Butyric Acid Production by *Clostridium tyrobutyricum* in Sugar Mixtures and Corncob Hydrolysate Containing Arabinose

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The objective of this study was to understand arabinose effects on butyric acid fermentation by *Clostridium tyrobutyricum* in sugar-supplemented media and biomass hydrolysate. Compared to glucose and xylose, the conversion of arabinose to butyric acid was less efficient with a longer lag phase (4 d) and fermentation period (6 d), and a lower butyric acid yield of 0.26 g/g and 0.15 g/g in 5 g/L and 10 g/L of arabinose media, respectively. However, the addition of 2 g/L or 5 g/L of arabinose to the baseline medium that contained glucose (15 g/L) and xylose (15 g/L) enhanced butyric acid synthesis, resulting in 16.1 g/L and 20.3 g/L of butyric acid, respectively. Further increase in arabinose to 10 g/L in the medium of 10 g/L of glucose and 10 g/L of xylose showed inhibitory effects on *C. tyrobutyricum*, which suggested that high concentrations of arabinose (> 10 g/L) were not desirable. Dilute-acid pretreated corncobs that contained xylose (19 g/L) and a small amount of arabinose (2 g/L) were a feasible substrate for butyric acid production by *C. tyrobutyricum*, and resulted in 10.6 g/L butyric acid, which was slightly lower than in the mimic medium (11.3 g/L).

Keywords: Clostridium tyrobutyricum; Butyric acid; Corncob; Xylose; Lignocellulosic biomass; Fermentation; Arabinose

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INTRODUCTION

Plant biomass is an abundant and relatively inexpensive renewable resource that has gained great attention in the last few decades (Mohan *et al*. 2006; Burhenne *et al*. 2013). In addition to biofuels, plant biomass can also be used to produce petrochemical products such as butyric acid (Nhien *et al.* 2016). Among varieties of biomass, the non-edible lignocellulosic biomass is more favorable because it does not compete with human food (Patel *et al.* 2015). Lignocellulose is mainly composed of cellulose, hemicellulose, and lignin, with small amounts of pectin, protein, extractives, and ash (Jorgensen *et al.* 2007). In the microstructure of lignocellulose, cellulose and hemicellulose are tangled together to form a carbohydrate polymer, which is cross-linked with lignin (Jorgensen *et al.* 2007; Toquero and Bolado 2014). The composition of these three constituents can vary from one plant species to another (Kumar *et al.* 2008). Hardwoods are known to have greater proportional amounts of cellulose, whereas forage biomass contains higher levels of hemicelluloses (Sun and Cheng 2002).

In general, cellulose, hemicellulose, and lignin account for 35% to 50%, 20% to 50%, and 12% to 35%, respectively, of the dry weight of lignocellulosic biomass (Kumar *et al.* 2008). Cellulose consists of long, crystalline polymers of glucose. After pretreatment and enzymatic hydrolysis of cellulose, glucose can be obtained for biofuel production. Degradation of hemicellulose is the easiest of the three main components of biomass because of its random amorphous structure. Converting cellulose and hemicellulose to renewable fuels and chemicals has been extensively studied (Martien and Amador-Noguez 2016).

Some work has been reported to convert cellulose and part of hemicellulose (mainly xylose) to butyric acid by *C. tyrobutyricum* (Zhu *et al*. 2002; Zhu and Yang; 2004; Jiang *et al.* 2009; Mitchell *et al*. 2009; Luo *et al.* 2017). Butyric acid is a short-chain fatty acid (C_3H_7COOH), which has many applications, including use in the production of butanol, ethyl butyrate, and butyl butyrate (Al-Shorgani *et al.* 2012). It may also be used to treat hemoglobinopathies, cancer, and gastrointestinal diseases (Dwidar *et al.* 2012). It is also a source material for plastics production, especially cellulose acetate butyrate plastics (Cao *et al.* 2011; Dwidar *et al.* 2012). In addition, plasticizers, surfactants, and textile auxiliaries also use butyric acid as an important ingredient (Liu *et al.* 2013). In the form of esters and salts, butyric acid can be utilized as a fragrance and flavoring agents in foods, beverages, and cosmetics (Dwidar *et al.* 2012; Liu *et al.* 2013).

Most of the abovementioned work has focused on converting glucose or xylose to butyric acid by various microbes, especially *C. tyrobutyricum*. As an important component of many agricultural residues, arabinose is also a notable pentose, but less attention has been paid to the fermentation of arabinose. Research exploring arabinose and their effects on fermentation media by *C. tyrobutyricum* has not been reported to the best of the authors' knowledge.

In contrast, converting hemicellulose in real biomass, specifically corncobs, to butyric acid has not been reported. Corn is one of the most important plants worldwide. Every part of corn has been used extensively to produce biofuels (mainly ethanol) and bioproducts. Corncobs, which generally contain approximately 45% cellulose, 35% hemicelluloses, and 15% lignin on a dry basis (Sun and Cheng 2002), can be utilized as sustainable feedstock for biofuel and biochemical production because it does not interfere with the food chain of the ecosystem. Many reports are available in the literature dealing with the use of corncobs for the production of animal and poultry hovel (Tsai *et al.* 2001), energy (Mullen *et al.* 2010), proteins (Perotti and Molina 1988), carbon adsorbents (Tsai *et al.* 2001), ethanol (de Carvalho Lima *et al.* 2002), xylitol (Dominguez *et al.* 1997; Latif and Rajoka 2001), and biochar (Mullen *et al.* 2010). However, no report of using corncobs for butyric acid production was found when searching the literature.

Thus, this study had two objectives: to understand the effects arabinose has on butyric acid fermentation by *C. tyrobutyricum* in either arabinose-alone media or in glucose-xylose mixtures, and to evaluate the performance of corncob hydrolysate (hemicellulose) in butyric acid fermentation by *C. tyrobutyricum*. The bacteria *C. tyrobutyricum* is a gram-positive, obligate anaerobe. It is one of the most promising strains for butyric acid production due to its high butyric acid selectivity, yield, and productivity, and great tolerance to high product concentration (Zigova and Sturdik 2000; Liu and Yang 2006; Najafpour 2006; Song *et al.* 2010).

EXPERIMENTAL

Bacteria Strain and Growth Media

The *C. tyrobutyricum* (ATCC 25755) was obtained from American Type Culture Collection (ATCC, Manassas, VA). The synthetic medium (recipe provided by ATCC) with sugar supplements was used for the inoculation and culture experiments. In all of the experiments, D-glucose (CAS 50-99-7) was obtained from Fisher Scientific (Hampton, NH, USA). The D-xylose (CAS 58-86-6) and L-arabinose (CAS 5328-37-0) were obtained from Cascade Analytical Reagents & Biochemicals (Corvallis, OR, USA). They were added to the synthetic medium at varying initial concentrations (Table 1).

Table 1. Experimental Design of Arabinose-supplemented Media Test

Medium Type		Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)
Arabinose alone	Control 1	5, 10	-	-
	Control 2	-	5, 10	-
	Treatments	-	-	5, 10
Arabinose-supplemented	Control	15	15	-
	Treatments	15	15	2, 5
Sugar mixtures	Control	15	15	-
	Treatment	10	10	10
Biomass	Corncob hydrolysate	2	19	2
	Mimic medium	2	19	2

In arabinose-alone experiments, media with 5 g/L or 10 g/L of arabinose were tested. The purpose was to understand the effects of arabinose as the sole carbon source in the culture medium on butyric acid fermentation. Glucose- or xylose-alone media with the same sugar concentration were also studied as the controls. In arabinose-supplemented experiments, arabinose of 2 g/L or 5 g/L was added to the glucose and xylose mixture. The control had no arabinose in the medium. The purpose of these experiments was to understand the effect of small amounts of arabinose on glucose/xylose fermentation, but arabinose was not the main carbon source. In sugar mixture experiments, the treatment medium contained 10 g/L each of glucose, xylose, and arabinose. The control contained 15 g/L each of glucose and xylose without arabinose. Thus, the total sugar was 30 g/L for both the control and the treatment. The purpose was to understand the effect of arabinose as a major carbon source in the culture medium along with glucose and xylose.

In biomass experiments, corncobs were hydrolyzed to obtain sugars from hemicellulose. The corncobs were obtained from Kaytee Products Inc. (Chilton, WI) and ground using a Retsch SM2000 rotary cutting mill (Retsch Inc., Newtown, PA) with a 1.0 mm screen. The hydrolysate was prepared by a diluted acid treatment of 50 g of corncobs in 50 mL of 5% sulphuric acid (vol/vol) and autoclaved at 121 ºC for 30 min. The solid and liquid phases were separated by filtration (Fisherbrand™, Coarse cellulose fiber papers remove particles > 25 μm). The hydrolysate was stored at 4 ºC until use. The corncob mimic medium was prepared to match corncob hydrolysates and contained xylose (19 g/L), arabinose (2 g/L), and glucose (2 g/L).

All of the media also contained yeast extract (5 g/L), ammonium sulfate ($(NH_4)_2SO_4$, 3 g/L), dipotassium phosphate (K_2HPO_4, 1.5 g/L), magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$, 0.6 g/L), and ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$,

0.03 g/L), and were sterilized at 121 °C for 25 min before inoculation. All chemicals were purchased from Fisher Scientific.

Fermentation process

All media were adjusted to a pH of 6.4 daily by adding ammonium hydroxide (NH_4OH, Fisher Scientific). All experiments were performed in 500-mL Erlenmeyer flasks containing 270 mL of culture medium and 30 mL of bacteria seeds. The flasks were capped with air-lock valves. After inoculation, the bottles were flushed with N_2 gas to ensure anaerobic conditions. The culture was maintained at 37 °C on a reciprocating shaker (Fisher Scientific) at 150 rpm with three replicates.

Methods

The cell density was analyzed by measuring the optical density (OD) of a cell suspension at the wavelength of 620 nm (OD_{620}) with a spectrophotometer (Model 340, Sequoia-Turner Corp., Santa Clara, CA, USA). A high-performance liquid chromatography (HPLC) system (Agilent Technologies 1200 Series, Agilent Technologies, Santa Clara, CA, USA) was used to analyze the organic compounds, including xylose, glucose, arabinose, lactate, butyrate, and acetate in the fermentation broth. The HPLC system consisted of an auto-sampler, an ion-exclusion rezex organic acid column (Phenomenex, Torrance, CA, USA) in a column oven at 45 °C, a diode array detector, and an evaporative light scattering detector. The mobile phase was 5 mM sulfuric acid (H_2SO_4, HPLC grade, Fisher Scientific) at a flow rate of 0.6 mL/min.

A statistical analysis was performed to determine the level of significance. Multiple one-way analyses of variance (ANOVA) tests were conducted to evaluate the effect of the initial sugar concentration on butyric acid production. The concentration of the initial sugar was used as the independent variable, while the butyric acid peak concentration, yield, or productivity was used as the dependent variable. All of the results were expressed as the mean ± standard deviation ($n \leq 3$). Tukey's adjustment was applied to the general linear model for the determination level of significance ($P < 0.05$) among various treatments. The software SAS Version 9.1.3 (SAS Institute Inc., Cary, NC) was used for all statistical analysis testing.

RESULTS AND DISCUSSION

Fermentation in Arabinose-alone Media

When *C. tyrobutyricum* was cultured in low concentrations of arabinose (5 g/L or 10 g/L), it grew very slowly and had a long lag phase of approximately 4 d, and arabinose was not used until after day 3 (Fig. 1A). In contrast, the lag phase in the glucose media was 1 day, and in the xylose media was 2 d (Luo *et al.* 2017). It was also apparent that cell growth increased with an increased arabinose concentration, but butyric acid synthesis was approximately the same for 5 g/L and 10 g/L of arabinose (Fig. 1B).

Compared to glucose or xylose media, arabinose conversion to butyric acid was much less efficient (Table 2). The conversion and effects of glucose and xylose on butyric acid production by *C. tyrobutyricum* was reported in a previous study (Luo *et al.* 2017). In both 5 g/L and 10 g/L sugar-supplemented media, xylose resulted in the highest butyric acid concentration and yield, and arabinose was the poorest. In terms of butyric acid

productivity, glucose was the best sugar because of its much shorter fermentation time (2 d in glucose *vs.* 4 d in xylose and 6 d in arabinose media to reach peak butyric acid concentration), and arabinose was again the poorest. The results suggested that arabinose alone at concentrations higher than 5 g/L in the medium was not suitable for butyric acid fermentation by *C. tyrobutyricum*. However, when other sugars are present in the culture medium, the bacteria is capable of converting arabinose to butyric acid (see next section).

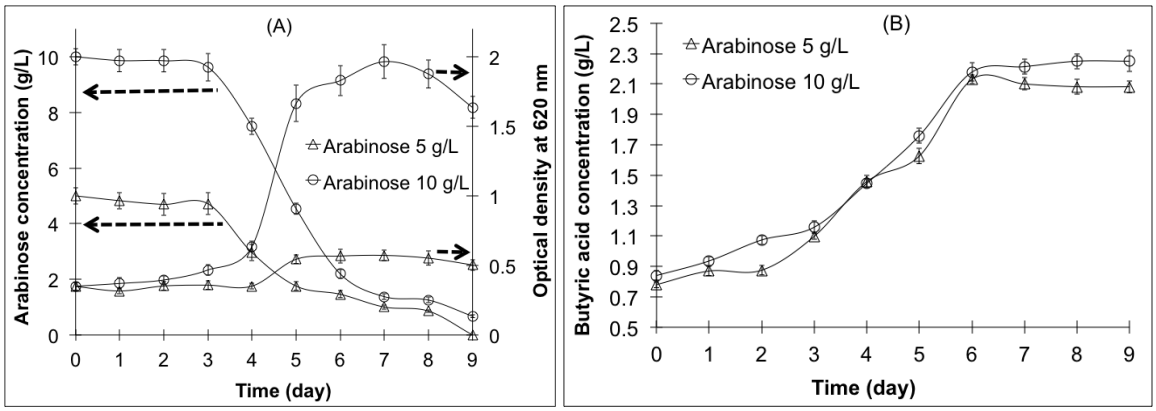

Fig. 1. Growth and sugar consumption (A) and butyric acid concentration (B) of *C. tyrobutyricum* in arabinose-alone media

Table 2. Final Butyric Acid Concentration, Yield, and Productivity in Single-sugar Media

Medium Type	5 g/L Sugar			10 g/L Sugar		
	Peak BA Concentration [#] (g/L)	BA yield (g/g)	BA Productivity (g/L-d)	Peak BA Concentration[#] (g/L)	BA Yield (g/g)	BA Productivity (g/L-d)
Glucose media	1.57 ± 0.13 (b)	0.31 ± 0.03 (b)	0.79 ± 0.07 (a)	3.41 ± 0.18 (b)	0.34 ± 0.02 (b)	1.71 ± 0.09 (a)
Xylose media	2.25 ± 0.21 (a)	0.45 ± 0.04 (a)	0.56 ± 0.05 (b)	4.17 ± 0.23 (a)	0.42 ± 0.02 (a)	1.04 ± 0.05 (b)
Arabinose media	1.30 ± 0.11 (c)	0.26 ± 0.02 (c)	0.22 ± 0.02 (c)	1.41 ± 0.13 (c)	0.15 ± 0.01 (c)	0.24 ± 0.02 (c)

[#]Butyric acid concentration was adjusted by deducting the initial butyric acid coming from the seed inoculum; different letters following the numbers indicate significant differences.

Fermentation in Arabinose-supplemented Media

In this experiment, the mixture of 15 g/L of glucose and 15 g/L of xylose was chosen as the baseline medium because such a mixture resulted in efficient butyric acid production (Luo *et al.* 2017). When supplemented with arabinose of 2 g/L or 5 g/L, *C. tyrobutyricum* reached the maximum OD of approximately 1.8 on day 4. This was similar to the baseline medium without arabinose (Fig. 2A), which implied that the addition of small amounts of arabinose (2 g/L or 5 g/L) to the glucose and xylose mixture did not significantly affect bacterial growth (Tukey's test, $p > 0.05$).

The butyric acid production was quite different among the three media, especially when the medium was supplemented with 5 g/L arabinose. The butyric acid concentration

increased with no significant change (Tukey's test, $p > 0.05$) in the fermentation time (Fig. 2B). As shown, the medium with 2 g/L or 5 g/L arabinose produced 16.1 g/L and 20.3 g/L butyric acid, respectively, while the baseline resulted in only 14.5 g/L butyric acid. This increase in butyric acid was possible only when arabinose stimulated or enhanced butyric acid synthesis from glucose and xylose. This phenomenon has not been reported before and is yet to be fully understood. The authors' previous work showed that mixtures of glucose and xylose all had better results than the single sugar media having the same total sugar concentration (Luo *et al.* 2017). This suggested that mixing the two sugars could promote bacteria growth and butyric acid generation. It seems a similar mechanism might also have an important function when adding small amounts of arabinose to the glucose and xylose mixture, which could promote butyric acid synthesis by *C. tyrobutyricum*.

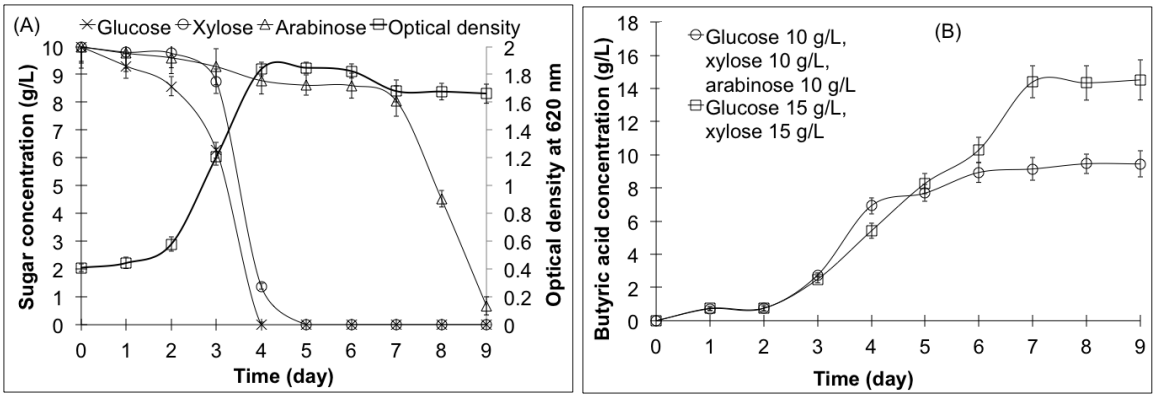

Fig. 2. Growth (A) and butyric acid synthesis (B) of *C. tyrobutyricum* in arabinose-supplemented media

The sugar consumption in the three media was as expected. Glucose was used first, followed by xylose. Arabinose was not used until glucose and xylose were almost depleted (Fig. 3A). This was consistent with previous studies (Hahn-Hägerdal 2007; Luo *et al.* 2017). It has been known that the affinity of the hexose transporters for xylose is one to two orders of magnitude lower than the affinity for glucose (Kotter and Ciriacy 1993). Therefore, in sugar mixtures, xylose is usually consumed only after the depletion of hexose sugars.

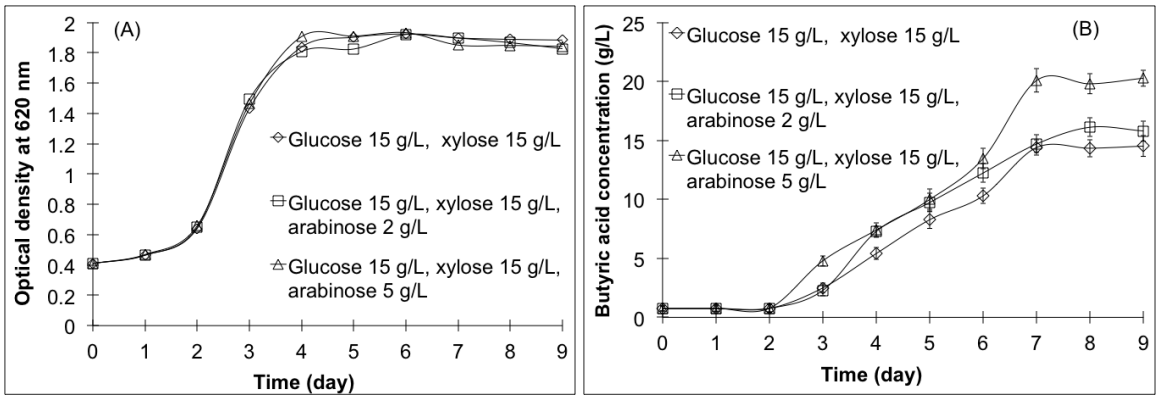

Fig. 3. Sugar consumptions in 2 g/L (A) and 5 g/L (B) arabinose-supplemented media

Although arabinose and xylose are both pentoses from hemicellulose, it is known that L-arabinose has a different metabolic pathway from D-xylose in bacteria (Fig. 4). L-arabinose and D-xylose are isomerized to L-ribulose and D-xylulose, which are then phosphorylated to L-ribulose-5-phosphate and D-xylulose-5-phosphate, respectively. It takes another step to convert L-ribulose-5-phosphate to D-xylulose-5-phosphate. This extra step may require some extra energy and not occur when glucose or xylose are present.

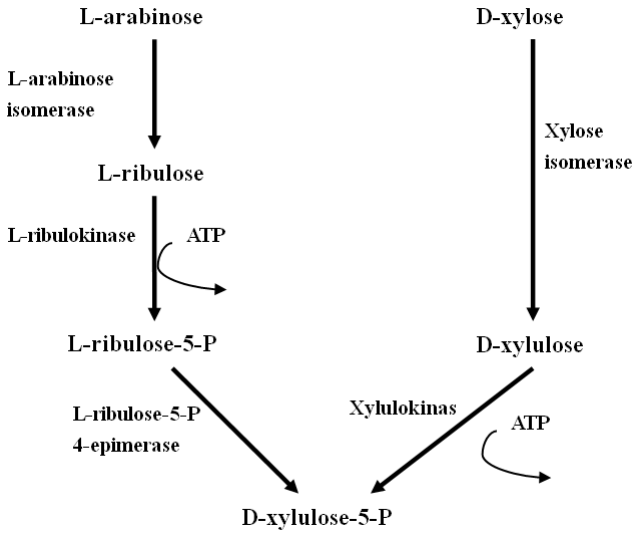

Fig. 4. The metabolic pathway of xylose and arabinose by bacteria (Hahn-Hagerdal 2007). ATP = adenosine triphosphate.

Fermentation in Glucose-xylose-arabinose Mixtures

A logical idea was to see if higher arabinose concentrations in a glucose/xylose mixture would also support butyric acid synthesis. The *C. tyrobutyricum* was cultured in the baseline medium with 15 g/L glucose and 15 g/L xylose, and in the treatment the medium contained 10 g/L glucose, 10 g/L xylose, and 10 g/L arabinose. Thus, the total amount of sugars was kept the same in both media. Interestingly, the treatment reached maximum OD similarly to the control (Fig. 5A *vs.* 3A); however, the OD did not increase after day 5 when glucose and xylose were both exhausted.

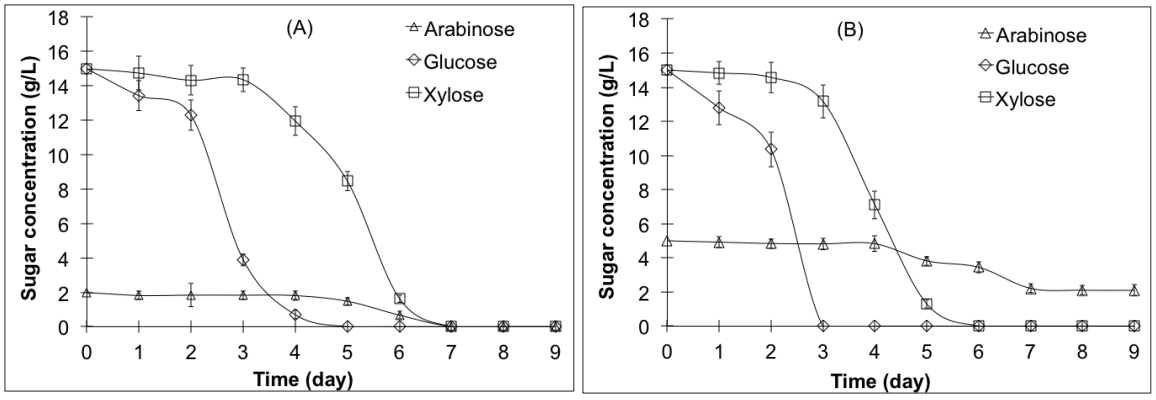

Fig. 5. Growth and sugar consumption (A) and butyric acid synthesis (B) of *C. tyrobutyricum* in glucose/xylose/arabinose mixtures

The sugar consumption curves showed that glucose was exhausted in 4 d and xylose in 5 d, while arabinose was not used at all until after day 6. This again suggested that arabinose at high concentrations (≥10 g/L) was not a desirable sugar for *C. tyrobutyricum* growth in the presence of glucose or xylose.

The bacteria in the 30 g/L three-sugar mixture medium produced 9.5 g/L butyric acid in 9 d, whereas bacteria in the medium with 15 g/L glucose and 15 g/L xylose produced 14.5 g/L butyric acid in 7 d (Fig. 5B). It was apparent that arabinose at 10 g/L was much less efficient than glucose and xylose in the conversion to butyric acid. The beneficial effects similar to 2 g/L or 5 g/L of arabinose added into the butyric acid synthesis were not observed. Instead, adverse effects were evident. It suggested that arabinose at 10 g/L or higher in a glucose and xylose mixture had inhibitory effects on butyric synthesis by *C. tyrobutyricum*, similar to in an arabinose-alone media.

Fermentation in Corncob Hydrolysate and Mimic Medium

The main sugar in the corncob hydrolysate was xylose, with small amounts of arabinose and glucose (Table 1). The synthetic mimic medium had the same sugar amounts as the hydrolysate. Compared to the mimic medium, *C. tyrobutyricum* in the corncob hydrolysate had a longer lag phase (3 d *vs.* 1 d in the mimic medium). This occurred because the acid-pretreated lignocellulose not only released sugar monomers in the hemicellulose, but it also generated other substances, such as phenolic compounds, furan derivatives, and aliphatic acids (Larsson *et al.* 2000; Ranatunga *et al.* 2000; Nilvebrant *et al.* 2003), which all could adversely affect bacterial growth during the initial stage of cultivation. Cell growth at the end of the cultivation was similar in the two media, which indicated that the bacteria adapted to the inhibitors in approximately 3 d (Fig. 6A). Although the same amount of bacteria inoculum was added to the two culture media, the initial OD value of corncob hydrolysate was higher than that of the mimic medium due to the darker color of the hydrolysate at the beginning.

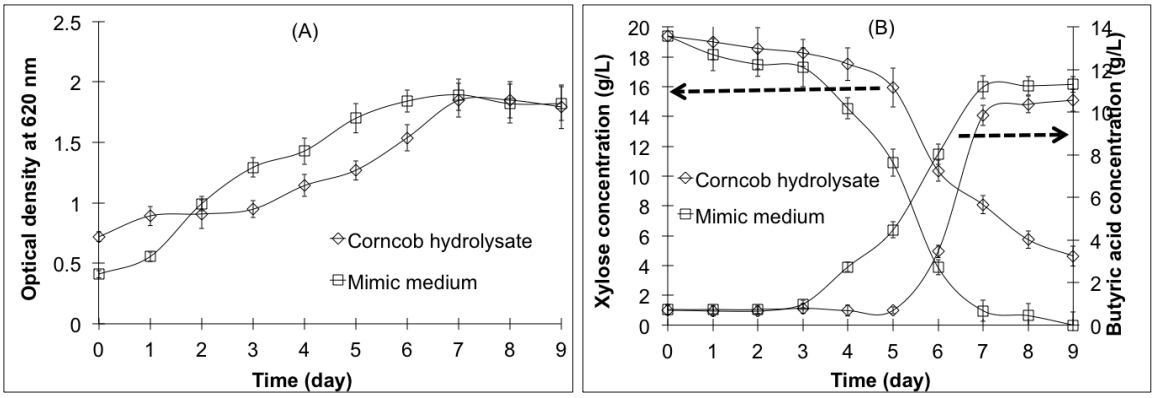

Fig. 6. Growth (A) and xylose consumption and butyric acid synthesis (B) of *C. tyrobutyricum* in corncob hydrolysate and its mimic medium

Sugar consumption in the hydrolysate was slower than in the mimic medium (Fig. 6B), which was consistent with cell growth. On day 9, all of the xylose in the mimic medium was exhausted, while 4.6 g/L of xylose still remained in the corncob hydrolysate. The main product of both media was butyric acid. At the end of the cultivation, the concentration of butyric acid in the corncob hydrolyses reached 10.6 g/L, whereas the

mimic medium reached 11.3 g/L (Fig. 6B). Although the hydrolysate resulted in slightly lower butyric acid production, its xylose to butyric acid conversion efficiency was actually higher than in the mimic medium if xylose was assumed to be the sole carbon source for butyric acid synthesis. It is hypothesized that some of the non-xylose compounds that were generated during the pretreatment process might have been metabolized by the bacteria and converted to butyric acid. This was in accordance with the growth curve (Fig. 6A) that the bacteria was initially inhibited but caught up in the later phase. These results indicated that the acid-treated corncob hydrolysate was an effective substrate for butyric acid fermentation by *C. tyrobutyricum*, and no further treatment of the hydrolysate was necessary.

CONCLUSIONS

1. Arabinose alone was not an effective carbon source for butyric acid synthesis by *C. tyrobutyricum*. Compared to glucose- or xylose-alone with the same concentration, xylose alone resulted in the lowest butyric acid concentration, yield, and productivity.
2. Arabinose addition at low concentrations (2 g/L or 5 g/L) to glucose and xylose mixtures resulted in enhanced butyric acid production by *C. tyrobutyricum*.
3. A high concentration of arabinose (10 g/L) in the glucose and xylose mixture had inhibitory effects on butyric acid synthesis by *C. tyrobutyricum*.
4. Acid-pretreated corncob hydrolysate was an effective substrate for butyric acid fermentation by *C. tyrobutyricum*, and no further treatment of the hydrolysate was necessary.

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REFERENCES CITED

Al-Shorgani, N. K. N., Ali, E., Kalil, M. S., and Yusoff, W. M. W. (2012). "Bioconversion of butyric acid to butanol by *Clostridium saccharoperbutylacetonicum* N1-4 (ATCC 13564) in a limited nutrient medium," *Bioenergy Research* 5(2), 287-293. DOI: 10.1007/s12155-011-9126-6

Burhenne, L., Messmer, J., Aicher, T., and Laborie, M. P. (2013). "The effect of the biomass components lignin, cellulose and hemicellulose on TGA and fixed bed pyrolysis," *Journal of Analytical and Applied Pyrolysis* 101, 177-184. DOI: 10.1016/j.jaap.2013.01.012

Cao, Y., Li, H. Q., and Zhang, J. (2011). "Homogeneous synthesis and characterization of cellulose acetate butyrate (CAB) in 1-allyl-3-methylimidazolium chloride (AmimCI) ionic liquid," *Industrial and Engineering Chemistry Research* 50(13), 7808-7814. DOI: 10.1021/ie2004362

de Carvalho Lima, K. G., Takahashi, C. M., and Alterthum, F. (2002). "Ethanol production from corn cob hydrolysates by *escherichia coli* KO11," *Journal of Industrial Microbiology Biotechnology* 29(3), 124-128. DOI: 10.1038/sj.jim.7000287

Dominguez, J. M., Cao, N., Gong, C. S., and Tsao, G. T. (1997). "Dilute acid hemicellulose hydrolysates from corn cobs for xylitol production by yeast," *Bioresource Technology* 61(1), 85-90. DOI: 10.1016/S0960-8524(97)84703-7

Dwidar, M., Park, J. Y., Mitchell, R. J., and Sang, R. I. (2012). "The future of butyric acid in industry," *The Scientific World Journal* Article ID 471417. DOI: 10.1100/2012/471417

Hahn-Hägerdal, B. (2007). "Towards industrial pentose-fermenting yeast strains," *Applied Microbiology and Biotechnology* 74(5), 937-953. DOI: 10.1007/s00253-006-0827-2

Jiang, L., Wang, J., Liang, S., Wang, X., Cen, P., and Xu, Z. (2009). "Butyric acid fermentation in a fibrous bed bioreactor with immobilized *Clostridium tyrobutyricum* from cane molasses," *Bioresource Technology* 100(13), 3403-3409. DOI: 10.1016/j.biortech.2009.02.032

Jorgensen, H., Kristensen, J. B., and Felby, C. (2007). "Enzymatic conversion of lignocellulose into fermentable sugars: Challenges and opportunities," *Biofuels, Bioproducts and Biorefining* 1(2), 119-134. DOI: 10.1002/bbb.4

Kotter, P., and Ciriacy, M. (1993). "Xylose fermentation by *Saccharomyces cerevisiae*," *Applied Microbiology and Biotechnology* 38(6), 776–783. DOI: 10.1007/BF00167144

Kumar, R., Singh, S., and Singh O. V. (2008). "Bioconversion of lignocellulosic biomass: Biochemical and molecular perspectives," *Journal of Industrial Microbiology & Biotechnology* 35(5), 377-391. DOI: 10.1007/s10295-008-0327-8

Larsson, S., Quintana-Sainz, A., Reimann, A., Nilvebrant, N., and Jonsson, L. J. (2000). "Influence of lignocellulose-derived aromatic compounds on oxygen-limited growth and ethanolic fermentation by *saccharomyces cerevisiae*," *Applied Biochemistry and Biotechnology* 84(1), 617-632. DOI: 10.1385/ABAB:84-86:1-9:617

Latif, F., and Rajoka, M. I. (2001). "Production of ethanol and xylitol from corn cobs by yeasts," *Bioresource Technology* 77(1), 57-63. DOI: 10.1016/j.biortech.2013.06.023

Liu, X., and Yang, S. T. (2006). "Kinetics of butyric acid fermentation of glucose and xylose by *Clostridium tyrobutyricum* wild type and mutant," *Process Biochemistry* 41, 801-808. DOI: 10.1016/j.procbio.2005.10.009

Liu, S., Bischoff, K. M., Leathers, T. D., Qureshi, N., Rich, J. O., and Hughes, S. R. (2013). "Butyric acid from anaerobic fermentation of lignocellulosic biomass hydrolysates by *Clostridium tyrobutyricum* strain RPT-4213," *Bioresource Technology* 143, 322-329. DOI: 10.1016/j.biortech.2013.06.015

Luo, G., Zhang, L., Chen, T., Geng, Y., and Yuan, W. (2017). "Butyric acid fermentation in xylose and glucose by *Clostridium tyrobutyricum*," *BioResources* 12(2), 2930-2940. DOI: 10.15376/biores.12.2.2930-2940

Martien, J. I., and Amador-Noguez, D. (2016). "Recent applications of metabolomics to advance microbial biofuel production," *Current opinion in Biotechnology* 43, 118-126. DOI: 10.1016/j.copbio.2016.11.006

Mitchell, R. J., Kim, J. S., Jeon, B. S., and Sang, B. I. (2009). "Continuous hydrogen and butyric acid fermentation by immobilized *Clostridium tyrobutyricum* ATCC 25755: Effects of the glucose concentration and hydraulic retention time," *Bioresource Technology* 100(21), 5352-5355. DOI: 10.1016/j.biortech.2009.05.046

Mohan, D., Pittman, C. U., and Steele, P. H. (2006). "Pyrolysis of wood/biomass for bio-oil: A critical review," *Energy & Fuels* 20(3), 848-889. DOI: 10.1021/ef0502397

Mullen, C. A., Boateng, A. A., Goldberg, N. M., Lima, I. M., Laird, D, A., and Hicks, K. B. (2010). "Bio-oil and bio-char production from corn cobs and stover by fast pyrolysis," *Biomass Bioenergy* 34(1), 67-74. DOI: 10.1016/j.biombioe.2009.09.012

Najafpour, G. D. (2006). "Immobilization of microbial cells for the production of organic acid and ethanol," *Biochemical Engineering and Biotechnology* 8, 199-227. DOI: 10.1016/B978-044452845-2/50008-2

Nhien, L. C., Long, N. V. D., and Lee, M. (2016). "Design and optimization of the levulinic acid recovery process from lignocellulosic biomass," *Chemical Engineering Research and Design* 107, 126-136. DOI: 10.1016/j.cherd.2015.09.013

Nilvebrant, N. O., Persson, P., Reimann, A., de Sousa, F., Gorton, L, and Jonsson, L. J. (2003). "Limits for alkaline detoxification of dilute-acid lignocellulose hydrolysates," *Applied Biochemistry and Biotechnology* 107(1), 615-628. DOI: 10.1385/ABAB:107:1-3:615

Patel, A., Sindhu, D. K., Arora, N., Singh, R. P., Pruthi, V., and Pruthi, P. A. (2015). "Biodiesel production from non-edible lignocellulosic biomass of *Cassia fistula* L. fruit pulp using oleaginous yeast *Rhodosporidium kratochvilovae* HIMPA1," *Bioresource Technology* 197, 91-98. DOI: 10.1016/j.biortech.2015.08.039

Perotti, N. I., and Molina, O. E. (1988). "Corn cob as a bacterial substrate for the production of forage protein," *Biological Wastes* 26(2), 125-131. DOI: 10.1016/0269-7483(88)90157-7

Ranatunga, T. D., Jervis, J., Heim, R. McMillan, J. D., and Wooley, R. J. (2000). "The effect of overliming on the toxicity of dilute acid pretreated lignocellulosics: The role of inorganics, uronic acids and ether-soluble organics," *Enzyme and Microbial Technology* 27(3-5), 240-247. DOI: 10.1016/S0141-0229(00)00216-7

Song, H., Eom, M. H., Lee, S., Lee, J., Cho, J. H., and Seung, D. (2010). "Modeling of batch experimental kinetics and application to fed-batch fermentation of *Clostridium tyrobutyricum* for enhanced butyric acid production," *Biochemical Engineering Journal* 53, 71-76. DOI: 10.1016/j.bej.2010.09.010

Sun, Y., and Cheng, J. (2002). "Hydrolysis of lignocellulosic materials for ethanol production: A review," *Bioresource Technology* 83, 1-11. DOI: 10.1016/S0960-8524(01)00212-7

Toquero, C., and Bolado, S. (2014). "Effect of four pretreatments on enzymatic hydrolysis and ethanol fermentation of wheat straw. Influence of inhibitors and washing," *Bioresource Technology* 157, 68-76. DOI: 10.1016/j.biortech.2014.01.090

Tsai, W. T., Chang, C. Y., Wang, S. Y., Chien, S. F., and Sun, H. F. (2001). "Cleaner production of carbon adsorbents by utilizing agricultural waste corn cob," *Resources, Conservation, and Recycling* 32(1), 43-53. DOI: 10.1016/S0921-3449(00)00093-8

Zhu, Y., and Yang, S. T. (2004). "Effect of pH on metabolic pathway shift in fermentation of xylose by *Clostridium tyrobutyricum*," *Journal of Biotechnology* 110(2), 143-157. DOI: 0.1016/j.jbiotec.2004.02.006

Zhu, Y., Wu, Z., and Yang, S. T. (2002). "Butyric acid production from acid hydrolysate of corn fiber by *Clostridium tyrobutyricum* in a fibrous-bed bioreactor," *Process Biochemistry* 38(5), 657-666. DOI: 10.1016/S0032-9592(02)00162-0

Zigova, J., and Sturdik, E. (2000). "Advances in biotechnological production of butyric acid," *Journal of Industrial Microbiology and Biotechnology* 24, 153-160. DOI: 10.1038/sj.jim.2900795

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