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Selective conversion of carbon monoxide to hydrogen by anaerobic mixed culture

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HIGHLIGHTS

- Efficiently fermentative CO conversion was obtained by anaerobic granular sludge.
- Addition of chloroform was necessary to achieve selective conversion of CO to H_2.
- Stable and efficient H_2 production from CO was obtained in a continuous reactor.
- Gas recirculation was crucial to increase the CO conversion efficiency.
- The abundance of known CO-utilizing bacteria enriched in the reactor was very low.

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ABSTRACT

A new method for the conversion of CO to H_2 was developed by anaerobic mixed culture in the current study. Higher CO consumption rate was obtained by anaerobic granular sludge (AGS) compared to waste activated sludge (WAS) at 55 °C and pH 7.5. However, H_2 was the intermediate and CH_4 was the final product. Fermentation at pH 5.5 by AGS inhibited CH_4 production, while the lower CO consumption rate (50% of that at pH 7.5) and the production of acetate were found. Fermentation at pH 7.5 with the addition of chloroform achieved efficient and selective conversion of CO to H_2. Stable and efficient H_2 production was achieved in a continuous reactor inoculated with AGS, and gas recirculation was crucial to increase the CO conversion efficiency. Microbial community analysis showed that high abundance (44%) of unclassified sequences and low relative abundance (1%) of known CO-utilizing bacteria *Desulfotomaculum* were enriched in the reactor.

1. Introduction

The challenge in fossil fuel shortage and the threat in atmosphere pollution are becoming serious in the fast developing countries, and they lead us to search for the alternative cleaner and sustainable energy sources. The renewable energy from biomass is attracting more attention. Although there are biological methods for the conversion of biomass into bioenergy, a significant part of the biomass (e.g. lignocellulosic materials) is difficult to be biodegraded. The non-biodegradable biomass can be converted to synthesis gas (syngas) by thermo-chemical gasification (Guiot et al., 2011).

Syngas is a mixture of mainly CO and H_2, which can be used as fuel directly (Luo et al., 2013). However, the low energy density and toxicity of CO limit its application (Haddad et al., 2014). Alternatively, CO in syngas can be converted to H_2, which will increase the amount of H_2 in syngas and provide a cheap source of H_2. After further purification and separation, H_2 can be used as clean fuel or raw material for industry.

The conversion of CO to H_2 by anaerobic microorganisms has been reported previously (Henstra et al., 2007; Sokolova et al., 2009). The detailed reaction processes of carboxydotrophic hydrogenogenesis are shown in the following reactions:

$$CO + H_2O \rightarrow H_2 + CO_2 \quad \Delta G^{o\prime} = -20\ \text{kJ/mol CO} \tag{1}$$

$$CO + H_2O \rightarrow CO_2 + 2e^- + 2H^+ \tag{2}$$

$$2H^+ + 2e^- \rightarrow H_2 \tag{3}$$

Molecular H_2 is formed by the biological water–gas shift reaction (1). More specifically, the CO dehydrogenase (CODH) provides electrons and protons derived from H_2O for the electron transformation by reaction (2). At the same time, the hydrogenase supplies energy

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for cell growth through reaction (3) (Phillips et al., 1994) and reduces the protons to form H_2.

Carboxydothermus hydrogenoformans, *Desulfotomaculum carboxydivorans* et al. (Table S1) are able to metabolize CO to H_2 by biological water–gas shift reaction (Parshina et al., 2005; Tiquia-Arashiro, 2014), and the process has special advantages over chemical catalytic process, which generally requires high temperature or pressure and has low product selectivity (Henstra et al., 2007). The temperature and pressure needed in the biological reaction are moderate, inducing an energy saving in the operation. Besides, the high specificity of enzymes enable a higher product yield with fewer by-products evolved during the process. Furthermore, most biocatalysts can tolerate trace amounts of contaminants such as sulfur and chorine (Mohammadi et al., 2011).

Until now, there are only studies on H_2 production from CO by pure microorganisms (Haddad et al., 2014; Jung et al., 2002; Kim et al., 2015; Younesi et al., 2008). The conversion of CO to H_2 by mixed culture has not been investigated until now, which has the potential advantages including non-sterilized conditions and utilization of wastewater as nutrients. It is possible to enrich one or more of the pure microorganisms listed in Table S1 in a mixed culture if the operation conditions are available. The conversion of CO to CH_4 or acetate by mixed culture has been reported before (Alves et al., 2013; Guiot et al., 2011), and H_2 was observed as an intermediate during the mixed culture conversion of syngas, especially in thermophilic conditions (Guiot et al., 2011). The activities of hydrogenotrophic methanogens ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$) and homoacetogens ($4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O$) need to be fully inhibited (Luo et al., 2011a) in order to achieve selective conversion of CO to H_2 by the mixed culture. Other scientific questions are also needed to be considered. First, CO is both substrate and inhibitor to microorganisms and its effect on the CO conversion efficiency by mixed culture is still unknown (Oelgeschlager and Rother, 2008). Second, CO has low solubility in water, and the gas–liquid mass transfer may limit its conversion in a continuously operated reactor. Therefore, the methods to overcome the gas–liquid mass transfer limitation have to be investigated (Yasin et al., 2015). In addition, mixed culture fermentation may involve various microorganisms that could achieve CO conversion, and it is necessary to characterize the microbial community compositions in the mixed culture.

Based on the above considerations, the present study aimed at developing a new biological process for H_2 production from CO by anaerobic mixed culture. Specifically, the effects of inoculum sources, pH, methods to inhibit hydrogen consuming microorganisms, and CO partial pressures on H_2 production from CO were investigated to achieve selective conversion of CO to H_2. Moreover, a continuous reactor was operated to study the performance of continuous production of H_2 from CO, and also gas recirculation was tested to increase the gas–liquid mass transfer. The microbial community composition in the long-term operated reactor was analyzed by high-throughput sequencing of the 16S rRNA genes.

2. Methods

2.1. Inoculum sources

Two different inocula were tested in order to compare their potentials to convert CO into H_2. One was the waste activated sludge (WAS) (pH = 6.4 ± 0.2, TSS = 15 ± 0.1 g/L, VSS = 11.7 ± 0.1 g/L) obtained from Quyang wastewater treatment plant (Shanghai, China), and the other one was anaerobic granular sludge (AGS) (pH = 7.5 ± 0.5, TSS = 133.4 ± 4.6 g/L, VSS = 103.3 ± 2.5 g/L) obtained from an up-flow anaerobic sludge blanket (UASB) reactor treating papermaking wastewater in Longchen Paper CO., LTD (Jiangsu, China).

2.2. H_2 production potential from CO by mixed culture

Four batch experiments were carried out. In batch experiment 1, both WAS and AGS were tested for their potentials to convert CO to H_2. The inocula were diluted by basic medium (prepared according to a previous publication (Angelidaki and Sanders, 2004)) to 100 mL with a final VSS concentration 10 g/L. The mixture also contained 50 mM phosphate buffer saline (pH 7.5) to keep a constant pH. The 100 mL mixtures were added to 320 mL serum bottles, and the pH of the mixtures were then adjusted to 7.5 by 2 M NaOH. The bottles were closed with butyl stoppers and aluminum crimps to make them air tight, and they were subsequently purged with N_2 for 2 min to maintain anaerobic conditions. CO was injected into the closed bottles to achieve CO partial pressure 0.2 atm in the gas phase. Finally, all the bottles were incubated in a shaker at 55 °C. The shaker was controlled at 300 rpm to overcome the gas–liquid mass transfer limitation. Bottles without CO were used as control to determine H_2 production from endogenous respiration. During the experiments, the gas composition (CO, H_2 and CH_4) in the headspace of each bottle was measured every day, and the liquid samples were collected and analyzed for the possible presence of volatile fatty acids (VFA) every two days. All the tests were prepared in triplicate. In batch experiment 2, the effect of different pH (5.5 and 7.5) on the H_2 production from CO was conducted by AGS based on the results from batch experiment 1. In batch experiment 3, three different methods (heat pretreatment of the inoculum (120 °C, 1 h), the addition of 2-bromoethanesulfonic acid (BES) (10 mM) and the addition of chloroform (5 mM)), were investigated to inhibit the hydrogen consumption to increase the H_2 production from CO. The methods were chosen according to the previous studies focusing on fermentative hydrogen production from organic wastes/wastewater (Bundhoo et al., 2015; Luo et al., 2010) and also our preliminary experiments. CO is both substrate and inhibitor for microorganisms, and therefore the effect of different CO partial pressures (0.05, 0.1, 0.2, 0.4 and 0.8 atm) on H_2 production from CO was tested in batch experiment 4. 320 mL serum bottles with 100 mL mixture containing both inoculum and nutrients were used. The stability of H_2 production process was also studied by successively refreshing the CO in the headspace of each bottle. The experimental procedure for batch experiments 2, 3, and 4 was similar to batch experiment 1.

2.3. H_2 production from CO in a continuous reactor

A lab-scale UASB reactor with 1L working volume was used to study the performance of continuous H_2 production from CO by anaerobic mixed culture. The reactor was inoculated with AGS, and the VSS concentration in the reactor was 10 g/L. The temperature and pH in the bioreactor were controlled at 55 °C and pH 7.5, respectively. 2 M NaOH was used to adjust the pH. Basic medium containing chloroform (5 mM) was fed to the UASB reactor every two days (100 mL/2d) in order to provide nutrients and inhibit hydrogen-consuming microorganisms. Three experimental phases were set to study the effect of gas recirculation and increased CO loading rate on the CO conversion efficiency. In phase I, pure CO was continuously pumped into the reactor through a gas diffuser at a flow rate of 1 L/d with no gas circulation. The volume and concentration of H_2 and CO in the collected gas, as well as the VFA concentration in the liquid were measured periodically. From day 22 onwards (phase II), gas recirculation was implemented with a recirculation flow rate of 1 L/h. After the reactor achieved a steady state, the CO loading rate was doubled while the gas recirculation