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Mixed culture syngas fermentation and conversion of carboxylic acids into alcohols

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HIGHLIGHTS

- Higher alcohols production using mixed culture from syngas was investigated.
- Ethanol, *n*-propanol, *n*-butanol were produced by a mixed culture.
- CP15 or mixed culture did convert carboxylic acids to respective alcohols.
- There was an increased productivity by the CP15 and *Clostridium propionicum* mixed culture.
- Mixed culture converted 50% more acids to alcohols than CP15 alone.

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ABSTRACT

Higher alcohols such as *n*-butanol and *n*-hexanol have higher energy density than ethanol, are more compatible with current fuel infrastructure, and can be upgraded to jet and diesel fuels. Several organisms are known to convert syngas to ethanol, but very few can produce higher alcohols alone. As a potential solution, mixed culture fermentation between the syngas fermenting *Alkalibaculum bacchi* strain CP15 and propionic acid producer *Clostridium propionicum* was studied. The monoculture of CP15 produced only ethanol from syngas without initial addition of organic acids to the fermentation medium. However, the mixed culture produced ethanol, *n*-propanol and *n*-butanol from syngas. The addition of propionic acid, butyric acid and hexanoic acid to the mixed culture resulted in a 50% higher conversion efficiency of these acids to their respective alcohols compared to CP15 monoculture. These findings illustrate the great potential of mixed culture syngas fermentation in production of higher alcohols.

1. Introduction

Efforts to develop renewable energy are driven by the negative impacts of satiating an ever-increasing global consumption of energy with the burning of fossil fuels (Atsumi et al., 2008). The costs associated with transitioning from fossil to renewable transportation fuels will be minimized if major changes in existing infrastructures can be avoided. Ethanol is a renewable transportation fuel (i.e. biofuel) that can be directly blended with gasoline. Ethanol, however, is hygroscopic and has corrosive characteristics that translate into increased transportation costs because it must be transported mainly by trucks instead of existing gasoline pipelines (Tyner, 2010). Higher alcohols such as *n*-butanol and *n*-hexanol are candidates to replace ethanol due to their higher energy density and lower water solubility than ethanol. *n*-Butanol is less hygroscopic than ethanol and it has a 29% higher volumetric energy density than ethanol (ethanol 21 MJ/L vs *n*-butanol 27 MJ/L) (Mann et al., 2006; Atsumi and Liao, 2008). *n*-Propanol is an important chemical for ink, polymer and pharmaceutical industries (Demirer and Speece, 1998). *n*-Propanol has also been considered as a candidate for the replacement of gasoline (Simmons, 2011). *n*-Hexanol has low miscibility with water and less volatility than ethanol or *n*-butanol and can be blended with biodiesel or gasoline (Yeung and Thomson, 2013). Thus, these higher alcohols have more potential than ethanol as "drop-in" biofuels, and they also can be converted to jet fuels and chemicals (Harvey and Meylemans, 2011).

The hybrid gasification-syngas fermentation technology for the production of fuels and chemicals is on the verge of commercialization. In this process, syngas is produced by gasification of

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biomass or municipal solid waste followed by conversion of syngas components (mainly CO, H_2 and CO_2) to liquid fuels and chemicals (Wilkins and Atiyeh, 2011). Several reports have been published on the production of ethanol and higher alcohols such as *n*-butanol using syngas fermentation by *Clostridium carboxidivorans*, "*Clostridium ragsdalei*", and *Eubacterium limosum* ("*Butyribacterium methylotrophicum*") (Shen et al., 1999; Tanner, 2008; Maddipati et al., 2011; Ukpong et al., 2012; Ramachandriya et al., 2013). "*C. ragsdalei*" also converted acetone to isopropanol during syngas fermentation (Ramachandriya et al., 2011).

Previous studies of syngas fermentation for the production of liquid fuels have been focused on the use of monocultures. Mixed cultures of sulfate reducing bacteria, methanogenic archaea and homoacetogenic bacteria using H_2/CO_2 or CO have been reported to produce methane during anaerobic digestion of sludge (Esposito et al., 2003; Sipma et al., 2004). In another study, a mixed culture containing *Rhodospirillum rubrum*, *Methanobacterium formicicum* and *Methanosarcina barkeri* was reported to convert CO, CO_2 and H_2 to methane via synergy among the three bacteria (Klasson et al., 1990).

Alkalibaculum bacchi strain CP15 is capable of producing ethanol at a yield that is over 43% higher than *A. bacchi* strains CP11^T and CP13 (Liu et al., 2012). It was found that the cost of the syngas fermentation medium for strain CP15 can be reduced by over 27% by removing [N-*tris* (hydroxymethyl)methyl]-3-aminopropanesulfonic acid (TAPS buffer) as well as replacing the yeast extract (YE), minerals and vitamins with corn steep liquor (CSL) (Liu et al., 2014). 78% more ethanol was produced in 20 g/L CSL medium than in the YE medium using strain CP15, indicating the potential of CSL use as a cost-effective nutrient for large scale fermentation. Additionally, strain CP15 can grow to a high cell mass during continuous syngas fermentation with cell recycling (Liu et al., 2014). During this continuous syngas fermentation in CSL medium, *n*-propanol and *n*-butanol production was observed, which has not been reported previously for strain CP15 (Allen et al., 2010; Liu et al., 2012). These alcohols were produced from a serendipitous mixed culture formed at the late stages of a continuous fermentation as confirmed by 16S rRNA gene sequencing. The mixed culture consisted largely of *A. bacchi* CP15 (56%) and *Clostridium propionicum* (34%), with the remaining 10% made up of 4 other *Clostridium* species (Liu et al., 2014).

C. propionicum is known to consume amino acids and ferments lactate via the acrylate-CoA pathway, converting lactate into propionate and acetate (Cardon and Barker, 1946; Tholozan et al., 1992). *C. propionicum* was reported to not consume carbohydrates (Cardon and Barker, 1946; O'Brien et al., 1990). There have been no previous reports of *n*-propanol production during syngas fermentation via mixed culture. This study investigated the production of ethanol, *n*-propanol and *n*-butanol during syngas fermentation using the mixed culture in YE and CSL media in a 3 L fermentor. The ability of a monoculture of *A. bacchi* strain CP15 and a mixed culture of mainly *A. bacchi* CP15 and *C. propionicum* to convert carboxylic acids into their corresponding alcohols was also examined.

2. Methods

2.1. Microorganisms

The mixed culture was obtained from the fermentor at the end of the continuous syngas fermentation as reported previously (Liu et al., 2014). The mixed culture was transferred to yeast extract (YE) medium and maintained under syngas (40% CO, 30% CO_2 and 30% H_2) and used as the inoculum source for all experiments performed in the present study. The monoculture of *A. bacchi* strain CP15 and mixed culture were maintained on YE medium with an initial pH 8.0 under anaerobic condition at 37 °C. The YE medium preparation was described previously (Liu et al., 2012). Inocula of strain CP15 and the mixed culture were prepared by sub-culturing twice to reduce the lag phase of growth. The inocula of the mixed culture were from the same mother culture and were prepared in YE medium and incubated at 37 °C. Syngas made of 40% CO, 30% CO_2 and 30% H_2 was used. This was done to ensure consistency in the initial composition of the mixed culture used in all experiments. Fermentations with either strain CP15 or the mixed culture were inoculated with 10% (v/v) inocula.

2.2. Semi-continuous fermentation in a 3 L fermentor using mixed culture

A 3 L fermentor (Bioflo 110, New Brunswick Scientific Co., Edison, NJ, USA) with 2.5 L working volume was used in a semi-continuous fermentation (i.e., only continuous syngas feed). Two six-blade Rushton impellers separated by a distance equal to the impeller diameter were mounted on an agitator shaft as suggested by Bakker et al. (1994). Four baffles were used to avoid vortices.YE medium and 20 g/L CSL medium without TAPS buffer were used. The YE medium also contained YE, minerals, vitamins and trace metals as descried previously (Liu et al., 2012). The 20 g/L CSL was used to replace YE, vitamins and minerals in the YE medium. All media contained 5 g/L $NaHCO_3$ as a buffer, 2.5 mL/L of 4% cysteine sulfide solution as a reducing agent and 1 mL/L of 0.1% resazurin solution as a redox indicator. The compositions of the minerals, trace metals and vitamins stock solutions were reported previously (Tanner, 2007). The medium in the fermentor was sterilized at 121 °C for 30 min and allowed to cool to room temperature by purging 18 sccm (standard cubic centimeter per minute) N_2 at 150 rpm agitation for 3 h. The medium was then purged for 8 h with 18 sccm syngas (38% CO, 28.5% H_2, 28.5% CO_2 and 5% N_2 by volume, Stillwater Steel Co., Stillwater, OK, USA). Before inoculation, the medium was reduced by adding 2.5 mL/L 4% cysteine sulfide. The fermentor was inoculated with 10% (v/v) of the mixed culture. The inlet gas flow rate was controlled by a thermal mass flow controller (Porter, Hatfield, PA, USA). The pH of the medium during the fermentation was controlled above 6.1 by the addition of 7% $NaHCO_3$ based on preliminary results, which showed a substantial decrease in H_2 conversion at lower pH values. When foam in the fermentor was 1.3 cm above the level of liquid, 0.2 mL of 5% antifoam (Antifoam B emulsion, Sigma–Aldrich, St. Louis, MO, USA) was added. The fermentation temperature was controlled at 37 °C via a heating jacket (New Brunswick Scientific Co.). A condenser and a bubbler controlled at 5 °C by a refrigerated recirculator (1156D, VWR International, West Chester, PA, USA) were used to condense the vapor leaving the exhaust gas line. Liquid and gas samples were withdrawn from the reactor periodically to measure pH, cell mass and product concentrations, and gas composition in the exhaust gas line.

2.3. Conversion of carboxylic acids into alcohols in bottle fermentations

Fed-batch fermentations in 250 mL bottles (Wheaton, NJ, USA) with 100 mL working volume were used in the conversion of carboxylic acids into alcohols. The fermentation medium used was the YE medium. The carboxylic acids used included propionic acid, butyric acid, hexanoic acid and lactic acid. Each carboxylic acid was added separately to the medium at the beginning of fermentation at an initial concentration around 1.5 g/L. A control treatment contained all medium components but no carboxylic acid. The initial pH of the medium was adjusted to pH 7.5 by adding sterilized 2 N KOH, and each bottle was inoculated with 10% (v/v) of either strain CP15 or the mixed culture. The syngas used contained 40%