Bioresource Technology 102 (2011) 6494–6501

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Ethanol production from syngas by *Clostridium* strain P11 using corn steep liquor as a nutrient replacement to yeast extract

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ARTICLE INFO

Article history:
Received 20 December 2010
Received in revised form 15 March 2011
Accepted 16 March 2011
Available online 21 March 2011

Keywords:
Clostridium
Corn steep liquor
Ethanol
Syngas fermentation
Yeast extract

ABSTRACT

The feasibility of replacing yeast extract (YE) by corn steep liquor (CSL), a low cost nutrient source, for syngas fermentation to produce ethanol using *Clostridium* strain P11 was investigated. About 32% more ethanol (1.7 g L^{-1}) was produced with 20 g L^{-1} CSL media in 250-mL bottle fermentations compared to media with 1 g L^{-1} YE after 360 h. Maximum ethanol concentrations after 360 h of fermentation in a 7.5-L fermentor with 10 and 20 g L^{-1} CSL media were 8.6 and 9.6 g L^{-1}, respectively, which represent 57% and 60% of the theoretical ethanol yields from CO. Only about 6.1 g L^{-1} of ethanol was obtained in the medium with 1 g L^{-1} YE after 360 h, which represents 53% of the theoretical ethanol yield from CO. The use of CSL also enhanced butanol production by sevenfold compared to YE in bottle fermentations. These results demonstrate that CSL can replace YE as the primary medium component and significantly enhance ethanol production by *Clostridium* strain P11.

1. Introduction

The dominant biofuel in the US is ethanol made via yeast-based fermentation of corn starch, which is a well-developed technology. Ethanol made via fermentation of synthesis gas (syngas) produced from gasification of non-edible feedstocks such as biomass and municipal solid wastes is relatively a new technology. Syngas, primarily containing CO, CO_2, and H_2, can be fermented to ethanol and acetic acid by acetogenic organisms such as *Clostridium ljungdahlii* (Klasson et al., 1992; Phillips et al., 1994; Younesi et al., 2005), *Clostridium autoethanogenum* (Abrini et al., 1994), *Clostridium carboxidivorans* P7 (Ahmed and Lewis, 2007; Rajagopalan et al., 2002), and *Clostridium* strain P11 (Huhnke et al., 2010; Saxena, 2008). These microorganisms utilize the reductive acetyl-CoA pathway, also known as the "Wood–Ljungdahl" pathway, for the synthesis of acetyl-CoA, conservation of energy and growth and production of acetic acid and ethanol (Wood et al., 1986). The overall reactions for ethanol and acetic acid production from H_2 and CO are (Vega et al., 1989):

$$6CO + 3H_2O \rightarrow C_2H_5OH + 4CO_2 \quad (1)$$

$$6H_2 + 2CO_2 \rightarrow C_2H_5OH + 3H_2O \quad (2)$$

$$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2 \quad (3)$$

$$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O \quad (4)$$

When equal moles of CO and H_2 are supplied, theoretically two-thirds of the carbon from CO can be converted to ethanol and the remaining carbon is accounted for in CO_2 production as seen by combining Eqs. (1) and (2). However, all the carbon supplied in the form of CO theoretically can be converted to acetic acid by combining Eqs. (3) and (4).

Butanol production has also been reported from CO (Shen et al., 1999; Worden et al., 1991) or a mixture of CO, CO_2 and H_2 (Babu et al., 2010; Datar et al., 2004; Liou et al., 2005; Rajagopalan et al., 2002) by only three bacteria. *Butyribacterium methylotrophicum* was reported to produce between 0.3 and 1.4 g L^{-1} butanol from pure CO (Shen et al., 1999; Worden et al., 1991). However, *Clostridium* strain P11 produced 0.3 g L^{-1} butanol from a simulated syngas mixture (Babu et al., 2010) and *C. carboxidivorans* produced 1.3 g L^{-1} butanol from syngas derived from cellulosic feedstock (Rajagopalan et al., 2002). The overall reactions for butanol production from H_2 and CO are (Rajagopalan et al., 2002):

$$12CO + 5H_2O \rightarrow C_4H_9OH + 8CO_2 \quad (5)$$

$$12H_2 + 4CO_2 \rightarrow C_4H_9OH + 7H_2O \quad (6)$$

A recent literature review on biomass-derived syngas fermentation into biofuel describes the factors that affect syngas

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doi:10.1016/j.biortech.2011.03.047

fermentation, which include type of biocatalyst and growth media, mass transfer, reactor configuration and operating conditions (Munasinghe and Khanal, 2010).

Composition of the fermentation medium plays an important role in cell and product yields. The development of a low-cost fermentation medium alternative containing all essential nutrients required for cell growth and product formation would reduce the overall cost of the fermentation process. Standard medium for *Clostridium* strain P11 is composed of yeast extract, vitamins, minerals, trace metals and reducing agent (Saxena and Tanner, 2010). Apart from the reducing agent, yeast extract (YE) is the most expensive component. Some inexpensive nutrients that could replace YE are corn steep liquor (CSL), hydrolyzed cotton seed flour, hydrolyzed soy flour and ethanol stillage (Witjitra et al., 1996).

CSL is a major by-product from the corn wet milling industry. It is rich in vitamins, minerals, amino acids and proteins (Azeredo et al., 2006; Kadam and Newman, 1997). It has been used as growth medium for mannitol production by *Lactobacillus intermedius* (Racine and Saha, 2007), and solvent production by *Saccharomyces cerevisiae* (Kadam and Newman, 1997), *Clostridium beijerinckii* (Parekh et al., 1999) and *Zymomonas mobilis* (Lawford and Rousseau, 1997). The cost of bacto-yeast extract from Difco Laboratories (Detroit, MI) is \$157.49 kg^{-1} (Racine and Saha, 2007), while the cost of CSL on an industrial scale is \$0.07 kg^{-1} (Lawford and Rousseau, 1997). As per 2010, the industrial price of spray dried YE is \$9.2 kg^{-1}, while the cost of industrial CSL is \$0.18 kg^{-1}. The incorporation of CSL at 20 g L^{-1} in the fermentation medium resulted in elimination of a few growth factors from standard *Clostridium* strain P11 medium (Saxena, 2008). However, this study did not investigate growth and product kinetics of strain P11 in CSL media. In addition, tests were done in 160-mL serum bottles with 10 mL of medium and only CO was fed to strain P11, which was different from the syngas composition that potentially will be used for ethanol production on a large scale.

Corn steep liquor (CSL) is selected in the present study as an alternative to yeast extract (YE) for syngas fermentation because it is rich in nutrients and lower in cost compared to YE. Accordingly, the main objective of this study was to determine whether CSL is a potential alternative nutrient source to YE in syngas fermentation medium and to evaluate growth and product kinetics of *Clostridium* strain P11 using CSL as compared to YE.

2. Methods

2.1. Microorganism

Clostridium strain P11 (ATCC PTA-7826) provided by Dr. Ralph Tanner, University of Oklahoma, was maintained on standard yeast extract medium. *Clostridium* strain P11 is highly sensitive to O_2; and hence, the fermentation was performed under strict anaerobic conditions. In order to reduce the lag phase and also to ensure that viable cells were inoculated into the medium, cells from the stock inoculum were passaged twice prior to inoculation. Cell passaging and syngas fermentation were performed in 250-L serum bottles with 100 mL working volume. After cell passaging, the subculture at 10% (v/v) was transferred to the fermentation serum bottles and the 7.5-L fermentor to follow syngas fermentation.

2.2. Fermentation media

Three media formulations developed by Saxena (2008) were used (Table 1). The standard medium contained yeast extract, minerals, trace metals, vitamins, morpholinoethanesulfonic acid (MES), resazurin and cysteine sulfide. The second medium (10 g L^{-1} CSL) had the same components as the standard medium except yeast extract was replaced with CSL. The third medium (20 g L^{-1} CSL) only contained trace metals, MES, resazurin, ammonium chloride and cysteine sulfide. Yeast extract (Difco Laboratories, Detroit, MI) and CSL (Sigma–Aldrich, St. Louis, MO) were used as complex nitrogen and nutrient sources in the fermentation media. The compositions of minerals, vitamins and trace metal stock solutions are shown in Table 2. Unless stated, all media components were purchased from Sigma–Aldrich (St. Louis, MO).

In order to produce consistent results, CSL from the same batch was used throughout the study. CSL contained about 50% solids. Before the addition of CSL into the fermentation medium, the solids from crude CSL were removed by centrifugation at 13,000 rpm for 10 min. Only the liquid portion of the crude CSL was used in the syngas fermentation experiments to avoid interference of solids with measurement of P11 growth. The presence or removal of solids from CSL did not affect ethanol and acetate formation by strain P11 (Saxena, 2008). In addition, removal of solids from CSL did not affect the nutritional requirements of *Zymomonas* ethanol

Table 1
Composition of media formulations used for syngas fermentation (Saxena, 2008).

	Standard yeast extract 1 g L^{-1}	CSL[a] 10 g L^{-1}	CSL[a] 20 g L^{-1}
Yeast extract (g)	1	–	–
Corn steep liquor (g)	–	10	20
Minerals (mL)	30	30	–
Trace metals (mL)	10	10	10
Vitamins (mL)	10	10	–
MES[b] (g)	10	10	10
0.1% Resazurin (mL)	1	1	1
Ammonium chloride (g)	–	–	1.25
4% Cysteine sulfide (mL)	10	10	2.5
Total volume (L)	1	1	1

[a] CSL = corn steep liquor.
[b] MES = morpholinoethanesulfonic acid.

Table 2
Composition of trace metal, vitamin and mineral stock solutions (Huhnke et al., 2010).

Trace metal stock solution	g L^{-1}
Cobalt chloride	0.20
Ferrous ammonium sulfate	0.80
Manganese sulfate	1.00
Nickel chloride	0.20
Nitrilotriacetic acid	2.00
Sodium molybdate	0.02
Sodium selenate	0.10
Sodium tungstate	0.20
Zinc sulfate	1.00
Mineral stock solution	g L^{-1}
Ammonium chloride	100
Calcium chloride	4
Magnesium sulfate	20
Potassium chloride	10
Potassium phosphate	10
Sodium chloride	80
Vitamin stock solution	mg L^{-1}
p-(4)-Aminobenzoic acid	5
d-Biotin	2
Calcium pantothenate	5
Folic acid	2
MESNA[a]	10
Nicotinic acid	5
Pyridoxine	10
Riboflavin	5
Thiamine	5
Thioctic acid	5
Vitamin B12	5

[a] MESNA = mercaptoethanesulfonic acid.