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Research paper

High efficient ethanol and VFA production from gas fermentation: Effect of acetate, gas and inoculum microbial composition

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ABSTRACT

In bioindustry, syngas fermentation is a promising technology for biofuel production without the use of plant biomass as sugar-based feedstock. The aim of this study was to identify optimal conditions for high efficient ethanol and volatile fatty acids (VFA) production from synthetic gas fermentation. Therefore, the effect of different gases (pure CO, H_2, and a synthetic syngas mixture), media (acetate medium and acetate-free medium), and biocatalyst (pure and mixed culture) were studied. Acetate was the most dominant product independent on inoculum type. The maximum concentration of volatile fatty acids and ethanol was achieved by the pure culture (*Clostridium ragsdalei*). Depending on the headspace gas composition, VFA concentrations were up to 300% higher after fermentation with *Clostridium ragsdalei* compared to fermentation with mixed culture. The preferred gas composition with respect to highest VFA concentration was pure CO (100%) regardless of microbial composition of the inoculum and media composition. The addition of acetate had a negative impact on the VFA formation which was depending on the initial gas composition in head space.

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1. Introduction

The extensive consumption of fossil fuels, high energy prices and the need for reducing greenhouse gas emissions have drawn increasing attention for development of alternative and sustainable energy. Biological fermentation of syngas is a promising emerging technology, expected to play a vital role in achieving this goal. This technology not only produces biofuels and valuable chemicals but also contributes to environmental pollution control. Syngas which essentially consists of a mixture of carbon monoxide, hydrogen, and carbon dioxide derived from the gasification of solid fuels (i.e. coal, petroleum coke, and oil shale), and biomass, or reforming of natural gas, and from manufacturing waste gases, particularly from steel production [1,2]. Syngas platform for biofuels production is a two stage process, where syngas is first produced by gasification and then syngas can be fermented to biofuels. This platform has several advantages over the conventional biochemical methods for biofuel production, such as high specificity of the biocatalyst, and utilization of the whole biomass including lignin [3]. Fermentation of syngas for the production of biofuels can be performed at ambient temperature and pressure and does not require any costly pretreatment of the feed gas or costly metal catalysts [4].

The most promising bacterial groups for fermenting syngas are acetogens. These unique obligatory anaerobic bacteria have the ability to fix CO_2 or CO using H_2 as an electron donor or CO alone and produce alcohols and organic acids [5]. These microorganisms undergo a set of enzyme catalyzed reactions through an irreversible, non-cyclic pathway known as Wood-Ljungdahl pathway to convert syngas into acetyl-CoA [3]. Acetyl-CoA can subsequently be used for ATP generation needed for microbial anabolism and formation of acetate, ethanol and other byproducts during the later stages of the pathway [6]. Several mesophilic pure cultures especially within the species *Clostridium* have been used for syngas fermentation [7]. Prominent among these is *Clostridium ragsdalei*, which has been successfully used for syngas fermentation [8,9]. However still important challenges need to be addressed before commercial application. Gas-liquid mass transfer limitations, syngas quality, microbial catalysts and product recovery are the major

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issues to be addressed in order to make syngas fermentation more economically feasible [10].

Recently mixed culture fermentation has gained attention due to several advantages compared to pure culture, such as process robustness during continuous processes and no need for highly sterile conditions [11]. However, systematic comparison of pure and mixed culture syngas fermentation to alcohols and/or acids, which could permit developing efficient biofuels and biochemicals processes, has not be made so far.

In addition to the microbial catalysts, syngas composition in terms of H_2/CO ratio is also an important factor that significantly affects the output of the syngas fermentation process [12]. It has been recently reported that some *Clostridia* species could further reduce volatile fatty acids (VFA) to their corresponding alcohols by using syngas as electron donor [13]. Thus, it is of importance to investigate the influence of presence of VFA (e.g., acetate) on the gas fermentation processes [14].

Based on the points highlighted above, the main objective of this study was to identify the optimal conditions (media, gas composition and microbial catalyst) for high efficient alcohol and VFA production from synthetic gas fermentation. Moreover process performance for VFA and ethanol production during pure culture and mixed cultures fermentation were compared.

Table 1
Experimental design for effect of media, feed gas and microbial composition during the fermentation process.

Treatment levels	Factors		
0	Media	Culture	Gas composition
1	AFM	Pure culture	Syngas (60%CO: 35% H_2:5%CO_2)
2	AAM	Mixed culture	Pure CO (100%)
			Pure H_2 (100%)
1	0	0	0
2	0	1	0
3	0	0	1
4	0	1	1
5	1	0	0
6	1	1	0
7	1	0	1
8	1	1	1
9	1	0	2
10	1	1	2

2. Materials and methods

2.1. Microorganisms

Clostridium ragsdalei P11 (No. 15248, Deutche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany) was used as a pure culture for syngas fermentation process in this study. Digested manure (treated cattle manure and wheat straw) obtained from the effluent of a lab scale anaerobic thermophilic continuous stirred reactor (CSTR) at Technical University of Denmark was used as anaerobic mixed microbial inoculum for syngas fermentation. The digested manure was pre-treated with heat at 90 °C for 15 min to inactivate methanogenic archaea and to enrich for anaerobic spore-forming bacteria [15].

2.2. Experimental design

An experimental scheduled (Table 1) was designed to test the effect of three different factors on the fermentation process. The variables tested included two different inocula: pure culture (*C. ragsdalei*) and mixed culture, two media compositions: acetate amended medium (AAM) and acetate free medium (AFM), and three feed gas compositions: Syngas (±60% CO, ± 35% H_2, ±5% CO_2); 100% CO and 100% H_2 (added only in the acetate amended medium). In the following paper the gas compositions will be referred to as: synthetic syngas (SS), CO and H_2. The response variable for each treatment combination was the concentration of ethanol, acetate and the other VFA obtained during 10 days of fermentation.

2.3. Inoculum preparation

To reduce the lag phase and ensure adaptation to the syngas, *C. ragsdalei* and mixed culture inocula were propagated under strict anaerobic conditions three times prior to inoculating the batch experiment [16]. Each inoculum was prepared in 250 ml serum bottles with 100 ml of ATCC 1754 medium [2] and 10% (v/v) inoculum in each passage. The bottles were pressurized to 1.5 bar with (60% CO, 5% CO_2 and 35% H_2) syngas mixture by volume and kept at 37 °C. The inoculation of a new batch was made when the previous

Table 2
ΔG° of possible fermentation reactions by clostridium and mixed cultures under standard condition.

Chain elongation (Incl. wood-ljungdal pathway)				
Stoichiometry		#	ΔG°[a] (kJ/mol)	Reference
Ethanol	$6CO + 3H_2O \rightarrow CH_3CH_2OH + 4CO_2$	1	−217.4	[1]
	$3CO + 3H_2 \rightarrow CH_3CH_2OH + CO_2$	2	−157.2	[1]
	$2CO + 4H_2 \rightarrow CH_3CH_2OH + H_2O$	3	−137.1	[1]
	$2CO_2 + 6H_2 \rightarrow CH_3CH_2OH + 3H_2O$	4	−97.0	[2]
Acetate	$4CO + 2H_2O \rightarrow CH_3COO^- + H^+ + 2CO_2$	5	−154.6	[1]
	$2CO + 2H_2 \rightarrow CH_3COO^- + H^+$	6	−114.5	[1]
	$2CO_2 + 4H_2 \rightarrow CH_3COO^- + H^+ + 2H_2O$	7	−54.8	[3]
Propionate	$HCOO^- + CH_3CH_2OH \rightarrow CH_3CH_2COO^- + H_2O$	8	−65.4	[3]
Butyrate	$2CH_3COO^- + H^+ + 2H_2 \rightarrow CH_3(CH_2)_2COO^- + 2H_2O$ −*OOHal.*, 2016))	9	−88.0	[3]
	2008*fluents g cattle manure and wheatstreaw terial yngas suggested by literature. gher volumes than what is*			
	$CH_3COO^- + CH_3CH_2OH \rightarrow CH_3(CH_2)_2COO^- + H_2O$	10	−38.5	[3]
	$10CO + 4H_2O \rightarrow CH_3(CH_2)_2COO^- + H^+ + 6CO_2$	11	−420.8	[2]
	$10H_2 + 4CO_2 \rightarrow CH_3(CH_2)_2COO^- + H^+ + 6H_2O$	12	−220.2	[2]
Valerate	$2CH_3CH_2COO^- + H^+ + 2CO_2 + 6H_2 \rightarrow CH_3(CH_2)_3COO^- + H_2O$	13	−142.8	[3]
	$2CH_3CH_2COO^- + H^+ + CH_3COO^- + H^+ \rightarrow CH_3(CH_2)_3COO^- + 2H_2O$	14	−88.0	[3]
Reduction of acetate to ethanol				
Ethanol	$CH_3COO^- + H^+ + 2CO \rightarrow CH_3CH_2OH + 2CO_2$	15	−62.5	[4]
	$CH_3COO^- + H^+ + 2H_2 \rightarrow CH_3CH_2OH + H_2O$	16	−22.3	[5]

[a] ΔG values were calculated using enthalpy and entropy values under standard condition i.e. 0 °C, 1atm, 1 M or 1 atm partial pressure liquid and gaseous concentration respectively.

was reaching cell optical density (OD_{660}) of about 0.5. The third cultivation was used to inoculate the batch experiment.

2.4. Experimental batch setup

Through this study, the effect of two different media: acetate free medium (AFM) and acetate amended medium (AAM) on the gas fermentation process was studied. The both medium was based on ATCC medium 1754 with the exception that yeast extract and fructose were omitted to minimize the interference of organic compounds during the fermentation [2]. The only difference between the two media was the addition of acetate in (AAM) to a final concentration of 13 mM along with the other chemicals before adjustment of the pH. All the fermentative experiments were carried out in triplicate using 320 ml serum bottles with a liquid volume of 22 ml. The bottles with the media were sealed with butyl rubber stopper and aluminum cap and then sterilized at 121 °C and 15 psi for 20 min. After sterilization, 150 mM MES (2-(N-Morpholino) ethanesulfonic acid sodium salt) buffer solution, vitamin and reducing agents were added using 0.2 μm sterilized filter while flushed with N_2 to keep anaerobic conditions. Adding MES to a concentration of 150 mM and vitamins after autoclaving was inspired by Perez et al. [14]. The detailed composition of trace metals, vitamin and reducing agents stock solutions are presented in Supplementary Information (SI). The initial pH of the medium was adjusted to 6 ± 0.2 with 1 M KOH and 1 M HCl solutions. Fermentations with either *C. ragsdalei* or the mixed culture were inoculated with 10% (v/v) inocula. All fermentation serum bottles were incubated horizontally (to maximize gas-liquid mixing) at 37 °C with a constant agitation of 120 rpm on an orbital shaker for 10 d. Three different gas compositions (Synthetic syngas consisting of 60% CO, 35% H_2, 5% CO_2), 100% CO and 100% H_2 (only added in the acetate amended medium) were injected to the headspace of the fermentation bottles to a final pressure of 1.5 bar. For biotic controls the cultures were kept under 100% N_2 gas phase instead of the other gases.

2.5. Analytical methods

Samples (2 ml) were retrieved from the fermentation broth (to avoid disturbance of the bacteria) on day 4 and 10 for VFA analysis along with optical density and pH measurements. The pH was measured by a digital PHM210 pH meter connected to the Gel pH electrode (pHC3105−8; Radiometer analytical). The optical density (OD) was determined using Spectronic 20D+ (Thermoscientific, Soeborg, Denmark). Cell samples were collected in 4 ml cuvettes from the Serum bottles and the OD was measured at 600 nm. After measuring the pH and the optical density, the fermentation samples were prepared as previously described for VFA and alcohols determination using a gas chromatograph (GC) (Shimadzu GC-2010, Kyoto, Japan), equipped with a flame ionization detector (FID) and a FFAP fused-silica capillary column, 30 m × 0.53 mm I.D., film thickness 1.0 μm, using nitrogen as a carrier gas [17]. The oven temperature was initially set at 50 °C for 3.5 min and then increased 25 °C/min to 130 °C followed by 10 °C/min to 210 °C, and kept at final temperature for 10 min. The injection port and detector temperatures were 150 °C and 230 °C, respectively. Headspace gas samples were collected using sample lock gas tight syringes every 48 h from the serum bottle reactors and analyzed for CO_2 consumption using a GC (MicroLab, Arhus, Denmark) with a paralleled column of 1.1 m × 3/16 "Molsieve 137 and 0.7 m × 1/4" chromosorb 108. Hydrogen concentration was measured by GC-TCD with N_2 as carrier gas. It was fitted with a 4.5 m^3 mms-m stainless column packed with Molsieve SA (10/80), and the temperature of the injector, detecor and oven were 190 °C, 110 °C and 90 °C, respectively. Detection limit for H_2 and CO_2 was 0.06%. All chemicals were purchased from Sigma−Aldrich, unless stated otherwise.

2.6. Statistical analyses and calculation of kinetic parameters

Analysis of variance (ANOVA) and Duncan's new multiple range test (MRT, $p < 0.05$) were used for multiple comparisons via MSTATC (Michigan State University, East Lansing, MI, USA). Gibbs free energy changes at standard conditions (ΔG°) of possible fermentation reactions by *Clostridium* and mixed cultures are shown in Table 2. According to Coma et al. [18] ΔG is dependent on the concentrations of initial reactants and products through the equation:

$$\Delta G = \Delta G^{o} + RT \ln(Q) \quad (1)$$

where R is a constant (0.00829 kJ/mol*K), T is the absolute temperature (K) and;

$$Q = \frac{[C]^{c}[D]^{d}}{[A]^{a}[B]^{b}} \quad (2)$$

Which applies for the general reaction:

$$aA + bB \leftrightarrow cC + dD$$

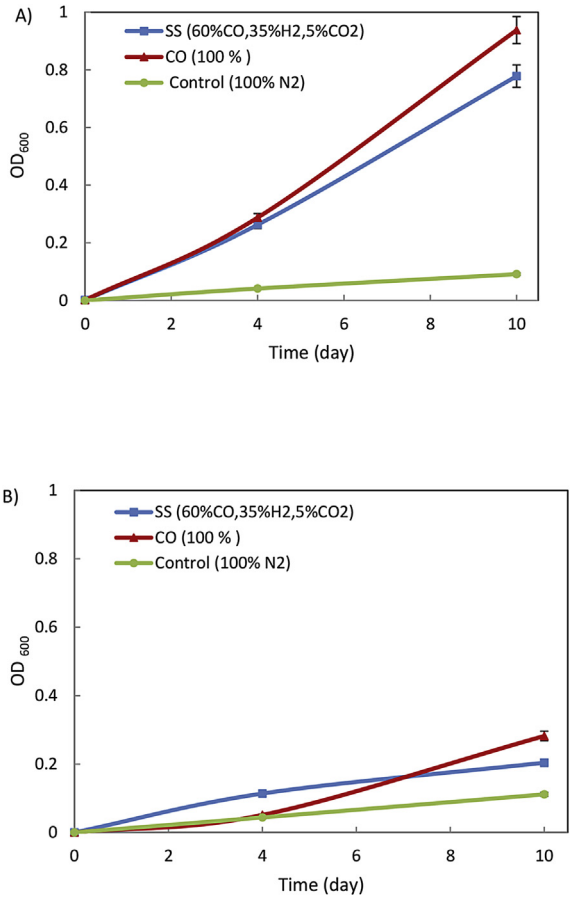

Fig. 1. Growth profile (optical density, OD_{600}) of: A) pure and B) mixed culture with acetate free media.

3. Results and discussion

3.1. Growth of pure and mixed culture with synthetic media

The growth profile of *C. ragsdalei* and mixed culture cells in terms of optical density (OD_{600}) in acetate free medium is shown in Fig. 1. The maximum growth of *C. ragsdalei* and mixed cultures was observed under the headspace of CO, which was 21% and 38%, respectively, higher than that under synthetic syngas (SS): (60% CO, 5% CO_2 and 35% H_2) (Fig. 1). It is more thermodynamically favorable using CO as electron donor than other electron donors (e.g., H_2) independent of pH, ionic strength, gas partial pressure, and electron carrier pairs and therefore the bacteria would potentially gain more energy for cell growth by using CO [19,20]. The difference between biotic controls (with only N_2 in headspace) and other samples (with fermentation gases) was most significant for pure culture ($p < 0.05$). It was clear that the fermentation gasses had a positive effect on bacterial growth. Results showed that OD_{600} of the mixed culture was, significantly lower ($p < 0.05$) (74% and 70%, for SS and CO, respectively) compared with the *C. ragsdalei*. Meanwhile there was no significant difference ($p > 0.05$) in OD_{600} between samples with and without acetate.

3.2. Fermentative ethanol and VFA production

Fig. 2 compares the production profiles of ethanol and VFA formed by the *C. ragsdalei* under different gas compositions. The metabolites produced during fermentation were ethanol, acetate, propionate, butyrate and valerate. The most dominant product by pure culture fermentation in all gas composition was acetate. The maximum concentration of acetate (17 mmol/l) was observed under a headspace of only CO after 10 days. In comparison, the final concentration of acetate under headspaces of SS was 26% lower. Previous studies have also pointed out CO as the preferred reductant over H_2 [19,20]. This could explain the higher formation of acetate under a headspace of CO than the mixture of CO, H_2 and CO_2.

Production of ethanol was observed in both experiments with SS and CO headspace (Fig. 2A and B) with a maximum concentration 1.4 and 1.6 mmol/l after only 4 days of inocubation whereafter

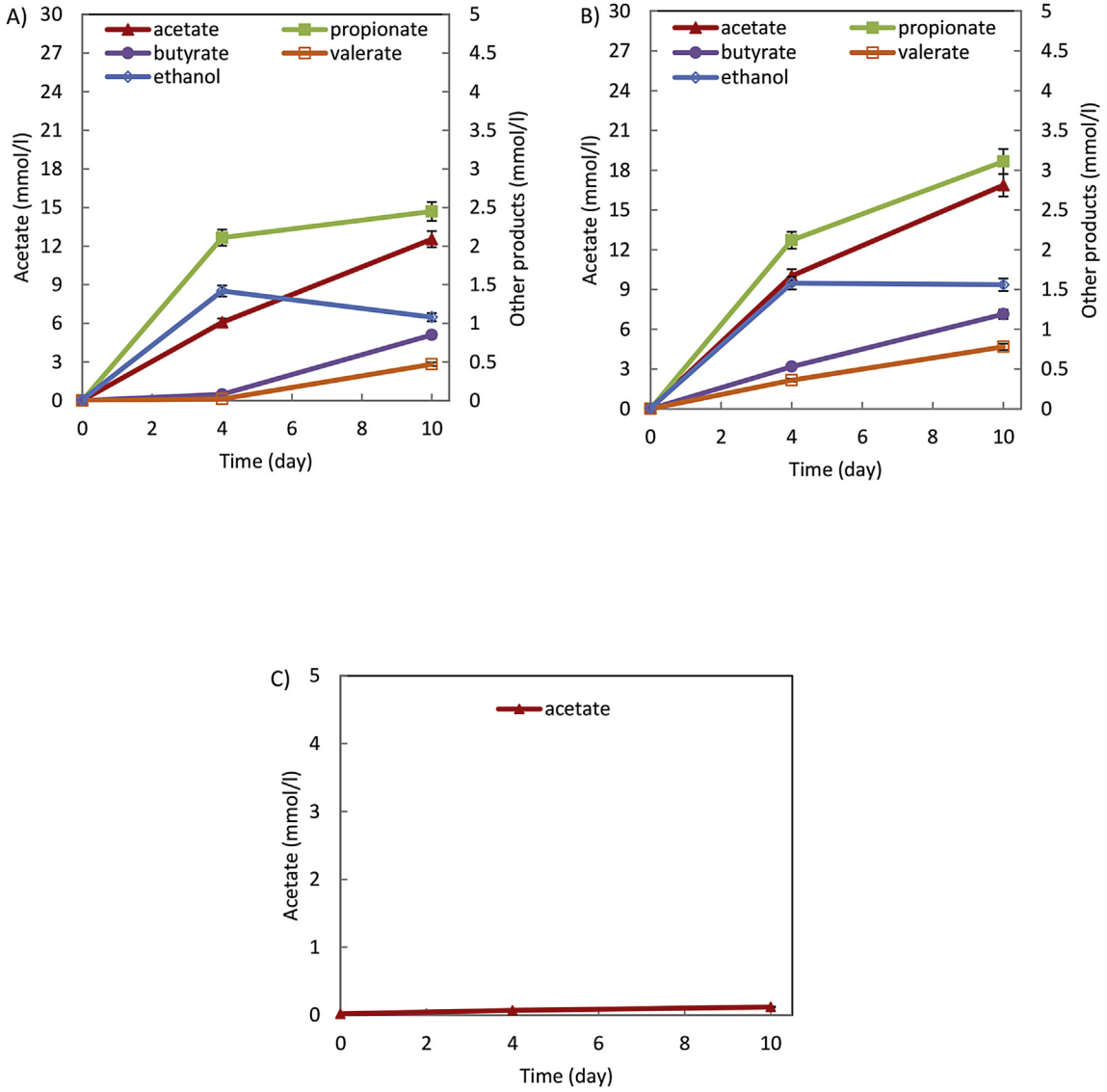

Fig. 2. Ethanol and VFA production profile of the pure culture with acetate free media observed under different gas composition: (A) Synthetic syngas, (B) pure CO and (C) control gas consisting of only N_2.

the concentration of ethanol decreased. This could indicate that ethanol was consumed for the production of higher chained fatty acids through chain elongation. According to Table 2 production of ethanol should be thermodynamically more favorable than acetate. A simultaneous production and consumption of ethanol could therefore explain the relatively low concentrations. From Table 2 (reactions 1–4) it can be seen that ethanol formation with CO is thermodynamically more favorable than with H_2. This was also supported by Liu et al. [21] stating that most favorable reactions are with low H_2:CO ratios.

The current study confirmed that *C. ragsdalei* can grow and build components needed for growth directly from syngas in yeast free medium (Figs. 1A and 2), which is contradicted with previous studies [22]. The productivity observed in the current study was a bit lower compared to other studies [23–25], indicating the importance of the yeast for enhancing the productivity of *C. ragsdalei*. Gao et al. [25] observed a significantly lower acetate and ethanol concentration in a yeast free medium compared to a yeast medium and confirmed that the components in yeast such as carbohydrates and vitamins could contribute to better growth of *C. ragsdalei* and thus lead to a higher concentration of products.

Propionate was the second most dominant product during fermentation with *C. ragsdalei*. It was produced in all compositions in concentrations higher than 2 mmol/l after 4 days. The maximum concentration of propionate was observed under a headspace of CO with a value of 3.1 mmol/l. Only few pathways for propionate production have been reported for *Clostridium* species. Coma et al. [18] suggested that propionate could be formed by chain elongation of formate with ethanol as reductant (Table 2, reaction 8). Formate has been found to be one of the products by microbial fermentation of syngas [26]. Formate and ethanol were assumed to be produced simultaneously and used as precursors for propionate formation. According to Eqs. (1) and (2) the formation of propionate (reaction 8) should become more favorable with ethanol being formed simultaneously. Another pathway for propionate production is through the acrylate pathway however this has only been described for very few bacterial species [5].

Maximum production of butyrate and valerate were also observed under a headspace of CO with a concentration of 1.2 and 0.8 mmol/l, respectively. The final concentration of butyrate and valerate under the headspace of SS was 29% and 40% lower than that under CO. This again suggests that oxidizing enzymes might

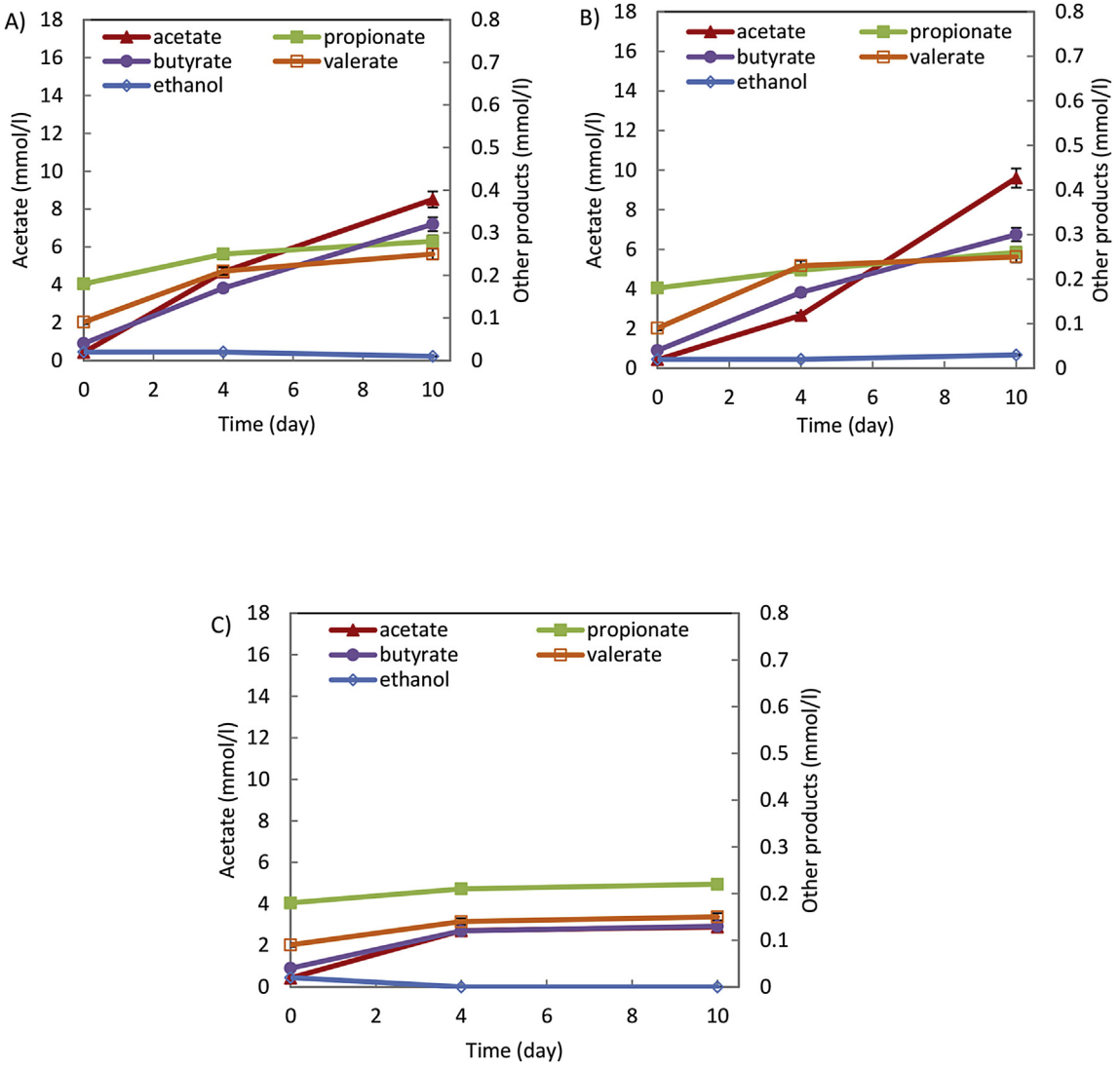

Fig. 3. Ethanol and VFA production profile of the mixed culture with acetate free media observed under different gas composition: (A) Synthetic syngas, (B) pure CO and (C) control gas consisting of only N_2.

have been inhibited by the presence of both CO and H_2. Overall a headspace of CO showed to be the most favorable gas composition with respect to final concentrations of VFA. The concentration of VFA produced under CO was 35% higher than that under headspaces of SS. Fermentation with *C. ragsdalei* showed negligible formation of products under the control (Fig. 2C), which indicated the importance of having either CO or H_2 as reductant for product formation to occur.

The production of ethanol and VFA using mixed culture was also investigated. The most dominant product in both gas compositions was acetate, while no ethanol was produced under the headspace of CO and SS (Fig. 3). The maximum acetate concentration was achieved under a headspace of CO which was 13% higher than under headspaces of SS. This clearly indicated that CO was preferred over mixture of CO and H_2 (as syngas) for acetate production by the mixed culture. The maximum acetate concentration reported by Singla et al. [11] through a syngas fermentation with TERI SA1 anaerobic mixed cultured was five and four-fold that produced under SS and CO headspace in the current study. Beside the absence of yeast on the fermentation medium through the present study, the heating treatment of the inoculum could be another possible reason for the lower productivity of the mixed culture compared to other studies. The adverse effect of the high temperature on the microbial community has been previously reported [27]. For example, it can disrupt the chemical bonds of the cell wall and membrane, solubilize the cell components and alter microbial proteins. No significant difference ($p > 0.05$) in final concentrations of other VFA was observed during fermentation under a headspace of SS and CO. These results were also confirmed by other researchers who reported the ability of acetogens to grow chemolithotrophically on CO alone or syngas and ferment them to acetate as a main microbial end product [5,11]. Overall mixed culture clearly showed to prefer headspaces of CO with respect to final concentrations of VFA.

3.3. The effect of acetate on gas fermentation

Fermentation by *C. ragsdalei* in all three gas compositions with acetate amended medium (Fig. 4A, B, C) showed that acetate was the most dominant product. The net acetate production under the headspace of CO at the end of batch run was 77% and 131% higher than that achieved under synthetic syngas (SS) and H_2 headspace, respectively. This maximum production was 22% lower compared to fermentation without addition of acetate. This suggested a negative effect on acetate formation when it is already present in the medium. The negative effect could be explained by thermodynamic considerations due to the higher initial acetate concentration. From Eqs. (1) and (2) it can be seen that when the initial

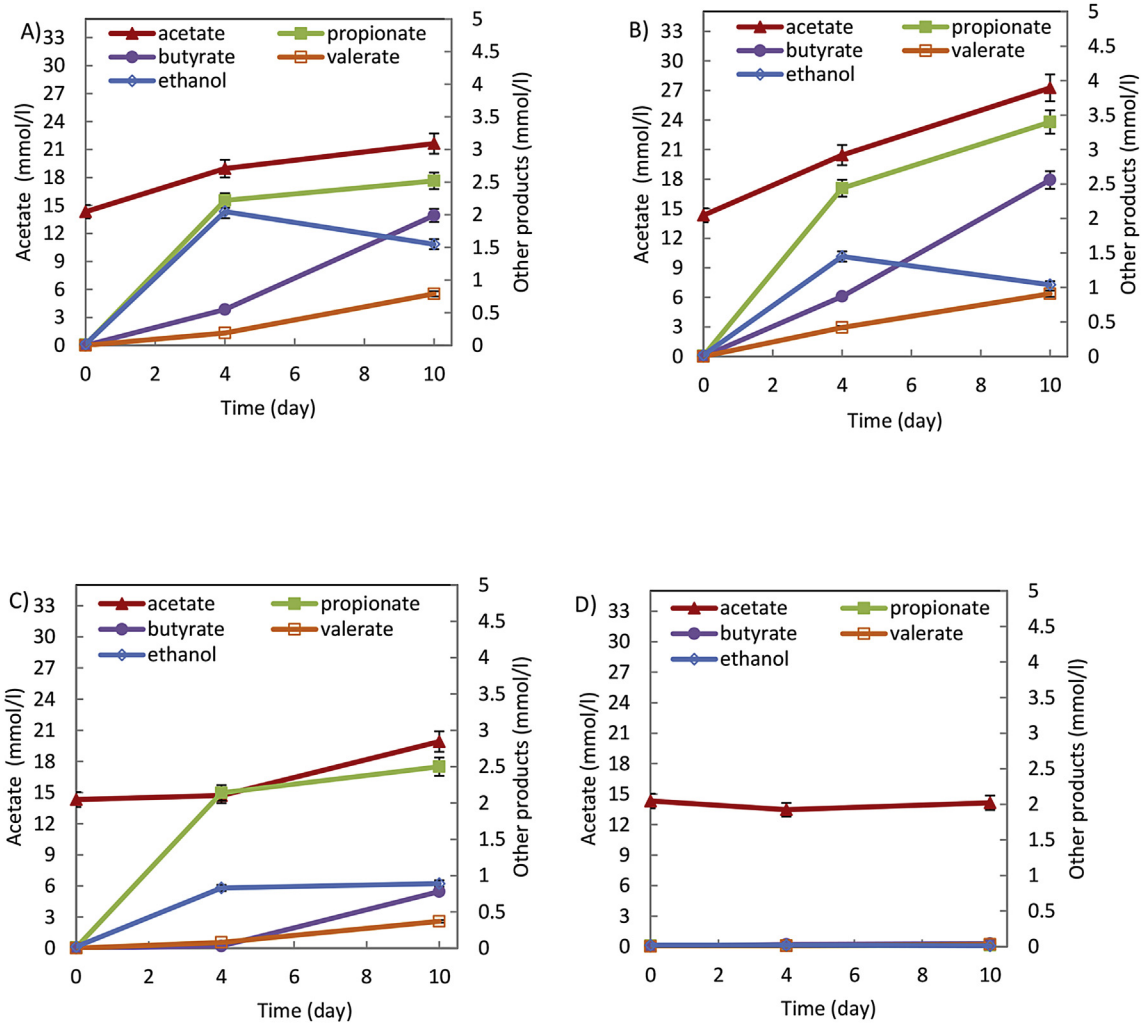

Fig. 4. Ethanol and VFA production profile of the pure culture with acetate amended media observed under different gas composition: (A) Synthetic syngas, (B) pure CO, (C) pure H_2 and (D) control gas consisting of only N_2.

concentration of a product (in this case acetate) is high, the ΔG for the reaction will increase making the reaction less exergonic. Fig. 4A and B showed that the addition of acetate during pure culture fermentation under headspaces of SS and CO resulted in a significant increase ($p < 0.05$) of butyrate formation. The maximum concentration of butyrate (2.5 mmol/l) was observed under the headspace of CO, which was 29% and 228% higher than that produced under SS and H_2 headspace. The highest concentration of butyrate measured under a headspace of CO and SS was 115% and 134% significantly higher ($p < 0.05$) compared to the same experiment without addition of acetate. This increase in butyrate concentrations with *C. ragsdalei* could be explained by that chain elongation reactions were becoming more favorable as a result of initial presence of acetate. It has not been so far documented that *C. ragsdalei* have the ability to perform reverse β-oxidation for butyrate formation. Results from this study however indicate that this could be a possibility. According to calculations of the actual Gibbs free energy change (ΔG) at real fermentation conditions (Eqs. (1) and (2)), high acetate concentrations would result in increase of ΔG for formation of acetate from syngas, (reaction 5 in Table 2), while the ΔG would decrease for reduction of acetate to ethanol with CO as reductant (in reaction 15). Consequently the formation of butyrate from ethanol and acetate (reaction 10) would also become more favorable potentially explaining the increase in formation. Alternatively the increased concentration of butyrate as a result of adding acetate, could be due to that the formation of acetate directly from CO became less favorable (reaction 5), resulting in an alternative reaction, i.e. direct butyrate formation from CO (reaction 11) occurring instead. An increase in butyrate concentration of more than 100% was considered a significant improvement. Two other studies however reported significantly concentrations of butyrate (229% [8] and 287% [2]) compared to our study. One reason could be that our media had MES concentrations as we wished an effective inhibition of methanogenesis. Phillips et al. [2] used no MES buffer, while Ramachandriya et al. [8] employed less than 30% of the concentration used in the present study. MES buffer has been reported to have a negative effect on product formation [25,28] and the high concentration used in this study was therefore considered likely to have inhibited further increase of butyrate production. However more valuable products (butyrate and valerate) increased as a result of acetate addition but the overall concentration of VFA was lower compared to experiment without acetate addition. Under headspaces of CO and SS, the decrease in the concentration of all VFA produced was 16% and 28%, respectively compared to when no acetate was added. When the headspace was filled with SS, the addition of acetate increased the

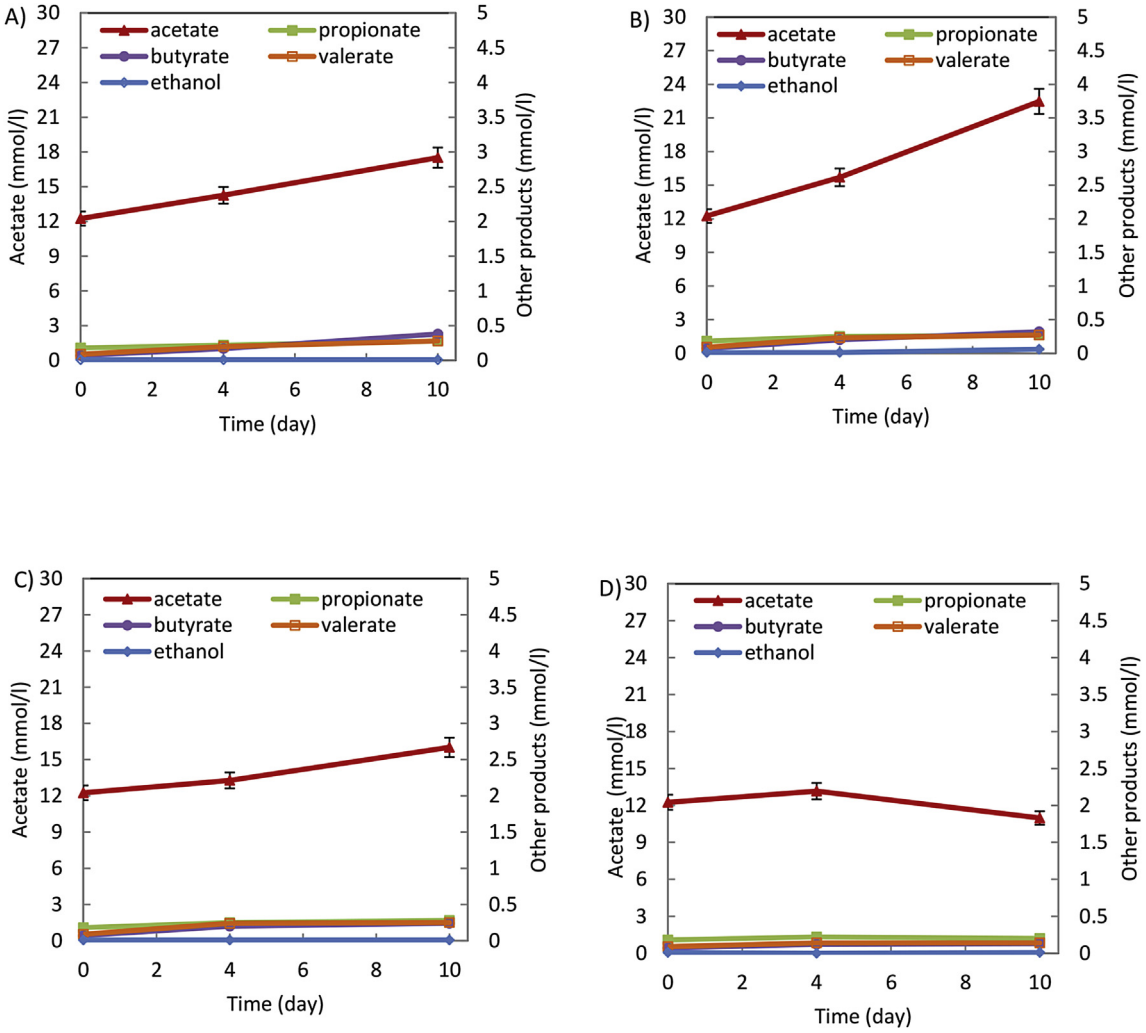

Fig. 5. Ethanol and VFA production profile of the mixed culture with acetate amended media observed under different gas composition: (A) Synthetic syngas, (B) pure CO, (C) pure H_2 and (D) control gas consisting of only N_2.

rate of formation of ethanol compared to that without acetate addition. The maximum concentration of ethanol (2.0 mmol/l) produced by *C. ragsdalei* was observed under a headspace of SS after day 4 of inoculation, which was 41% and 130% higher than ethanol produced under CO and H_2, respectively. Final concentrations of ethanol in headspaces of SS and CO however did not clearly change as a result of acetate addition. This could be due to that ethanol was directly used as reductant during chain elongation as well as an increase in pH (Supplementary Information (SI)). The addition of acetate during fermentation resulted in a positive effect on ethanol formation under all the gases conditions (Fig. 4C). According to Eqs. (1) and (2) the reduction of acetate to ethanol (reaction 16 in Table 2) would also become more favorable with a higher initial concentration of acetate.

The effect of acetate addition on the gas fermentation was also investigated with mixed culture. Acetate was the main metabolite produced from mixed culture fermentation (Fig. 5). The maximum concentration of acetate by mixed culture fermentation was observed under a headspace of CO. The presence of acetate under the headspace of CO resulted in 11% increase in net acetate production compared to experiment without acetate. While, the addition of acetate showed negative effect under headspaces of SS, where the net acetate production was 35% lower compared to the same experiment without acetate. The formation of propionate, butyrate and valerate with mixed culture was slightly higher with acetate addition (Fig. 5). The addition of acetate had no effect on ethanol under any of the gas compositions during mixed culture fermentation. This was assumed to be due to increasing pH throughout inoculation (Supplementary Information (SI)). According to previous studies [29,30] increasing pH inhibits formation of alcohol while chain elongation is enhanced. Besides, Jankowska et al. [31] observed higher production of fatty acids at high pH (with maximum concentrations around pH 10–11). Furthermore, it was observed that longer inoculation times (10–15 days) the higher concentrations were achieved. Thus, a high initial pH could therefore potentially have enhanced the production of fatty acids in this study.

4. Future research direction for syngas fermentation

Results obtained from the present study can pave the way towards sustainable valorization of environmental pollutants for commercial production of biochemical and biofuels. Gaseous pollutants (e.g. CO/CO_2) that are considered as a significant environmental loading for many industrial institutions (e.g. petrochemical, electric power, and iron smelting plants) can be used as an efficient substrate for production of these valuable products. Fermentation of these gases using biological effluent (digested manure or sludge) from biogas or wastewater plants and wastes rich in VFA (e.g., brewery wastewaters) as a cheap source of inoculum and fermentation medium can significantly contribute in the reduction of environmental pollution and the high cost of biofuel production.

5. Conclusions

Through this study, the optimal conditions (media, gas composition and biocatalyst) for high efficient fermentative production of VFA and ethanol have been identified. Acetate was the most dominant product independent of inoculum type. CO was the preferred gas composition with respect to highest concentrations of VFA regardless of media and microbial composition. Fermentation of CO in acetate-free medium by *C. ragsdalei* produced up to 3 times higher VFA compared to mixed culture. The maximum concentration of ethanol was also achieved under the headspace of CO by *C. ragsdalei*, while no ethanol was produced by mixed culture fermentation in any of the both gas compositions. Addition of acetate through the pure culture fermentation resulted in lower production of VFA compared to experiment without acetate. While, the effect of added acetate in mixed culture fermentation varied depending on the headspace composition.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biombioe.2017.06.020.

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