consumption and may lower the bacterial activity (Henstra et al., 2007). Increasing the specific gas-liquid interfacial area can diminish poor gas solubility. Zhang et al. (2013b) proposed a mesophilic hollow-fiber membrane biofilm reactor (HfMBR) for the in-situ consumption of H_2 and CO_2, in which the dominant bacteria were *Clostridium* spp. and the product was a mixture of acetate, butyrate, caproate, and caprylate. In a thermophilic HfMBR (55 °C), acetate comprised more than 99% of total VFAs from H_2 and CO_2, whereas no caproate was produced (Wang et al., 2017). HfMBR also presents other advantages, such as lower energy consumption and smaller footprints (Martin and Nerenberg, 2012). The biofilm that formed on the outer surface of HfMBR may also enhance bacterial resistance to CO toxicity in HfMBR. However, studies on syngas (CO and H_2) MCF in HfMBR such as metabolites and dominant bacteria are rarely reported.

Moreover, metabolite production in gas fermentation is generally considered to be thermodynamically controlled (Liew et al., 2016; Richter et al., 2016b). Generally speaking, mesophilic temperatures favor bioreactions in syngas fermentation, except for the production of caproate (Table 1). The Gibbs free energy (ΔG′) at 35 °C (−87.8 kJ/mol) is higher than that at 55 °C (−77.4 kJ/mol) in Eq. (1), which indicates that more energy is generated at 35 °C (Zhang et al., 2013c). Comparing with acetate, medium-chain fatty acids with longer carbon chain and lower oxygen/carbon ratio could gain higher energy density (Steinbusch et al., 2011). On the other hand, all ΔG′ values of acetate and ethanol production from CO (Eqs. (3) and (4)) are lower than those from CO_2 and H_2 (Eqs. (1) and (2)), allowing bacteria to obtain more energy from CO utilization. However, the effect of temperature on metabolite distributions in syngas (CO/H_2) fermentation in HfMBR remains unclear.

Based on above consideration, HfMBR provides an important pathway for syngas of CO and H_2 conversion by mixed culture, however, the metabolite spectra and the dominant bacteria in biofilm are still needed to be revealed. Therefore, the aims in the present study were to 1) investigate the metabolite spectra from syngas (CO/H_2) MCF in mesophilic and thermophilic HfMBRs under both batch and continuous-flow modes; 2) analyze the dominant bacteria in the biofilms by the Illumina high-throughput sequencing.

Table 1
Change in Gibbs free energy of main bioreactions in syngas (CO/H_2) fermentation.

Bioreactions	ΔG′ (kJ/mol) [a]	
	35 °C	55 °C
Acetate production from CO_2 and H_2: $4H_2 + 2CO_2 \rightarrow C_2H_3O_2^- + H^+ + 4H_2O$ (Eq. (1))	−87.8	−77.4
Ethanol production from CO_2 and H_2: $6H_2 + 2CO_2 \rightarrow C_2H_5OH + 3H_2O$ (Eq. (2))	−96.0	−79.1
Acetate production from CO: $4CO + 2H_2O \rightarrow C_2H_3O_2^- + H^+ + 3CO_2$ (Eq. (3))	−172.2	−166.6
Ethanol production from CO: $6CO + 3H_2O \rightarrow C_2H_5OH + 4CO_2$ (Eq. (4))	−220.6	−212.9
Butyrate: $C_2H_3O_2^- + C_2H_5OH \rightarrow C_4H_7O_2^- + H_2O$ (Eq. (5))	−38.5	−37.8
Caproate: [b] $C_2H_3O_2^- + 2C_2H_5OH \rightarrow C_6H_{11}O_2^- + 2H_2O$ (Eq. (6))	−81.5	−89.1
Caproate: [b] $C_4H_7O_2^- + C_2H_5OH \rightarrow C_6H_{11}O_2^- + H_2O$ (Eq. (7))	−43.0	−51.3

[a] All calculated under standard conditions.
[b] The change in Gibbs free energy calculation was according to the method of Thauer et al. (1977).

2. Materials and methods

2.1. Set-up of HfMBR-A and HfMBR-B

The total working volume of HfMBR-A and HfMBR-B was 390 mL (Fig. S1). Initially, 40 mL inocula were collected from a mesophilic anaerobic digester (Zhang et al., 2013b). The total solids (TS) and volatile suspended solid (VSS) of the inoculum was 25.1 g/L and 10.7 g/L, respectively, and the ratio of VSS to TS was 0.43. The feeding syngas was composed of 60% H_2 and 40% CO. HfMBR-A was assembled in the laboratory with the hollow-fiber membranes purchased from Huilong Membrane Technology Development Co., Ltd. (Suzhou, China). HfMBR-B was purchased from Puresea Spring Membrane Technology Co., Ltd. (Tianjin, China). The parameters of HfMBR-A and HfMBR-B are summarized in Table S1. The physical characteristics of membranes in the 2 reactors were similar, except for the total surface area of the membrane, which was 0.10 m^2 in HfMBR-A and 0.24 m^2 in HfMBR-B. The inlet pressure was controlled at 0.1–0.15 atm to ensure no syngas detected in the headspace.

The bromoethanesulfonate (10 mmol/L) was initially added in HfMBR-A and HfMBR-B to inhibit methanogenesis. In the HfMBR-A batch experiment, pH was controlled at 6.0 ± 0.1 to inhibit the methanogens with a NaOH solution (1 mol/L) via automatic titration, and temperature was controlled at 35 ± 1 °C by using the tube that was wrapped around the main body of HfMBR and hot water flew through the tube from a water bath. Then, the temperature of HfMBR-A and HfMBR-B was gradually increased (0.5 °C/d) from 35 °C and was finally controlled at 55 ± 1 °C by using a water bath. The effect of the membrane area on VFAs production was evaluated by comparing the 2 reactors under thermophilic conditions. At last, HfMBR-B was operated in a continuous-flow mode after the batch mode. The hydraulic retention time (HRT) was controlled at 1.5 days (i.e., the influent rate of 260 mL/d) by a peristaltic pump and 1 mm internal diameter silicone tubing (BT100-2J, Longer pump, Baoding, China). The medium ingredients (in 1.0 L distilled water) were as follows: NH_4Cl, 500 mg; KH_2PO_4, 200 mg; Na_2SO_4, 40 mg; KCl, 50 mg; $CaCl_2$, 10 mg; $MgCl_2 \cdot 6H_2O$, 70 mg; $MnCl_2 \cdot 4H_2O$, 0.8 mg; $CoCl_2 \cdot 2H_2O$, 1.2 mg; $FeSO_4 \cdot 7H_2O$, 3.2 mg; $AlCl_3$, 0.5 mg; $NaMO_4 \cdot 2H_2O$, 0.1 mg; H_3BO_3, 0.2 mg; $NiCl_2 \cdot 6H_2O$, 0.5 mg; $CuCl_2 \cdot 2H_2O$, 1.1 mg; $ZnSO_4 \cdot 2H_2O$, 3.2 mg; and EDTA (Na^+), 3 mg (Zhang et al., 2013b).

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

The contents of H_2, CO, and methane in the headspace were analyzed using a gas chromatograph (Lunan model SP7890, Ruihong Chemical Industry Instrument Company Ltd., China) equipped with a thermal conductivity detector and a 1.5 m stainless steel column packed with 5 Å (0.5 nm) molecular sieve (Zhang et al., 2013b). For H_2 and CO determination, the temperatures of the injector, detector, and column were kept at 80, 100, and 50 °C, respectively, and N_2 was used as the carrier gas. While, for methane determination, the temperatures of the injector, detector, and column were kept at 170, 170, and 150 °C, respectively, and H_2 was used as the carrier gas.

The concentrations of VFAs, ethanol, and butanol, caproate and caprylate were measured by another gas chromatograph (Agilent 7890, CA) equipped with a flame ionization detector and a 10 m × 0.53 mm HP-FFAP fused-silica capillary column (Zhang et al., 2013b). The column operating temperature profiles were 70 °C for 3 min, then 20 °C/min to 180 °C, hold for 5.5 min. The injector and detector temperatures were 250 °C and 300 °C, respectively.

The HfMBR-B biofilm in the continuous mode was analyzed by SEM (SIRION 200, FEI, USA). The samples for SEM analysis were