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Continuous syngas fermentation for the production of ethanol, n-propanol and n-butanol

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HIGHLIGHTS

- 6 g/L of ethanol was obtained in continuous syngas fermentor with cell recycle.
- Opportune contamination of the fermentor resulted in production of higher alcohols.
- Mixed culture was mainly made of *A. bacchi* strain CP15 and *C. propionicum*.
- Mixed culture formed a maximum of 8 g/L ethanol, 6 g/L propanol and 1 g/L butanol.
- Mixed culture presents an opportunity for higher alcohols production from syngas.

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ABSTRACT

Syngas fermentation to fuels is a technology on the verge of commercialization. Low cost of fermentation medium is important for process feasibility. The use of corn steep liquor (CSL) instead of yeast extract (YE) in *Alkalibaculum bacchi* strain CP15 bottle fermentations reduced the medium cost by 27% and produced 78% more ethanol. When continuous fermentation was performed in a 7-L fermentor, 6 g/L ethanol was obtained in the YE and YE-free media. When CSL medium was used in continuous fermentation, the maximum produced concentrations of ethanol, n-propanol and n-butanol were 8 g/L, 6 g/L and 1 g/L, respectively. n-Propanol and n-butanol were not typical products of strain CP15. A 16S rRNA gene-based survey revealed a mixed culture in the fermentor dominated by *A. bacchi* strain CP15 (56%) and *Clostridium propionicum* (34%). The mixed culture presents an opportunity for higher alcohols production from syngas.

1. Introduction

Syngas fermentation is part of the hybrid thermochemical–biochemical process, also called gasification–syngas fermentation. In this process, feedstocks such as biomass or municipal solid waste are gasified into syngas (CO, H_2 and CO_2), which is then converted into biofuels and chemicals using microbial catalysts (Wilkins and Atiyeh, 2011). Syngas can be converted into ethanol using acetogens such as *Clostridium ljungdahlii*, *Clostridium ragsdalei*, "*Clostridium carboxidivorans*" and "*Clostridium autoethanogenum*" (Phillips et al., 1993; Wilkins and Atiyeh, 2011; Ukpong et al., 2012).

Decreasing the medium cost and increasing ethanol titer and productivity are important to improve the economic feasibility of the production of biofuels and chemicals using syngas fermentation technology. Low cost nutrients such as cotton seed extract (CSE) and corn steep liquor (CSL), have been used instead of yeast extract (YE) in syngas fermentation for ethanol production (Kundiyana et al., 2010; Maddipati et al., 2011). CSL is rich in proteins, vitamins, minerals and amino acids (Lawford and Rousseau, 1997). The industrial cost of CSL was reported to be \$0.18/kg, which is about 2% of the industrial price of YE, \$9.20/kg (Maddipati et al., 2011). The use of CSL as a replacement of YE, vitamins and minerals in a 7-L fermentor with "*C. ragsdalei*" resulted in 40% more ethanol production compared to YE medium (Maddipati et al., 2011), indicating the potential of CSL as a low cost nutrient for syngas fermentation.

High ethanol titer and productivity can be achieved through higher cell concentration and improving the mass transfer of the substrate gases CO and H_2 to cells. The highest reported ethanol concentration was 48 g/L, achieved using *C. ljungdahlii* during continuous syngas fermentation with cell recycle and 4 g/L cell mass

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concentration (Phillips et al., 1993). However, only 6.5 g/L ethanol was produced during continuous syngas fermentation using *C. ljungdahlii* without cell recycle and 2.3 g/L cell mass concentration (Mohammadi et al., 2012). This showed the advantage of cell recycle to obtain high cell and ethanol concentrations during syngas fermentation. When "*C. ragsdalei*" was used in a two-stage continuous syngas fermentation with cell recycle, a maximum ethanol yield of 15 g ethanol/g cells was obtained (Kundiyana et al., 2011), which was comparable to the yield (12 g ethanol/g cells) with *C. ljungdahlii* (Phillips et al., 1993). The ability to produce high concentrations of ethanol depends on the microorganism, syngas composition and fermentor operating conditions. *Eubacterium limosum* KIST612 only produced 0.3 g/L ethanol in a continuous fermentation using pure CO and cell recycle with a 4 g/L cell mass concentration (Chang et al., 2001).

The H_2:CO ratios in previously reported syngas fermentations were mostly below 0.75, which results in lower carbon conversion efficiency to ethanol or acetic acid as shown in Table 1 (Wilkins and Atiyeh, 2011). H_2 serves as an electron source and CO can be used as either an electron source or converted by the microorganism to ethanol or acetic acid. The biochemical reaction proceed in the direction of favored thermodynamics (i.e., when Gibbs free energy, ΔG°, <0). All reactions in Table 1 are thermodynamically feasible. However, ΔG° decreases as the H_2:CO ratio increases, which makes the reaction less favorable thermodynamically. The H_2:CO ratios are affected by the gasification operating conditions and feedstock used. A H_2:CO ratio of 2 can be produced when steam and pure O_2 are used in the gasification process (Turn et al., 1998), which can result in a theoretical carbon to ethanol conversion efficiency of 100%. In addition, an H_2:CO ratio of 2 was reported from gasification of dairy biomass (cow manure) using air (Gordillo and Annamalai, 2010), indicating the potential of dairy biomass for biofuels production.

Recently, *Alkalibaculum bacchi* strains CP11^T, CP13 and CP15 were found to grow at an initial pH 8.0 and convert syngas into ethanol and acetic acid in YE medium (Allen et al., 2010; Liu et al., 2012). In bottle fermentations, strain CP15 was found to be the most promising *A. bacchi* strain for ethanol production because of its higher growth and ethanol production rate and yield compared to CP11^T and CP13 (Liu et al., 2012). However, further process development is required for strain CP15 to increase its potential use in large scale ethanol production. This includes reducing the fermentation medium cost and investigating characteristics of CP15 at larger scale than fermentation bottles. The YE medium cost was relatively expensive at $10.53/L, mostly due to the high cost of the [Tris (hydroxymethyl) methyl]-3-amino propanesulfonic acid (TAPS) buffer. Thus, the first object of the present study was to reduce the cost of CP15 fermentation medium by removal of costly TAPS buffer and replacing YE with CSL. The second objective was to scale up the fermentation from bottle to a 7-L fermentor in continuous mode with cell recycling.

2. Methods

2.1. Microorganisms

A. bacchi strain CP15 was maintained under anaerobic condition in a standard YE medium at initial pH 8.0 and 37 °C. The medium preparation and compositions were reported previously (Liu et al., 2012). Strain CP15 inoculum was prepared by sub-culturing twice to reduce the growth lag phase. Inoculum size used was 10% (v/v).

2.2. Effect of medium composition

Four fermentation media were formulated without the addition of TAPS buffer with an objective to reduce medium cost (Table 2). The composition of the YE medium with 3× minerals was similar to standard YE medium but with 3-fold more minerals, which was also similar to the amount of minerals added in the YE medium for "*C. ragsdalei*" in a previous study (Maddipati et al., 2011). In the two CSL media, 20 g/L or 50 g/L CSL replaced YE, vitamins and minerals in the standard YE medium. In addition, 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 CSL (Sigma–Aldrich, St. Louis, MO, USA) used in the present study was centrifuged at 16,000*g* for 10 min using Accuspin Micro centrifuge (Fisher Scientific, Pittsburgh, PA, USA) to remove the solids before preparing the fermentation medium. The solids were removed to allow measurement of cell mass concentration in the fermentation broth. The fermentation was done in 250-mL serum bottles (Wheaton, NJ, USA) each containing 100 mL of medium. The syngas mixture contained 20% CO, 15% CO_2, 5% H_2 and 60% N_2 by volume, which was similar to producer gas generated from the Oklahoma State University gasification facility using switchgrass (Ahmed et al., 2006). The syngas was fed in the fermentation bottles every 24 h at 239 kPa. Fermentation

Table 1
Carbon to ethanol and acetic acid conversion efficiencies from syngas with various H_2:CO ratios.

	Stoichiometry	H_2:CO	Carbon conversion efficiency (%)	Product yield from CO (%)	Gibbs free energy, ΔG° (kJ/mol)
(1)	$6CO + 3H_2O \rightarrow C_2H_5OH + 4CO_2$	0	33.3	16.7	−217.4
(2)	$3CO + 3H_2 \rightarrow C_2H_5OH + CO_2$	1	66.7	33.3	−157.2
(3)	$2CO + 4H_2 \rightarrow C_2H_5OH + H_2O$	2	100.0	50.0	−137.1
(4)	$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2$	0	50.0	25.0	−154.6
(5)	$2CO + 2H_2 \rightarrow CH_3COOH$	1	100.0	50.0	−114.5

Table 2
Compositions of four media formulations used in bottle fermentations.

Medium components (per L)[a]	YE (g/L)	CSL (g/L)	Minerals (mL/L)[b]	Trace metals (mL/L)[b]	Vitamins (mL/L)[b]
Standard YE medium	1	–	10	10	10
YE medium with 3× minerals	1	–	30	10	10
20 g/L CSL medium	–	20	–	10	–
50 g/L CSL medium	–	50	–	10	–

[a] TAPS absent from all media; other components added in all media include 5% $NaHCO_3$, 2.5 mL/L of 4% cysteine sulfide and 1 mL/L of 0.1% resazurin.
[b] Compositions of mineral, trace metal and vitamin stock solutions are provided in Tanner (2007).