e-ISSN:2320-3528
p-ISSN:2347-2286

Research & Reviews: Journal of Microbiology and Biotechnology

Comparison of Ethanol Fermentation Efficiency: Syngases versus Microorganisms

Feng-Qin Wang[1#], Yan-da Zhang[1#], Bing-bing Sun[2], Hui Xie[1], Da-jiao Yang[1], Sen -Yang[1], Tian-bao Ren[3], Sheng Yang[2], An-Dong Song[1]*

[1]Key Laboratory of Enzyme Engineering of Agricultural Microbiology, Ministry of Agriculture, College of Life Science, Henan Agricultural University, Zhengzhou 450002, P. R. China
[2]Key Laboratory of Synthetic Biology, Chinese Academy of Sciences, Shanghai 200000, P. R. China
[3]National Tobacco Cultivation and Physiological and Biochemical Research Centre, Henan Agricultural University, Zhengzhou 450002, P. R. China
[#]Equally Contributed

Research Article

Received date: 16/06/2016
Accepted date: 18/08/2016
Published date: 28/08/2016

***For Correspondence**

An-Dong Song, Henan Agricultural University, Zhengzhou 450002, Henan, P.R. China

E-mail: songandong@henau.edu.cn

Keywords: Simulative syngas, Microorganisms, Ethanol, High-efficiency fermentation.

ABSTRACT

The anaerobic fermentation required for ethanol production from syngas appears to be a promising and competitive engineering application technology. The main challenge facing for syngas fermentation is the fact that it is often limited by low ethanol productivity. To evaluate the ability of different syngases and microorganisms to ferment ethanol and screen those efficient syngases and microorganisms, three simulative syngas mixtures and eight strains were investigated in 300 ml bottle fermentations. The results show that, both syngases and cultures affected ethanol production at a highly significant level ($p<0.01$). The maximum net ethanol concentrations (28.001, 23.871, 22.909 and 19.726 mg/L) were obtained with strains LP-*fm*4, *C. carboxidivorans* P7, B-*fm*4 and *C. ljungdahlii* using biomass-generated syngas, which yielded approximately two and three times more ethanol compared with strains *C. ljungdahlii*, B-*fm*4 (11.734, 10.300 mg/L) using corex-gas and strains *C. carboxidivorans* P7, *C. ragsdalei* P11 (9.937, 8.318 mg/L) using blast furnace gas, respectively. So, using biomass-generated syngas and strains LP-*fm*4、*C. carboxidivorans* P7、B-*fm*4 and *C. ljungdahlii* represented the best combination for fermenting ethanol efficiently compared with the other two. In addition, the maximum ethanol production per unit cell of both strains LP-*fm*4 and B-*fm*4 with biomass-generated syngas, *C. ragsdalei* P11 with blast furnace gas and B-*fm*4 with corex-gas were 1000.036, 881.103, 519.854 and 468.030 mg/L, respectively. This indicates that strains LP-*fm*4 and B-*fm*4 are the most promising for biomass-generated syngas fermentation, strains *C. ragsdalei* P11 and B-*fm*4 are potential candidates for blast furnace gas and corex-gas fermentations respectively.

INTRODUCTION

With the rapid growth in the world's population and the increasing development of industrialization, more and more energy resources are required eagerly. According to calculated data, the population of our planet for the year 2050 is estimated to be in excess of 9 billion [1]. In addition, fossil fuels containing oil and natural gas, are being rapidly depleted, total reverses to the end of year 2012 are approximately able to support the energy consumption for the next 51 and 56 years respectively [2]. On the

other hand, the burning of fossil fuels has caused an increase in greenhouse gas emissions that causes global warming, acid-rain and urban smog, etc. So these negative environmental consequences of fossil fuel consumption and concerns about petroleum supplies have spurred the search for new, more sustainable and renewable energy sources [3-6]. Ethanol is one of the most promising alternative biofuels, which provides a net energy gain, has environmental benefits and is economically competitive. For instance, the US biofuels industry is undergoing rapid growth and transformation, it has mandated the production of 36 billion gallons of biofuels by 2022 [7,8] while China will produce 500 million tons of ethanol based by 2015. Planned production of ethanol will expand to 1 billion tones by year 2020. However, this bioethanol is mostly derived from food sources of feedstock such as sugar, corn or starch, which may become a hidden threat to global food security in the future.

Luckily, fermentation of syngas or waste gas components to produce ethanol appears to be a promising alternative compared to existing chemical techniques [9-11]. Syngas is a mixture of principally CO, CO_2 and H_2, which can be produced by gasification of solid fuels (such as coal, oil shale, petroleum coke, biomass or organic wastes from daily life, etc) [9,12-16]. Moreover, with the growth in the iron and steel industries, more and more exhaust gases are also used as syngas through pre-treatment processes: these are directly emitted into the atmosphere generally. Compared to other conversion technologies, syngas fermentation offers several advantages [10,17]. However, the phenomenon facing current syngas fermentation is low ethanol productivity caused by low gas-liquid mass transfer, syngas composition and the fermentation microorganisms themselves. Of these three factors, the former has been studied more [18-21], and the latter two are relatively less. To date, steel waste gases such as blast furnace gas and corex gas have not been studied further as the amount of fermentation strains found is too small. Bacteria used in the hybrid conversion process are called acetogens, which are anaerobes that assimilate syngas fermenting ethanol via the Wood-Ljungdahl pathway. Examples of cultures used are *Clostridium ljungdahlii* [22,23], *Clostridium carboxidivorans* P7 [24,25], *Clostridium ragsdalei* P11 [26], *Clostridium autoethanogenums* [27], Alkalibaculum bacchi strains CP11T, CP13 [28], etc. Meanwhile, this research groups has isolated and enriched four dominant microflorae from animal faeces samples of alpaca, gibbon, lesser panda and papion with biomass syngas under strict anoxic conditions, these are A-*fm*4, G-*fm*4, LP-*fm*4, B-*fm*4, respectively.

To evaluate the ability of different syngases and microorganisms to ferment ethanol and screen the efficient syngases and microorganisms improving ethanol production in syngas fermentation, this study was designed to investigate three simulative syngas mixtures (biomass-generated syngas, blast furnace gas and corex gas) and eight strains (A-*fm*4, G-*fm*4, LP-*fm*4, B-*fm*4, *Clostridium autoethanogenums* DSM10061, *C. ljungdahlii*, *Clostridium carboxidivorans* P7 and *Clostridium ragsdalei* P11) in 300ml bottle fermentations.

MATERIALS AND METHODS

Microorganisms, Syngases and Media

The bacteria used in this study were A-*fm*4, B-*fm*4, G-*fm*4, LP-*fm*4, *Clostridium autoethanogenums* DSM10061, *C. ljungdahlii* (ATCC 55383), *Clostridium carboxidivorans* P7 and *Clostridium ragsdalei* P11 respectively. A-*fm*4, B-*fm*4, G-*fm*4 and LP-*fm*4 were isolated in a laboratory. The genomic DNA of the four samples was extracted and sequenced, from which their phylogenetic tree was constructed. Analysis shows that the main microorganism in the four dominant microflora are anaerobic bacterium, bacillus and coccus in A-*fm*4, bacillus and enterobacter in G-*fm*4 and B-*fm*4, and bacillus in LP-*fm*4. Like the *Clostridium autoethanogenums*, the four dominant microflora are gram-positive, and the main bacterium contained in the dominant microflora are rod-like. G-*fm*4 is mostly like DSM10061; both are rod-like, gram-positive and spore-forming. Moreover, *C. autoethanogenums* DSM10061 was acquired from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany), cultures *C. ljungdahlii* (ATCC 55383); *Clostridium carboxidivorans* P7 and *Clostridium ragsdalei* P11 were provided by The Key Laboratory of Synthetic Biology (Chinese Academy of Sciences, Shanghai, China). These strains were maintained on their respective growth media as reported previously [14], and allowed to grow under strict anoxic conditions at 37°C, at a pH of 5.75.

Three artificial syngas mixtures obtained from Yuan Zheng Development Company (Zhengzhou, China) were investigated in this study. The first syngas, a biomass-generated syngas, contained 85.5% CO, 10% H_2 and 4.5% CO_2 by volume; its composition simulates the gas from maize straw after gasification. The latter two syngases (blast furnace gas and corex gas), are by-products of the iron-steel industry, and simulate a gases from Baosteel (Baosteel Group Corporation, Shanghai, China). Blast furnace gas contained 22.4% CO, 23.6% CO_2, 3.3% H_2, 50.6% N_2 and 0.1% O_2 by volume. Corex gas containe 17.72% H_2, 45.23% CO, 33.17% CO_2, 1.68% CH_4, 2.20% N_2 and 100 to 120 ppm H_2S.

The fermentation medium [29] used contained (per litre) :10ml mineral solution [14], 10ml vitamin solution [14], 1.0 g NH_4Cl, 1.0g NaCl, 0.15 g $MgSO_4$, 0.1 g KH_2PO_4, 0.04 g $CaCl_2$, 2.0 g Tryptone, 0.3 g yeast extract, 10.0 g 2-(N-morpholino)ethanesulfonic acid (MES), 0.1 ml 0.1% resazurin solution, and 0.2 g/L cysteine-HCl for use as a reducing agent. The pH of the medium was adjusted to 4.5 using 10 M NaOH. Vitamin solution and cysteine sterilized by filtration, and then added to the medium after autoclaving. The medium was firstly prepared by boiling for a few minutes, while being degassed, and then cooled continuously in an YX-IIanaerobic box (Shanghai Medical Equipment Manufacturing Co., Ltd, Shanghai, China) for 24 hours to remove any oxygen. Finally, the media in bottles were sterilized at 121°C for 20 min. All experiments were carried out in triplicate.

Fermentation Runs

Batch fermentations were done in 300 ml serum bottles each containing 60 ml of fermentation medium. 10% (v/v) of inoculum was transferred to fresh media. The cultures were maintained under anaerobic conditions and agitated at 150 rpm on an QHZ-98A orbital shaker (Huamei Biochemical Instrument Company, Taicang City, China), inside an incubation chamber at 37°C, which were grown with three simulative syngas mixtures as the sole substrate. The syngas inlet volume was 240 ml, injected in each 300 ml serum bottle by syringe. Experiments were conducted every 24 h for 5 days, samples (2.0 ml) were collected to analyse pH and growth. Another 1.5 ml sample was centrifuged (10000 rpm, 10 min) to remove the cells; the supernatant was used to measure ethanol production.

Statistical Analysis

Analysis of variance (ANOVA) was undertaken using SPSS 16.0 software for ethanol production based on three simulative syngas mixtures and eight strains, it was used to determine if statistical significant differences exist in ethanol production, organisms, syngases, the maximum ethanol production and ethanol production per unit cell between the treatments with the strains and syngas mixtures used at the 95% confidence level. In addition, a t-test was also employed to determine whether or not values were significantly different for the maximum ethanol production and ethanol production per unit cell compared with the control strain *C. autoethanogenums* DSM10061. Ethanol production per unit cell [30] was calculated as follows:

$$\text{Ethanol production per unit cell} = \frac{\text{The maximum ethanol production}^* - \text{Initial ethanol production}^*}{\text{Maximum Cell mass}(\text{OD600})}$$

Where the maximum ethanol production*, Initial ethanol production* and Maximum cell mass (OD600)* are the mean of three observations, respectively.

Analytical Procedures

Growth was monitored by measuring optical density (OD) at 600 nm with a 722S spectrophotometer (Qinghua Scientific Instruments Co., Ltd., Shanghai, China). Ethanol concentration was analyzed using Agilent 7890A GC system (Agilent Technologies, Wilmington, DE, USA) with a flame ionization detector (FID) and an HP-FFAP capillary column (30 m × 0.32 mm × 0.3 μm) (Agilent Technologies, Wilmington, DE, USA). The sample size was 0.2 μL. Nitrogen was used as carrier gas at flow rate of 1.5 mL/min. The inlet port temperature was kept at 200°C with a split ratio 30:1. The initial oven temperature was set at 45°C with a holding time of 1.0 min. It was then increased at a ramping rate of 10°C/min to 80°C with a holding time of 0.5 min. The FID temperature was set to 250°C with hydrogen and air flow rates of 30 ml/min and 350 ml/min, respectively.

RESULTS

Cell Growth and Effect

The growth profiles of eight cultures using three syngas mixtures in batch fermentations are shown in **Figure 1.** It is observed that eight strains nearly had a decreasing trend during fermentation. **Figure 1A** shows there is a minor change in cell mass concentrations of the strains except for *Clostridium carboxidivorans* P7, which started to grow until one day had elapsed and attained a maximum concentration (OD600=0.061), then decreased rapidly until the fourth day. However, compared to biomass-generated syngas fermentation, eight organisms had a similar growth trend as shown in **Figures 1B and 1C.** Additionally, strains G-*fm*4, *C. autoethanogenums* DSM10061 and *Clostridium ragsdalei* P11 started to grow until the second, third and fourth days respectively **(Figure 1B).** For cultures A-*fm*4, B-*fm*4 and LP-*fm*4, they all reached their maximum cell level at the end of the second day, while control strain *C. autoethanogenums* DSM10061 reached the same piont after the fourth day **(Figure 1C).**

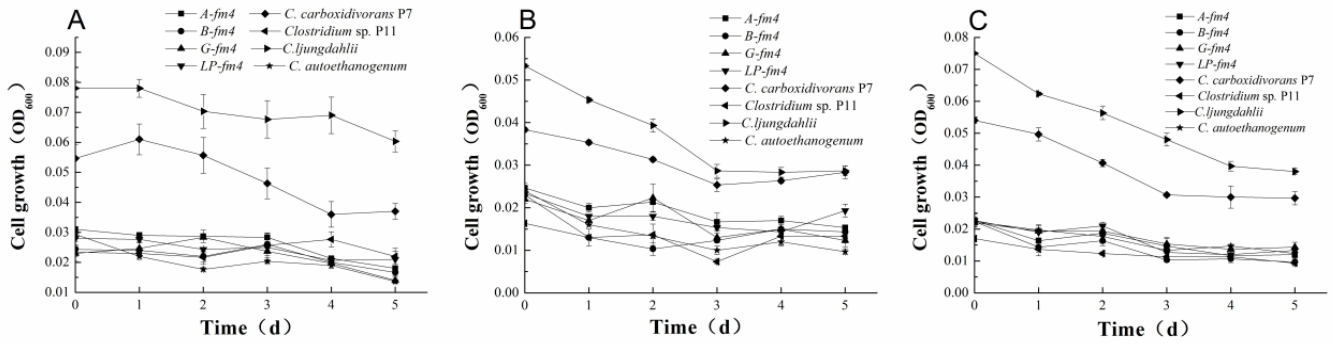

Figure 1. Growth of organisms in batch culture with syngases. A, eight cultures with biomass-generated syngas. B, eight cultures with blast furnace gas. C, eight cultures with corex gas. Error bars (n=3) represent plus and minus one standard deviation from the average of every experimental replicates.

Product Formation and Effect

Figure 2A shows the ethanol production profiles of eight strains using biomass-generated syngas in batch cultures. The maximum ethanol concentration (82.006 mg/L) was produced by *Clostridium ljungdahlii* at the end of the third day; this

e-ISSN:2320-3528
p-ISSN:2347-2286

concentration was significantly higher than in the other cases. The cultures, except *Clostridium ljungdahlii* could all attain their peak of ethanol concentration by the end of the fourth day. For organisms, *Clostridium carboxidivorans* P7, LP-*fm*4 and B-*fm*4, the ethanol concentration in batch fermentation were 56.386, 51.729 and 46.922 mg/L, respectively, which also increased significantly compared with strains A-*fm*4, G-*fm*4, *Clostridium ragsdalei* P11 and the control. Additionally, only strains A-*fm*4, B-*fm*4, LP-*fm*4, *Clostridium carboxidivorans* P7 and *Clostridium ragsdalei* P11 were observed to produce ethanol during fermentation in the eight cultures **(Figure 2B).** The strain *Clostridium carboxidivorans* P7 had the highest ethanol concentration at 38.282 mg/L in a manner similar to **Figure 2A. Figure 2C** shows that the ethanol concentration peaked at the end of the fourth day (53.866 mg/L) and subsequently decreased on the fifth day for all strains, it can be seen that the cultures producing ethanol mainly occurred as mid to late-batch fermentations. Statistical analysis indicated that ethanol production fermented by eight cultures using each kind of the three artificial syngas mixtures had a high significant effect (P<0.01), as shown in **Tables 1-3.**

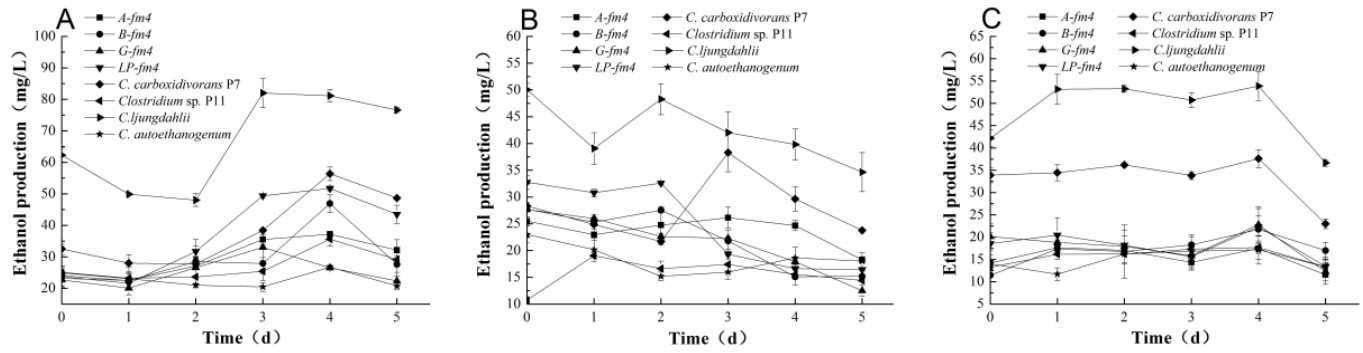

Figure 2. Ethanol production. A, eight cultures with biomass-generated syngas. B, eight cultures with blast furnace gas. C, eight cultures with corex gas. Error bars (n=3) represent plus and minus one standard deviation from the average of every experimental replicates.

Table 1. Chemical properties of the investigated topsoil samples (< 2 mm).

Soil	pH(H2O)	Total N	Total C	CECa	Available P	Available K
		------- g kg^{-1} -------		cmol+ kg^{-1}	---------- mg kg^{-1} ----------	
C	6.71	2.4	22.2	27.5	17.1	1964
F	5.54	3.1	26.3	5.3	66.7	137

[a]CEC, cation exchange capacity.

Table 2. Antibiotics concentration ($mgkg^{-1}$) in treated soils.

Treatments	Soil	Antibiotics	Concentration (mg kg^{-1})
C	C	none	0
CP10	C	Penicillin G	10
CP100	C	Penicillin G	100
CT10	C	Tetracycline hydrochloride	10
CT100	C	Tetracycline hydrochloride	100
F	F	none	0
FP10	F	Penicillin G	10
FP100	F	Penicillin G	100
FT10	F	Tetracycline hydrochloride	10
FT100	F	Tetracycline hydrochloride	100

Table 3. Populations of bacteria, fungi and actinomycetes in soil after penicillin and tetracycline treatments.

Treatment	1d	3d	7d	14d	27d
		Bacteria (10^7 cfu g^{-1} dry soil)			
C	73.5a	64.0a	65.2a	40.7a	44.0a
CP10	55.0b	48.0b	49.5b	40.5a	45.7a
CP100	33.5c	34.2c	38.5c	33.2b	42.2a
CT10	45.0b	43.7b	49.0b	23.7c	47.2a
CT100	34.7c	37.5c	41.5bc	32.0b	42.0a
F	72.5a	64.5a	55.7a	46.7a	48.5bc
FP10	41.2b	33.2b	29.0b	28.2b	60.0a
FP100	26.0c	23.7c	14.7c	25.5b	55.0ab
FT10	43.2b	39.7b	29.7b	26.7b	54.5ab
FT100	39.5b	39.2b	14.5c	20.0b	40.7c
		Fungi (10^4 cfu g^{-1} dry soil)			
C	40.0a	55.0a	47.5a	45.0a	42.5b
CP10	30.0b	45.0ab	37.5b	42.5ab	55.0a

CP100	17.5c	25.0c	40.0ab	35.0b	45.0ab
CT10	37.5a	40.0b	35.0bc	27.5c	35.0c
CT100	22.5c	20.0c	30.0c	42.5ab	45.0ab
F	35.0a	52.5a	45.0a	32.5a	20.0b
FP10	27.5b	27.5b	22.50b	25.0b	20.0b
FP100	15.0cd	9.0d	7.5b	7.5c	27.5a
FT10	20.0bc	27.5b	20.5b	27.5ab	22.5b
FT100	12.5d	20.0c	15.5b	20.0b	32.5a
		Actinomycetes (10^4 cfu g^{-1} dry soil)			
C	46.0a	40.2ab	39.0b	35.5a	45.7a
CP10	45.2a	37.0abc	44.5ab	34.5a	30.7b
CP100	50.7a	43.2a	37.7b	30.5a	23.5 c
CT10	56.5a	36.0bc	51.5a	38.0a	39.7b
CT100	51.7a	34.0c	37.0b	35.7a	35.7b
F	45.50a	40.5b	34.0a	33.2a	25.2a
FP10	35.2b	43.7ab	30.5a	32.0a	9.7c
FP100	43.2ab	52.2a	33.0a	33.0a	16.2b
FT10	24.0b	43.5ab	35.7a	26.2a	15.2b
FT100	44.7a	43.7ab	34.5a	27.2a	15.5b

Ethanol Production Capacity

Figure 3 compares ethanol production capacity between seven organisms and the control strain *Clostridium autoethanogenums* DSM10061 using three artificial syngas mixtures. Ethanol production is a process of accumulation during fermentations and the ethanol production per unit cell introduced could be used as a measure of the fermentative capability of unicellular ethanol production. In **Figure 3A**, significant differences (P<0.01) were observed in maximum ethanol production with strains B-*fm*4, LP-*fm*4, *Clostridium carboxidivorans* P7, *Clostridium ljungdahlii* and *Clostridium autoethanogenums* DSM10061. However, the ethanol production per unit cell with the control strain and *Clostridium ljungdahlii* was significantly lower than with B-*fm*4 and LP-*fm*4. Similarly, there were significant differences (P<0.01) in maximum ethanol production with strains B-*fm*4, G-*fm*4 LP-*fm*4, *Clostridium carboxidivorans* P7 and *Clostridium ljungdahlii* compared to the control while the ethanol production per unit cell with culture B-*fm*4 had become the highest **(Figure 3C).** Conversely, **Figure 3B** shows that only six strains could ferment ethanol, in which cultures *Clostridium carboxidivorans* P7 and *Clostridium ragsdalei* P11 had a higher ethanol production capacity.

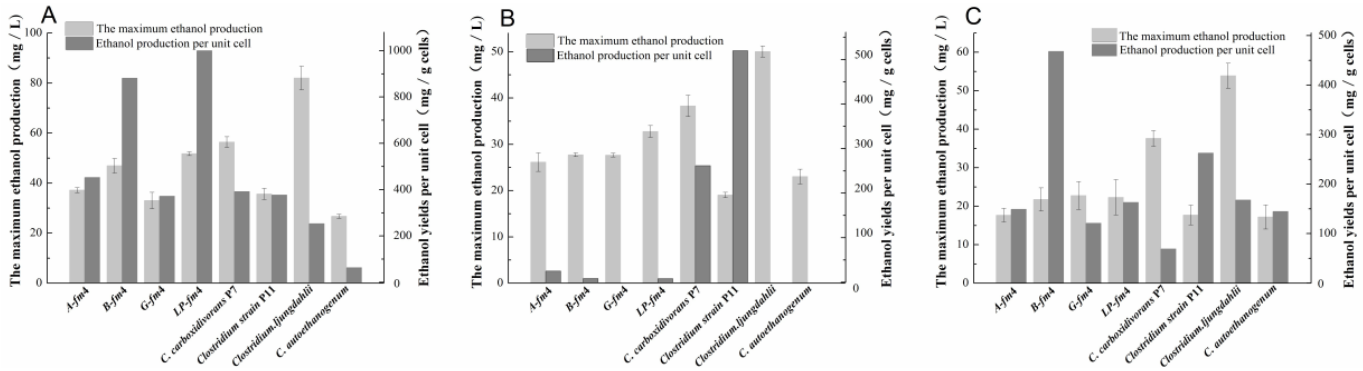

Figure 3. Comparison of the maximum ethanol production and ethanol production per unit cell for strains with syngases. A, eight cultures with biomass-generated syngas. B, eight cultures with blast furnace gas. C, eight cultures with corex gas. Error bars (n=3) represent plus and minus one standard deviation from the average of every experimental replicates.

Moreover, to compare ethanol production capacity of organisms with syngases in the present study distinctly, **Table 4** lists the net ethanol production fermented by syngases and cultures during fermentations. The maximum net ethanol concentrations (28.001, 23.871, 22.909 and 19.726 mg/L) were obtained with biomass-generated syngas and strains LP-*fm*4,*C. carboxidivorans* P7,B-*fm*4 and *C.ljungdahlii*, which was about twofold and threefold more ethanol compared with corex-gas and strains *C.ljungdahlii*, B-*fm*4 (11.734, 10.300 mg/L) and blast furnace gas and strains *C. carboxidivorans* P7 and *C. ragsdalei* P11 (9.937, 8.318 mg/L), respectively. However, the net ethanol levels of strains G-*fm*4, *Clostridium ljungdahlii* and *Clostridium autoethanogenums* DSM10061 were all zero. Furthermore, the difference in the effect of amounts of ethanol formed by the different organisms and syngases was highly significant (P<0.01) as shown in **Table 5**, which indicated that both played a major role in ethanol fermentations.

e-ISSN:2320-3528
p-ISSN:2347-2286

DISCUSSION

In this research, we firstly have evaluated the ability of different syngases and microorganisms to ferment ethanol and screened the efficient syngases and cultures using three simulative syngas mixtures and eight strains. Both played a major role in ethanol fermentations based on statistical analysis, which affected ethanol production highly significant ($p<0.01$). As shown by the data, it was found that the maximum net ethanol concentrations (28.001, 23.871, 22.909 and 19.726 mg/L, respectively) were obtained by strains LP-*fm*4、*C. carboxidivorans* P7 B-*fm*4 and *C. ljungdahlii* were generated when using biomass-generated syngas. Compared with strains *C. ljungdahlii*, B-*fm*4 using corex-gas and cultures *C. carboxidivorans* P7, *C. ragsdalei* P11 using blast furnace gas, the combination of using biomass-generated syngas and strains LP-*fm*4, B-*fm*4 and *C. ljungdahlii* will be optimal for efficient ethanol fermentation. As reported in results arising from this research, cultures LP-*fm*4 *C. carboxidivorans* P7 B-*fm*4 and *C. ljungdahlii* were known as ideal bacteria for the use of biomass syngas in ethanol fermentation. Additionally, the maximum ethanol production per unit cell of strains LP-*fm*4 and B-*fm*4 using biomass-generated syngas were 1000.036, and 881.103 mg/L, respectively **(Figure 3A)** while the cell concentrations of both organisms were lower than the other two strains, shown as **Figure 2A** . Strains LP-*fm*4 and B-*fm*4 with increasing cell concentrations in ethanol fermentations will in some way produce higher ethanol concentrations using biomass syngas in the future. So, these data laid the foundation for biomass-generated syngas fermentation, further research is required and this may have a great significance in promoting comprehensive utilisation of biomass resources and propelling ethanol fermentations with biomass syngas the forward.

In contrast, ethanol productions as fermented by strains using syngas mixtures with blast furnace gas and corex-gas were lower than with biomass syngas **(Figure 2 and Table 4)**, this may have had a close relationship to the components in syngases. For instance, Liu and Atiyeh studied ethanol production from two commercial syngas mixtures (Syngas I: 20% CO: 15% CO_2: 5% H_2 and 60% N_2: SyngasII : 40% CO: 30% CO_2 and 30% H_2) using three moderately alkaliphilic strains, results showed that strains produced more ethanol with syngasII than syngasI [28]. In this research, three syngas mixtures were used, which were biomass-generated syngas, blast furnace gas and corex gas. Their components varied, biomass syngas contained 85.5% CO, 10% H_2, 4.5% CO_2, and blast furnace gas contained 22.4% CO, 23.6% CO_2, 3.3% H_2, 50.6% N_2, 0.1% O_2 and corex gas contained 17.72% H_2, 45.23% CO, 33.17% CO_2, 1.68% CH_4, 2.20% N_2 and 100 to 120 ppm H_2S. These observations indicated that carbon monoxide was a major component in synthesis gas for fermenting ethanol efficiently. For example, it was seen that the ethanol productions of strain B-*fm*4 gradually increased using biomass syngas, blast furnace gas and corex gas, respectively, the concentrations of carbon monoxide in the syngases also increased **(Figure 3).** Similarly, as reported in the literature [12,26,28,31,32], the authors found that the maximum cell concentration and ethanol production were associated with increasing PCO. So, it was thought here that the high proportion of CO in syngas mixtures would benefit ethanol fermentations. Theoretically, all the carbon in CO is converted to ethanol at an H_2:CO ratio of 2 (Eq.(2) and (3)); but, only a third of the carbon in the CO is converted to ethanol without H_2 (Eq. (1)). Here, H_2 served as an electron source, which can be converted to reducing power by hydrogenase when H_2 is present.

Table 4. The net ethanol production fermented by syngases and organisms during culture.

Syngases[a]	The average value of net ethanol production during fermentation (mg /L)								
	Organisms[b]	1	2	3	4	5	6	7	8
BMG		14.018	22.909	10.411	28.001	23.871	10.548	19.726	1.833
BFG		0.632	0.189	0	0.184	9.937	8.318	0	0
CG		3.429	10.300	2.657	3.741	3.710	4.462	11.734	3.181

[a]Here, BMG, BFG and CG were simply called by biomass-generated syngas, blast furnace gas and corex gas respectively.

[b]The numbers 1 to 8 respectively represented cultures A-fm4, B-fm4, G-fm4, LP-fm4, *Clostridium carboxidivorans* P7, *Clostridium ragsdalei* P11, *Clostridium ljungdahlii* (ATCC 55383) and *Clostridium autoethanogenums* DSM10061 .

Table 5. ANOVA table: net ethanol production obtained by syngases and organisms

Source	Sum of squares	df	Mean square	F	$F_{0.01}$
Syngases	2611.894	2	1305.947	61.443**	4.98
Organisms	904.728	7	129.247	6.081**	2.95
Error	1317.757	62	21.253		
Total	4834.379	71			

$$6CO+3H_2O \rightarrow C_2H_2OH+4CO_2 \quad \triangle G^0 = -217.8 \text{ kJ/mol} \tag{1}$$

$$2CO+4H_2 \rightarrow C_2H_5OH+ H_2O \quad \triangle G^0 = -137.6 \text{ kJ/mol} \tag{2}$$

$$2CO+6H_2 \rightarrow C_2H_5OH+ 3H_2O \quad \triangle G^0 = -97.5 \text{ kJ/mol} \tag{3}$$

More carbon from CO will be converted to ethanol instead of CO_2 at this time. Conversely, never was an increase in both ethanol production and growth with higher hydrogen rate (H_2-rich condition) observed, which was different from the theoretical prediction made before carrying out the experiments. It was considered that the major causes leaded to the situation above contained two aspects, one was the presence of carbon monoxide in syngas mixtures, what was a known inhibitor of hydrogenase [31,33,34], and could inhibit the utilisation of hydrogen by the organism. Another was the pH during fermentations. The pH used in

this work was 4.5, which was considered relatively good for ethanol production [29,35]. However, other research [36] showed that the optimum pH for the in vitro measurements of hydrogenase activity was found at pH 8.5, and there was inactivity at pH values below 6.0 to be detected. So, the hydrogenase in the solvent-producing cells (grown at pH 4.5) was presumably present in an inactive form during this research. In addition, the results presented in **Figure 3B and Table 4** demonstrated that strains G-*fm*4, *C. ljungdahlii* and the control could not produce ethanol using blast furnace gas containing 0.1% O_2 by volume. This indicated that the three cultures were all too sensitive to oxygen to live while strains A-*fm*4, B-*fm*4, LP- *fm*4, *C. carboxidivoran*s P7 and *C. ragsdalei* P11 had some O_2 tolerance, which may be the first published evidence to this effect to date. Moreover, compared to organisms using biomass syngas, the ethanol production of strains using blast furnace gas was lower, which also suggested that the oxygen present in the syngas could inhibit growth and ethanol formation in fermentations especially as the concentration of O_2 was above the tolerable maximum.

For blast furnace gas and corex gas, low calorific value residual gases generated in iron and steel industries [37-39], these by-product gases are traditionally emitted into atmosphere, this causes air pollution. From the finding that the maximum net ethanol concentrations of strains *C. carboxidivoran*s P7 and *C. ragsdalei* P11 using blast furnace gas and cultures *C. ljungdahlii* and B-*fm*4 using corex gas were 9.937, 8.318 mg/L and 11.734, 10.300 mg/L, respectively, it deduced that the strains above could grow and produce ethanol based on these syngases. In addition, the maximum ethanol production per unit cell of strains *C. ragsdalei* P11 and B-*fm*4 **(Figures 3B and 3C)** indicated that both were considered as the ideal potential candidates for blast furnace gas and corex-gas fermentations respectively. To produce commercial quantities of ethanol, it may be possible to optimise the composition of the medium to obtain higher concentration strains, or deploy gas compositions based on the practical production needs. Otherwise, it is known that the temperature of these exhaust gases is relatively high, it would be interesting and meaningful to find a suitable thermo-phile for ethanol production from these waste gases. Furthermore, the amount of carbon dioxide in these waste gases will be increasing with the technological development of iron and steel industries, and CO_2 was also one of the main components in syngas. Hence, syngas fermentation will be applied to blast furnace gas and corex-gas more efficiently and become a promising and competitive method of reducing environmental pollution and promote the comprehensive utilization of industrial waste gas resources.

The syngases used in this work were all simulated mixtures, which only contained the key components of an actual syngas whose actual components were diverse and complex. For instance, biomass syngas is generated by the gasification of biomasses, and is a mixture of CO, H_2, CO_2, N_2, CH_4, NOX, O_2, CH_4, C_2H_2, C_2H_4, H_2S, NH_3 and tars, etc [9,31,40]. Besides, both blast furnace gas and corex-gas also contain: H_2, CO_2, N_2, H_2S, NOX, O_2, etc [41]. However, these impurities can potentially affect the fermentation process. In this work, it was shown that accumulated O_2 in the mixtures could inhibit cell growth and ethanol production **(Figures 1B and 3B)** and as and impurity, H_2S also reduced the ethanol production **(Figure 3C).** At the same time, Ahmed found that tars promoted cell dormancy of strain *C. carboxidivoran*s P7 and a redistribution of ethanol and acetic acid production [42], a similar result to that found by Guo [43] using strain *Clostridium autoethanogenum*s DSM10061. Ahmed also studied the effect of NO on the fermentation of biomass syngas with strain *C. carboxidivoran*s P7, the result showed that nitric oxide could inhibit hydrogenase and prevents utilisation of H_2 by this strain when the concentration of NO exceeded 40 ppm, it therefore acted as a non-competitive inhibitor of hydrogenase activity [44]. Ammonia (NH_3) is another component in syngas; it can rapidly convert to NH_4^+ following exposure of fermentation media to NH_3, which transports across the cell membrane to inhibit hydrogenase. A recent report [40] indicated that NH_4^+ is also a non-competitive inhibitor for hydrogenase activity with K NH_4^+ of (649 ± 35) mol.m^{-3}. Besides, methane (CH_4) is also present in the corex gas used in this work at a centration of 1.68% (v/v). However, the effect of CH_4 on cell growth and ethanol production has not been observed, nor was the utilisation of CH_4 has been detected here. Only a study using *C. ragsdalei* P11 showed that CH_4 was not consumed at contents up to 5% (v/v), which did not affect cell growth or ethanol production [31]. So, syngas components were complex and the effects of these impurities in syngas were not to be neglected, but whether or not other impurities have the similar effects on fermentation strains remains to be proven. Meanwhile, further research should quantify these impurities in syngases and assess their impact on the current experimental results.

CONCLUSIONS

Studies were conducted to evaluate the ability of different syngases and microorganisms to ferment ethanol, the most efficient syngases and cultures using three simulative syngas mixtures and eight strains in batch fermentations were selected. Findings from this study indicated that both syngases and cultures affected ethanol production to a significant extent ($p<0.01$). The ethanol production fermented by strains LP-*fm*4, *C. carboxidivoran*s P7, B-*fm*4 and *C. ljungdahlii* using biomass-generated methods was higher than the two other methods, strains *C. carboxidivoran*s P7 and *C. ragsdalei* P11 were fit for blast furnace gas fermentation as well as organisms *C.ljungdahlii*, and B-*fm*4, which were suited to corex gas fermentation under the experimental conditions described here. Compared with three simulated syngas mixtures used here, the high concentration of CO in syngas would benefit ethanol fermentations. Additionally, strains LP-*fm*4 and B-*fm*4 were the most promising for biomass-generated syngas fermentation; cultures *C. ragsdalei* P11 and B-*fm*4 are ideal potential candidates for blast furnace gas and corex-gas fermentations respectively according to the value of maximum ethanol production per unit cell. It is expected that the choice of strains fermenting syngas to ethanol production and the deployment of syngas components will further enhance ethanol yield for potential commercial use.

e-ISSN:2320-3528
p-ISSN:2347-2286

ACKNOWLEDGEMENTS

This research was financially supported by the program for New Century Excellent Talents in The University of The Ministry of Education of China (Grant no. NCET-12-0695) and the program for Innovative Research Team (in Science and Technology) in University of Henan Province (No.15IRTSTHN014).

REFERENCES

1. Lawson PA, et al. Anaerobes: a piece in the puzzle for alternative biofuels. Anaerobe. 2011;17:206-210.
2. Liew WH, et al. Review of evolution, technology and sustainability assessments of biofuel production. J Clean Prod. 2014; 71:11-29.
3. Demirbas A. Potential applications of renewable energy sources, biomass combustion problems in boiler power systems and combustion related environmental issues. Prog Energ Combust. 2005;31:171-92.
4. Vohra M, et al. Bioethanol production: Feedstock and current technologies. J Environ Chem Eng. 2014;2:573-584.
5. Daniell J, et al. Commercial Biomass Syngas Fermentation. Energies. 2012;5:5372-417.
6. Jason H, et al. Environmental, economic and energetic costs and benefits of biodiesel and ethanol biofuels. P Natl Acad Sci USA. 2006;103:11206-11210.
7. Hoekman SK. Biofuels in the US–challenges and opportunities. Renew Energ. 2009;34:14-22.
8. Latif H, et al. Trash to treasure: production of biofuels and commodity chemicals via syngas fermenting microorganisms. Curr Opin Biotech. 2014;27:79-87.
9. Abubackar HN, et al. Biological conversion of carbon monoxide: rich syngas or waste gases to bioethanol. Biofuel Bioprod Bior. 2011;5:93-114.
10. Munasinghe PC and Khanal SK. Biomass-derived syngas fermentation into biofuels: Opportunities and challenges. Bioresour Technol. 2010;101:5013-22.
11. Mohammadi M, et al. Bioconversion of synthesis gas to second generation biofuels: A review. Renew Sust Energ Rev. 2011;15:4255-4273.
12. Köpke M, et al. Fermentative production of ethanol from carbon monoxide. Curr Opin Biotech. 2011;22:320-325.
13. Munasinghe PC and Khanal SK. Syngas fermentation to biofuel: evaluation of carbon monoxide mass transfer coefficient (KLa) in different reactor configurations. Biotechnol Prog. 2010;26:1616-1621.
14. Rajagopalan S, et al. Formation of ethanol from carbon monoxide via a new microbial catalyst. Biomass Bioenerg. 2002;23:487-493.
15. Younesi H, et al. Ethanol and acetate production from synthesis gas via fermentation processes using anaerobic bacterium, Clostridium ljungdahlii. Biochem Eng J. 2005;27:110-119.
16. Chatterjee S, et al. Evaluation of support matrices for an immobilized cell gas lift reactor for fermentation of coal derived synthesis gas. J Ferment Bioeng. 1996;81:158-162.
17. Henstra AM, et al. Microbiology of synthesis gas fermentation for biofuel production. Curr Opin Biotech. 2007;18:200-206.
18. Lee PH, et al. Enhancement of carbon monoxide mass transfer using an innovative external hollow fiber membrane (HFM) diffuser for syngas fermentation: Experimental studies and model development. Chem Eng J. 2012;184:268-277.
19. Munasinghe PC and Khanal SK. Syngas fermentation to biofuel: Evaluation of carbon monoxide mass transfer and analytical modeling using a composite hollow fiber (CHF) membrane bioreactor. Bioresource Technol. 2012;122:130-136.
20. Zhang F, et al. Fatty acids production from hydrogen and carbon dioxide by mixed culture in the membrane biofilm reactor. Water Res. 2013;47:6122-6129.
21. Zhao Y, et al. Performance of a Carboxydothermus hydrogenoformans-immobilizing membrane reactor for syngas upgrading into hydrogen. Int J Hydrogen Energ. 2013;38:2167-2175.
22. Tanner RS, et al. Clostridium ljungdahlii sp. nov., an acetogenic species in clostridial rRNA homology group I. Int J Syst Bacteriol. 1993;43:232-236.
23. Cotter JL, et al. Ethanol and acetate production by Clostridium ljungdahlii and Clostridium autoethanogenum using resting cells. Bioproc Biosyst Eng. 2009;32:369-380.
24. Liou JSC, et al. Clostridium carboxidivorans sp. nov., a solvent-producing clostridium isolated from an agricultural settling lagoon, and reclassification of the acetogen Clostridium scatologenes strain SL1 as Clostridium drakei sp. nov. Int J Syst Evol Micr. 2005;55:2085-2091.
25. Ukpong MN, et al. Physiological response of Clostridium carboxidivorans during conversion of synthesis gas to solvents in a gas-fed bioreactor. Biotechnol Bioeng. 2012;109:2720-2728.

26. Kundiyana DK, et al. Effect of temperature, pH and buffer presence on ethanol production from synthesis gas by "Clostridium ragsdalei". Bioresource Technol. 2011;102:5794-5799.

27. Abrini J, et al. Clostridium autoethanogenum, sp. nov., an anaerobic bacterium that produces ethanol from carbon monoxide. Arch Microbiol. 1994;161:345-351.

28. Liu K, et al. Fermentative production of ethanol from syngas using novel moderately alkaliphilic strains of Alkalibaculum bacchi. Bioresource Technol. 2012;104:336-341.

29. Guo Y, et al. Medium optimization for ethanol production with Clostridium autoethanogenum with carbon monoxide as sole carbon source. Bioresource Technol. 2010;101:8784-8789.

30. Jiang Y and Shao WL. Primary study on ethanol production pathway in thermoanaerobacter ethanolicus . Journal of Nanjing Normal University (Natural Science). 2005;28:69-73.

31. Wilkins MR and Atiyeh HK. Microbial production of ethanol from carbon monoxide. Curr Opin Biotech. 2011;22:326-330.

32. Hurst KM and Lewis RS. Carbon monoxide partial pressure effects on the metabolic process of syngas fermentation. Biochem Eng J. 2010;48:159-165.

33. Bennett B, et al. Reversible carbon monoxide binding and inhibition at the active site of the Fe-only hydrogenase. Biochemistry. 2000;39:7455-7460.

34. Tibelius KH and Knowles R. Hydrogenase activity in Azospirillum brasilense is inhibited by nitrite, nitric oxide, carbon monoxide, and acetylene. J Bacteriol. 1984;160:103-106.

35. Nalakath AH, et al. Biological conversion of carbon monoxide to ethanol: Effect of pH, gas pressure, reducing agent and yeast extract. Bioresource Technol. 2012;114:518-522.

36. Andersch W, et al. Level of enzymes involved in acetate, butyrate, acetone and butanol formation by Clostridium acetobutylicum. Eur J Appl Microbiol Biot. 1983;18:327-332.

37. Liszka M, et al. Parametric study of HRSG in case of repowered industrial CHP plant. Energ Conver Manage. 2003;44:995-1012.

38. Chen WH, et al. An evaluation of hydrogen production from the perspective of using blast furnace gas and coke oven gas as feedstocks. Int J Hydrogen Energy. 2011;36:11727-11737.

39. Lampert K, et al. Thermoeconomical analysis of CO2 removal from the Corex export gas and its integration with the blast-furnace assembly and metallurgical combined heat and power (CHP) plant. Energy. 2010;35:1188-1195.

40. Xu D and Lewis RS. Syngas fermentation to biofuels: Effects of ammonia impurity in raw syngas on hydrogenase activity. Biomass Bioenerg. 2012;45:303-310.

41. Hou S, et al. Firing blast furnace gas without support fuel in steel mill boilers. Energ Convers Manag. 2011;52:2758-2767.

42. Ahmed A, et al. Effects of biomass-generated producer gas constituents on cell growth, product distribution and hydrogenase activity of Clostridium carboxidivorans P7T. Biomass Bioenerg. 2006;30:665-672.

43. Guo Y, et al. Study on composition effects of syngas and medium on ethanol production with C.autoethanogenum. Acta Energiae Solaris Sin. 2011;32:1370-1274.

44. Ahmed A and Lewis RS. Fermentation of biomass-generated synthesis gas: Effects of nitric oxide. Biotech Bioeng. 2007;97:1080-1086.