The Effects of Syringaldehyde and Vanillin on Butyric Acid Production by Fermentation Using *Clostridium tyrobutyricum*

Ying Liu,[a,b] Yingxi Geng,[b] Quan Zhou,[b] and Wenqiao Yuan [b,*]

Butyric acid is a valuable chemical that has various applications in the chemical, pharmaceutical, food, and biofuel industries. Its bio-fermentation from lignocellulosic materials may be affected by some unwanted substrates that are generated during biomass pretreatment processes. In the present study, the potential inhibitory effects of two phenolic aldehydes (syringaldehyde and vanillin) on butyric acid fermentation by *Clostridium tyrobutyricum* were evaluated. The toxicity of syringaldehyde and vanillin on cell growth, xylose consumption, and butyric acid production was dosage-dependent. The butyric acid productivity decreased significantly with increasing concentrations of syringaldehyde and vanillin. *C. tyrobutyricum* demonstrated a broad tolerance to both syringaldehyde and vanillin and only moderate reductions in the maximum cell density were observed with up to 2.4 g L^{-1} of syringaldehyde or vanillin in the medium. Both syringaldehyde and vanillin were assimilated by *C. tyrobutyricum*, and the metabolite products from vanillin caused considerable inhibition of the fermentation.

Keywords: Butyric acid; Clostridium tyrobutyricum; Inhibitors; Lignocellulosic hydrolysate; Syringaldehyde; Vanillin

*Contact information: a: Guangdong Engineering Research Centre for Marine Algal Biotechnology, Shenzhen Key Laboratory of Marine Bioresource and Eco-environmental Science, College of Life Sciences and Oceanography, Shenzhen University, Shenzhen, 518060 China; b: Department of Biological and Agricultural Engineering, North Carolina State University, Raleigh, NC, 27695 USA; *Corresponding author: wyuan2@ncsu.edu*

INTRODUCTION

With increasing concerns over the depletion of fossil fuels and their adverse environmental impacts, the development of bioenergy from alternative biomass feedstocks has attracted extensive research interest (Agbor *et al.* 2011). Specifically, as the most abundant and renewable organic source in the biosphere, lignocellulosic biomass has been widely investigated in the production of biofuels and other value-added products, such as ethanol, butanol, and butyric acid (Öhgren *et al.* 2006; Ezeji *et al.* 2007; Huang *et al.* 2011). Various monosaccharides could be released by the hydrolysis of lignocellulosic biomass and then be assimilated directly by microorganisms (Hendriks and Zeeman 2009; Agbor *et al.* 2011). Glucose and xylose represented a significant percentage of the fermentable sugars in lignocellulosic hydrolysate. Previous studies have shown that glucose is the preferred substrate in the metabolism of many microorganisms (Ezeji *et al.* 2007), whereas the bioconversion of xylose to chemicals has been limited by the preference of microorganisms.

In addition to sugars, some other compounds, *i.e.*, byproducts that may have potential inhibitory effects on the fermentation process, can also be released into the lignocellulosic hydrolysate. For instance, aliphatic acids and furaldehydes can be produced through sugar decomposition. Various phenolic compounds, such as syringaldehyde and vanillin, are generated from the partial breakdown of lignin (Palmqvist and Hahn-Hägerdal 2000). It has been reported that phenolic compounds could damage the integrity of biological membranes, consequently

impacting their abilities to serve as selective barriers and enzyme matrices and leading to significant reduction in sugar uptake and microbial cell growth (Palmqvist and Hahn-Hägerdal 2000; Mills *et al.* 2009). Numerous studies have investigated the inhibitory effects of phenolic compounds on alcohol (ethanol and butanol) fermentation regarding microbial cell growth, metabolism, and product titer (Ezeji *et al.* 2007; Li *et al.* 2017). However, little information is available about their impacts on butyric acid fermentation.

Butyric acid is a valuable substrate in the chemical, pharmaceutical, food, and biofuel industries (Larios *et al.* 2004; Jiang *et al.* 2010b; Li *et al.* 2013). It is well documented that butyric acid has promising therapeutic effects on cancer, hemoglobinopathies, and intestinal diseases (Canani *et al.* 2011; Lazarova *et al.* 2014). In addition, butyric acid can be used as a precursor to biofuels (Li *et al.* 2013). Compared to current petrochemical synthesis, the production of butyric acid from renewable bioresources by fermentation can fulfill the growing demands of using bio-based substances in food, cosmetics, and pharmaceuticals. In addition, environmental concerns raised by petroleum processing can also be eliminated in the case that chemical synthesis of butyric acid is replaced by microbial fermentation.

Among all the butyric acid-producing bacteria, *C. tyrobutyricum*, a gram-positive, rod-shaped obligate anaerobic bacteria, was shown to be a preferred species due to its superior ability of utilizing various carbohydrates and its excellent capacity to produce high yield and high productivity of butyric acid (Jiang *et al.* 2010a,b; Dwidar *et al.* 2013). It has been reported that *C. tyrobutyricum* can efficiently convert glucose, xylose and fructose, the main sugars found in lignocellulosic hydrolysates, into butyric acid (Zhu and Yang 2003; Huang *et al.* 2011; Luo *et al.* 2017; Chen *et al*. 2017). Besides, butyric acid production by *C. tyrobutyricum* is mostly at a higher level (two-fold or more) as compared to other butyric acid producing strains (Ma *et al.* 2015). Several researchers have demonstrated that phenolic compounds inhibited the cell growth and product formation of solventogenic clostridial strains, even at low concentrations (Ezeji *et al.* 2007; Ezeji and Blaschek 2008). Still, sufficient investigation on their effects on *C. tyrobutyricum* for butyric acid production is lacking. The objective of this study was to assess the effects of two phenolic aldehydes (syringaldehyde and vanillin) on *C. tyrobutyricum* by employing xylose as the carbon source in the production of butyric acid by fermentation.

EXPERIMENTAL

Strain and Cultivation

C. tyrobutyricum ATCC 25755 obtained from American Type Culture Collection (ATCC, Manassas, Virginia, USA) was used in the present study. The strain was maintained on a modified reinforced clostridial agar plate (ATCC medium 2107) at 4 °C anaerobically. The pre-culture medium contained the following compounds per liter of distilled water: 30 g glucose, 5 g yeast extract, 5 g peptone, 3 g $(NH_4)_2SO_4$, 1.5 g K_2HPO_4, 0.6 g $MgSO_4 \cdot 7H_2O$, and 0.03 g $FeSO_4 \cdot 7H_2O$ (Jiang *et al.* 2010b). The pH of the medium was adjusted to 6.4 by adding NH_4OH before sterilization. The *C. tyrobutyricum* cells that were grown in the pre-culture medium at 37°C for two days were used as the inoculum. In the fermentation medium, xylose was used as the carbon source, and the other components were the same as described above in the pre-culture medium. To initiate the fermentation, 20 mL of the inoculum was transferred into 180 mL of the fermentation medium.

Various amounts (0.12, 0.24, 0.36, and 0.48 g) of syringaldehyde and vanillin were added into the fermentation medium separately before sterilization, with final concentrations of 0.6, 1.2, 1.8, and 2.4 g L^{-1}. These concentrations were chosen based on findings from previous studies that

syringaldehyde and vanillin were generally at no more than 2 g/L in lignocellulosic hydrolysates (Cortez and Roberto 2010). Thus the concentration range of 0.5 to around 2 g/L has been frequently studied to evaluate their influence on xylose fermentation. Fermentation without syringaldehyde or vanillin addition served as the control (0 g L^{-1}). The batch fermentation was performed with a stable temperature of 37 °C under anaerobic conditions in a Forma Anaerobic System 1025 (Thermo Scientific, Grand Island, NY, USA). The fermentation process was monitored by measuring cell growth, xylose, butyric acid, syringaldehyde, and vanillin concentrations every 12 h. The pH was adjusted to 6.4 by NH_4OH or HCl every 12 h.

Analytical Methods

Cell growth of *C. tyrobutyricum* was analyzed by measuring the optical density (OD) of the fermentation broth with a microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) at a wavelength of 620 nm (OD_{620}). The concentrations of xylose, syringaldehyde, vanillin, and butyric acid were determined using high performance liquid chromatography (HPLC, Shimadzu Corporation, Kyoto, Japan). An Aminex HPX-87H column (300 × 7.8 mm, Bio-Rad, CA, USA) was used in the HPLC. For the quantification of xylose and butyric acid, 0.005 N sulfuric acid at a flow rate of 0.6 mL min^{-1} was used as the mobile phase, and the column temperature was set at 35°C. The detection of xylose and butyric acid was accomplished by a refractive index detector. While, to measure the concentrations of syringaldehyde and vanillin, a mixture of 84% of 0.01 N sulfuric acid and 16% of acetonitrile was used as the mobile phase. The flow rate and column temperature were set at 0.35 mL min^{-1} and 55°C, respectively. Syringaldehyde and vanillin were then detected by a UV detector at a wavelength of 254 nm. The pH of the fermentation broth was measured by an Accumet AP85 portable waterproof pH/conductivity meter (Fisher Scientific, Pittsburgh, PA, USA). All chemicals were purchased from Fisher Scientific unless specifically designated.

Butyric acid yield (Y) and productivity (P) were calculated using Eqs. 1 and 2, respectively,

$$Y = \frac{BA_f - BA_0}{XY_0 - XY_f} \quad (1)$$

$$P = \frac{BA_{max} - BA_0}{t} \quad (2)$$

where BA_f, BA_0, and BA_{max} are the final, initial, and maximum concentrations of butyric acid, respectively; XY_0 and XY_f are the initial and final concentrations of xylose, respectively, and t represents the fermentation in hours when the maximum butyric acid concentration was obtained.

Statistical Analysis

All treatments were carried out in triplicate, and the results were expressed as a mean ± standard deviation. Multiple one-way analysis of variance (ANOVA) was conducted using the SAS software (SAS Institute Inc., Cary, NC, USA) to evaluate the effects of syringaldehyde and vanillin on the cell-growth and metabolism of *C. tyrobutyricum*. Tukey's method was also used to calculate pairwise differences at a significance level of 0.05 for each treatment.

RESULTS AND DISCUSSION

Effects of Syringaldehyde and Vanillin on Cell Growth and Xylose Consumption

Syringaldehyde and vanillin exhibited similar effects on the growth and xylose utilization of *C. tyrobutyricum* (Fig. 1). Compared to the control, neither syringaldehyde nor vanillin caused

any notable effects on the growth of *C. tyrobutyricum* or xylose consumption at their lowest tested concentration (0.6 g L^{-1}). With an increase in the syringaldehyde or vanillin concentrations, a longer time was needed for *C. tyrobutyricum* to deplete the xylose and achieve the maximum cell concentration (Fig. 1). Moreover, the maximum cell concentration decreased by approximately 7% compared to the control when 1.2 or 1.8 g L^{-1} of syringaldehyde was supplemented to the fermentation. At the addition of 2.4 g L^{-1} syringaldehyde, a greater reduction (approximately 25%) in the highest cell density was observed (Fig. 1A). Regarding the fermentations supplemented with higher concentrations of vanillin (1.2, 1.8, and 2.4 g L^{-1}), the cell growth was also moderately inhibited and the maximum cell densities were 83.3%, 83.8%, and 77.9% of the value obtained in the control group, respectively (Fig. 1B).

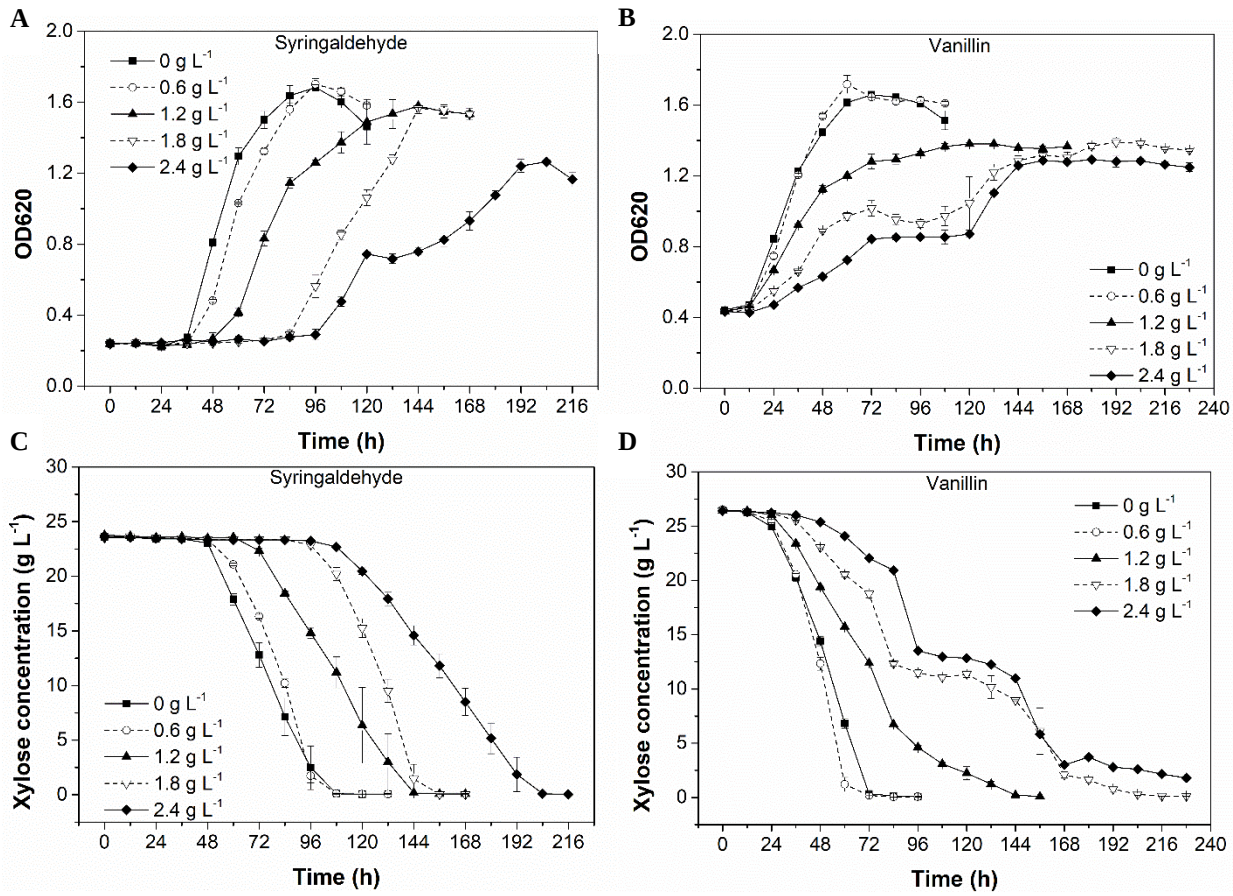

Fig. 1. The effect of syringaldehyde (**A**) and vanillin (**B**) on cell growth of *C. tyrobutyricum* represented by OD620 and the effect of syringaldehyde (**C**) and vanillin (**D**) on xylose consumption by *C. tyrobutyricum*

Within the tested concentrations from 0 to 2.4 g L^{-1} of syringaldehyde or vanillin, *C. tyrobutyricum* was able to recover from the inhibition stress and accumulated fairly large amounts of biomass, suggesting that *C. tyrobutyricum* had a broad tolerance range to these two phenolic aldehyde compounds, which are commonly found in lignocellulosic hydrolysates (Cortez and Roberto 2010). As reported previously, the inhibition extent of syringaldehyde or vanillin on microorganism growth was species-specific (Ezeji and Blaschek 2008). The cell growth of *C. beijerinckii* decreased by more than 76% with a concentration of 0.5 g L^{-1} vanillin in the medium (Guo *et al.* 2012). At a concentration of 2 g L^{-1} of vanillin, it was observed to be lethal to *Candida guilliermondii* (Kelly *et al.* 2008). On the other hand, syringaldehyde (1 g L^{-1}) showed slight toxic

effects on *C. beijerinckii*, inhibiting cell growth by only 3% (Cho *et al.* 2009). Meanwhile, the growth of *Thermoanaerobacterium thermosaccharolyticum* W16 was completely inhibited by adding 1.5 g L^{-1} of syringaldehyde (Cao *et al.* 2010).

In addition to the impact on the maximum cell concentration, inhibitor compounds can also affect cell fermentation by prolonging the lag phase. In the present study, different patterns of the lag phase were observed between the syringaldehyde and vanillin treatments. Syringaldehyde addition resulted in a concentration-dependent lag time in that the cell replication and xylose assimilation in 1.2, 1.8, and 2.4 g L^{-1} of syringaldehyde treatment groups started around 12 h, 48 h, and 60 h later compared to the control (Fig. 1A and Fig. 1C). No difference in the length of lag phase among the control and different concentrations of vanillin treatment groups was observed (Fig. 1B and Fig. 1D), suggesting that *C. tyrobutyricum* could rapidly adapt to the presence of vanillin.

Effects of Syringaldehyde and Vanillin on Butyric Acid Production

The dose of syringaldehyde and vanillin dependently affected butyric acid synthesis. In the fermentation treated with 0.6 g L^{-1} of syringaldehyde, the time profile of butyric acid synthesis was like that of the control (Fig. 2A), and the butyric acid yield and productivity were at the same level as compared to the control (Table 1). With an increase in syringaldehyde concentration, the butyric acid production was delayed. With 1.2, 1.8, and 2.4 g L^{-1} syringaldehyde in the medium, the highest butyric acid concentration decreased to 86.2%, 58.1%, and 23.4% of that in the control group, respectively (Fig. 2A). Particularly, the inhibitory effect of 2.4 g L^{-1} syringaldehyde on butyric acid production was so pronounced that no butyric acid accumulation was observed until 156 h of fermentation. Overall, due to the prolonged lag phase and reduced maximum butyric acid concentration, both the yield and productivity of butyric acid decreased significantly with increasing syringaldehyde concentration from 0.6 to 2.4 g L^{-1} (Table 1).

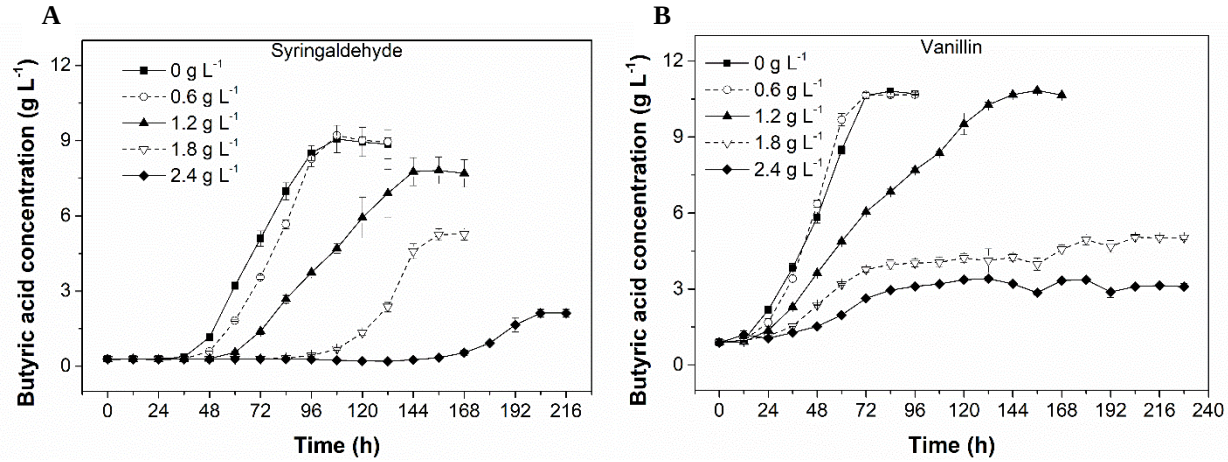

Fig. 2. The effect of syringaldehyde (**A**) and vanillin (**B**) on butyric acid production of *C. tyrobutyricum*

Regarding the vanillin treatments, low concentrations (0.6 and 1.2 g L^{-1}) of vanillin did not show significant effects on the highest butyric acid production of *C. tyrobutyricum* (Fig. 2B). At 0.6 g L^{-1} vanillin, the butyric acid yield and productivity were comparable to those in the control group (Table 1). On the other hand, with 1.2 g L^{-1} vanillin in the fermentation, the butyric acid concentration increased at a lower rate (Fig. 2B), resulting in a significantly reduced butyric acid productivity as compared to the control (Table 1). When high concentrations (1.8 and 2.4 g L^{-1}) of vanillin were added into the medium, the butyric acid production was severely inhibited. After the

lag phase, butyric acid concentration increased gradually from 36 h to 84 h, and then fluctuated in a narrow range (3.2 to 5.0 g L^{-1} and 3.0 to 3.4 g L^{-1}, respectively) for the rest of fermentation. The maximum butyric acid concentrations achieved with 1.8 and 2.4 g L^{-1} vanillin were only 46.5% and 31.5%, respectively, of that in control group (Fig. 2B).

Table 1. Butyric Acid Yield and Productivity at Varying Concentrations of Syringaldehyde or Vanillin

Inhibitor (g L^{-1})		Butyric acid yield (g g^{-1})	Butyric acid productivity (g L^{-1} h^{-1})
Syringaldehyde	0	0.374 ± 0.024 a	0.086 ± 0.003 a
	0.6	0.371 ± 0.006 a	0.083 ± 0.001 a
	1.2	0.319 ± 0.024 b	0.052 ± 0.004 b
	1.8	0.213 ± 0.010 c	0.032 ± 0.002 c
	2.4	0.078 ± 0.007 d	0.009 ± 0.001 d
Vanillin	0	0.371 ± 0.001 a	0.135 ± 0.001 a
	0.6	0.369 ± 0.003 a	0.136 ± 0.001 a
	1.2	0.371 ± 0.002 a	0.071 ± 0.001 b
	1.8	0.157 ± 0.003 b	0.040 ± 0.001 c
	2.4	0.090 ± 0.005 c	0.024 ± 0.000 d

Different letters following the numbers indicate significant differences (Tukey test, $p = 0.05$).

The comparison between syringaldehyde and vanillin treatments in this study revealed that these two compounds caused similar inhibitory effects on *C. tyrobutyricum* fermentation. The inhibition mechanisms of phenolic compounds on microorganisms have not been elucidated comprehensively in the literature (Ravindran and Jaiswal 2016). Nevertheless, it is known that they may alter the permeability of biological membranes and increase the cell fluidity, resulting in leakage of intracellular contents (such as proteins and RNAs) out of the cytoplasm, and thus the protein function and nutrient transport of microbial cells are impaired (Campos *et al.* 2009; Monlau *et al.* 2014). Generally, for compounds with the same functional group, their toxicity is mainly correlated to the hydrophobicity potentials, which are measured by log *P*, the partition coefficients in octanol-water (Klinke *et al.* 2004; Ibraheem and Ndimba 2013). With high hydrophobicity, inhibitors can readily penetrate cell membranes, thus causing a high level of toxicity. For example, butanol production by *C. beijerinckii* was completely inhibited when 1.0 g L^{-1} of vanillin (log P = 1.188) was added during fermentation, while syringaldehyde (log P = 0.863) at the same concentration only inhibited butanol production by 26% (Cho *et al.* 2009). Likewise, higher toxicity on cell growth and butanol production was also induced by vanillin than by syringaldehyde (Guo *et al.* 2012). However, the difference in inhibitory severity towards cell growth and butyric acid production between syringaldehyde and vanillin treatments was not apparent in the present study (Fig. 1, Table 1), suggesting that microorganisms may differ in their sensitivities to the presence of different inhibitors. Also, other than causing damage to the hydrophobic sites on the cell membrane, other detrimental actions of these phenolic aldehydes might also contribute to their inhibitory effects. Thus, the diffusion of syringaldehyde and vanillin through the *C. tyrobutyricum* cell membrane might not be the determining step of their toxic mechanism (Li *et al.* 2017).

In addition, with high concentrations of syringaldehyde or vanillin in the media, both the cell growth (Fig. 1A and 1B) and butyric acid yield (Fig. 2 and Table 1) of *C. tyrobutyricum* decreased with xylose eventually depleted in the medium (Fig. 1C and D). These results suggested

that besides butyric acid some other end products, such as acetic acid, lactic acid, and carbon dioxide, could have be generated from xylose fermentation, and the selectivity of butyric acid production by *C. tyrobutyricum* could have decreased due to inhibitory effects of syringaldehyde and vanillin.

Relationship Between Cell Growth and Butyric Acid Production

During the entire fermentation process treated with syringaldehyde, there was an exponential relationship between the cell density and the corresponding butyric acid concentration (Fig. 3A). That is, with an increment in cell density, more butyric acid was produced per OD unit. After the natural-log transformation of butyric acid concentration, a linear regression resulted in an R^2 = 0.915, with only a few data points outside of the 95% prediction limits (Fig. 3A inset). These outlying points were gathered from the 2.4 g L^{-1} syringaldehyde treatment group. As shown in Fig. 1A and Fig. 2A, the 2.4 g L^{-1} concentration of syringaldehyde in the fermentation, the cell replication started after 96 h of fermentation, whereas barely any butyric acid accumulation was observed until 144 h. Thus, some outlying points were caused by this asynchronous increment of cell growth and butyric acid production.

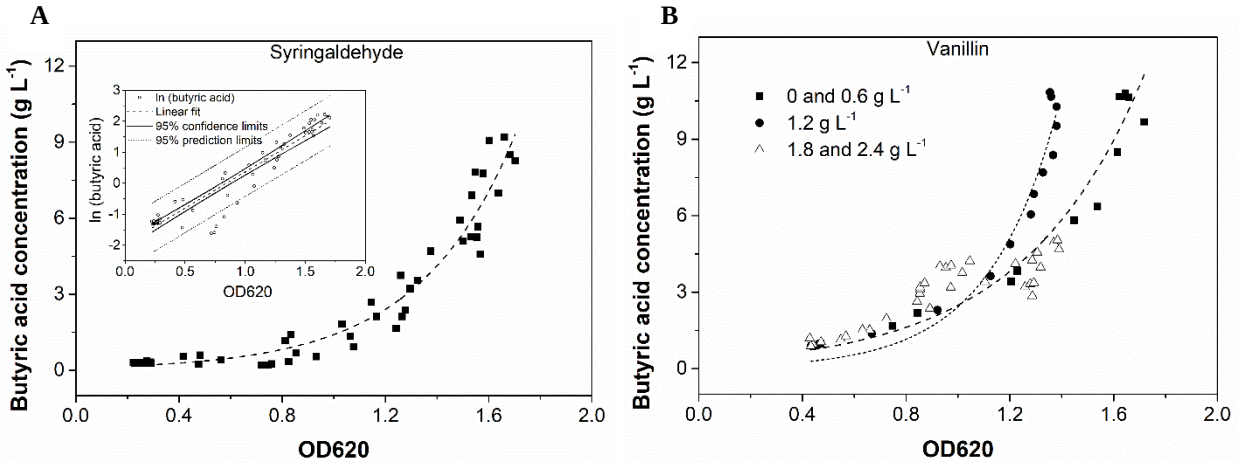

Fig. 3. The relationship between butyric acid accumulation and cell growth of *C. tyrobutyricum* with syringaldehyde (**A**) or vanillin (**B**) treatment. Inset in (**A**) shows that after natural-log transformation of the butyric acid concentrations, a linear regression was obtained.

Regarding the vanillin treatment, the relationship between the cell growth and butyric acid concentration was variable depending on the initial vanillin concentrations (Fig. 3B). An exponential relationship (R^2=0.988) was observed for groups with 0 and 0.6 g L^{-1} of vanillin. The data collected from the treatment group with 1.2 g L^{-1} of vanillin fit another exponential regression model (R^2=0.972), illustrating that 1.2 g L^{-1} of vanillin had more severe inhibitory effects on cell growth compared to that of the butyric acid production. Furthermore, when the vanillin concentration increased to 1.8 and 2.4 g L^{-1}, cell growth and corresponding butyric acid concentrations did not fit an exponential or linear model, showing that less butyric acid was produced when the same amount of biomass was accumulated.

The exponential relationships between cell growth and butyric acid concentration with syringaldehyde treatment or low concentrations (0 to 1.2 g L^{-1}) of vanillin treatment suggested that the cell growth rate increased at a slower pace than the increase in butyric acid production rate. This can be ascribed to end-product inhibition. As demonstrated in previous studies, butyric acid is known as an inhibitory product that can interfere with the maintenance of the functional pH gradient across the cell membrane (Zhu and Yang 2003). Thus, with the accumulation of butyric

acid, additional ATP must be consumed to pump out the extra protons to maintain a proper pH gradient. Similar results were reported that *C. tyrobutyricum* was sensitive to the fermentation product (butyric acid) inhibition and the specific growth rate of *C. tyrobutyricum* was dramatically reduced with increasing butyric acid concentration (Zhu and Yang 2003; Liu *et al.* 2006). On the other hand, the presence of outlying points from the 2.4 g L^{-1} syringaldehyde treatment group (Fig. 3A inset) and the low yield of butyric acid production in 1.8 and 2.4 g L^{-1} vanillin treatment groups indicated that, compared to the toxic effects on cell growth, high concentrations of syringaldehyde and vanillin inhibited butyric acid production to a larger extent. This result agreed with previous studies that syringaldehyde and vanillin were less toxic to cell growth compared to their effects on the end-products production (Ezeji *et al.* 2007; Cho *et al.* 2009). The interference of syringaldehyde and vanillin with dehydrogenase enzymes and the reactions involved in the glycolytic pathway, which are responsible for the production of a variety of products, could be the probable reason for the low yield of butyric acid production (Ezeji *et al.* 2007). Thus, further studies on the expression of corresponding enzymes in the presence of syringaldehyde or vanillin, would be of vital importance for the understanding of their inhibitory effects on end products production.

Assimilation of Syringaldehyde and Vanillin by *C. tyrobutyricum*

Within the tested concentration ranges, both syringaldehyde and vanillin were consumed completely by *C. tyrobutyricum*. Also, a longer time was required for the depletion of syringaldehyde or vanillin with a higher initial concentration (Fig. 4).

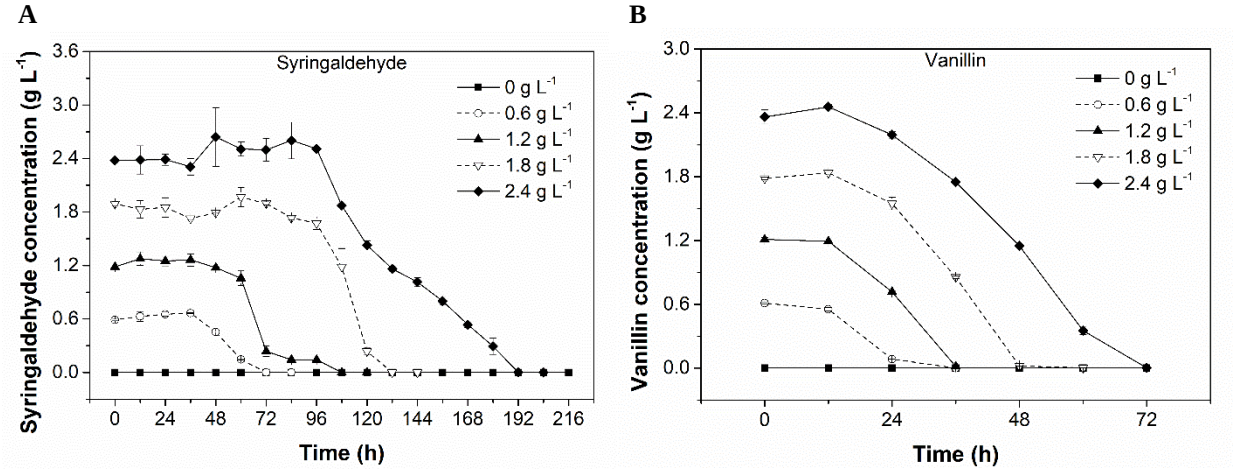

Fig. 4. The assimilation of syringaldehyde (A) and vanillin (B) by *C. tyrobutyricum* during the fermentation of xylose to butyric acid

Compared to vanillin, a longer lag phase for syringaldehyde assimilation was observed, and the lag phase was prolonged with the increase in syringaldehyde concentrations. These were probably caused by the lower hydrophobicity and larger molecular weight of syringaldehyde in comparison to vanillin. Hence, syringaldehyde penetrated into cells at a lower rate and consequently was metabolized over a longer period. Various microorganisms were able to metabolize syringaldehyde or vanillin, and convert them into the corresponding alcohols (syringic alcohol and vanillyl alcohol, respectively) or acids (syringic acid and vanillic acid, respectively) (Oliva *et al.* 2003; Cortez and Roberto 2010). These metabolites also belong to the group of phenolic compounds but are generally less toxic to yeast and bacteria strains than their phenolic aldehyde forms (Almeida *et al.* 2007; Zhang *et al.* 2016). As for syringaldehyde treatment in the present study, the toxicity of its metabolite products was not apparent. The *C. tyrobutyricum* cell

growth, xylose uptake, and butyric acid production were normal after the depletion of syringaldehyde (Fig. 1A, 1C, and 2A). However, with high initial vanillin concentrations (1.8 or 2.4 g L^{-1}), diauxic growth of *C. tyrobutyricum* was observed, showing that after the depletion of vanillin (at 48 or 72 h of fermentation, respectively. Fig. 4), a second lag phase was present (Fig. 1B). The xylose consumption also went through another lag phase (Fig. 1D). Moreover, the butyric acid production did not recover even after the depletion of vanillin (Fig. 2B). Thus, it can be speculated that high concentrations of metabolite products might be formed from the high initial concentrations of vanillin, and presented considerable inhibition on *C. tyrobutyricum* fermentation.

CONCLUSIONS

1. Syringaldehyde and vanillin showed similar inhibition effects on *C. tyrobutyricum* growth in xylose media.
2. At their lowest tested concentration (0.6 g L^{-1}) there were no significant effects on fermentation. At higher concentrations, moderate inhibition on cell growth was observed. Butyric acid yield and productivity were also reduced to a similar extent when the same concentration of syringaldehyde or vanillin was supplemented in the medium.
3. With 1.8 or 2.4 g L^{-1} of syringaldehyde/vanillin addition, a higher degree of inhibition was observed on butyric acid synthesis than on cell growth of *C. tyrobutyricum*.
4. Both syringaldehyde and vanillin could be assimilated by *C. tyrobutyricum* and the metabolite products from vanillin caused a considerable inhibition on butyric acid fermentation.

ACKNOWLEDGMENTS

This work was supported by USDA National Institute of Food and Agriculture, Hatch Project NC02613.

REFERENCES CITED

Agbor, V. B., Cicek, N., Sparling, R., Berlin, A., and Levin, D. B. (2011). "Biomass pretreatment: Fundamentals toward application," *Biotechnol. Adv.* 29(6), 675-685. DOI: 10.1016/j.biotechadv.2011.05.005

Almeida, J. R., Modig, T., Petersson, A., Hähn-Hägerdal, B., Lidén, G., and Gorwa-Grauslund, M. F. (2007). "Increased tolerance and conversion of inhibitors in lignocellulosic hydrolysates by *Saccharomyces cerevisiae*," *J. Chem. Technol. Biotechnol.* 82(4), 340-349. DOI: 10.1002/jctb.1676

Campos, F. M., Couto, J. A., Figueiredo, A. R., Tóth, I. V., Rangel, A. O. S. S., and Hogg, T. A. (2009). "Cell membrane damage induced by phenolic acids on wine lactic acid bacteria," *Int. J. Food. Microbiol.* 135(2), 144-151. DOI: 10.1016/j.ijfoodmicro.2009.07.031

Canani, R. B., Di Costanzo, M., Leone, L., Pedata, M., Meli, R., and Calignano, A. (2011). "Potential beneficial effects of butyrate in intestinal and extraintestinal diseases," *World J. Gastroenterol.* 17(12), 1519-1528. DOI: 10.3748/wjg.v17.i12.1519

Cao, G. -L., Ren, N. -Q., Wang, A. -J., Guo, W. -Q., Xu, J. -F., and Liu, B. -F. (2010). "Effect of lignocellulose-derived inhibitors on growth and hydrogen production by *Thermoanaerobacterium thermosaccharolyticum* W16," *Int. J. Hydrogen Energy* 35(24), 13475-13480. DOI: 10.1016/j.ijhydene.2009.11.127

Chen, T., Zhang, L., Luo, G., Geng, Y., and Yuan, W. (2017). "Butyric acid production by *Clostridium tyrobutyricum* in sugar mixtures and corncob hydrolysate containing arabinose," *BioResources* 12(4), 7931-7942. DOI: 10.15376/biores.12.4.7931-7942

Cho, D. H., Lee, Y. J., Um, Y., Sang, B. -I., and Kim, Y. H. (2009). "Detoxification of model phenolic compounds in lignocellulosic hydrolysates with peroxidase for butanol production from *Clostridium beijerinckii*," *Appl. Microbiol. Biotechnol.* 83(6), 1035-1043. DOI: 10.1007/s00253-009-1925-8

Cortez, D. V., and Roberto, I. C. (2010). "Individual and interaction effects of vanillin and syringaldehyde on the xylitol formation by *Candida guilliermondii*," *Bioresour. Technol.* 101(6), 1858-1865. DOI: 10.1016/j.biortech.2009.09.072

Dwidar, M., Kim, S., Jeon, B. S., Um, Y., Mitchell, R. J., and Sang, B. -I. (2013). "Co-culturing a novel *Bacillus* strain with *Clostridium tyrobutyricum* ATCC 25755 to produce butyric acid from sucrose," *Biotechnol. Biofuels* 6(1), 35. DOI: 10.1186/1754-6834-6-35

Ezeji, T., and Blaschek, H. P. (2008). "Fermentation of dried distillers' grains and solubles (DDGS) hydrolysates to solvents and value-added products by solventogenic clostridia," *Bioresour. Technol.* 99(12), 5232-5242. DOI: 10.1016/j.biortech.2007.09.032

Ezeji, T., Qureshi, N., and Blaschek, H. P. (2007). "Butanol production from agricultural residues: impact of degradation products on *Clostridium beijerinckii* growth and butanol fermentation," *Biotechnol. Bioeng.* 97(6), 1460-1469. DOI: 10.1002/bit.21373

Guo, T., Tang, Y., Zhang, Q. -Y., Du, T. -F., Liang, D. -F., Jiang, M., and Ouyang, P. -K. (2012). "*Clostridium beijerinckii* mutant with high inhibitor tolerance obtained by low-energy ion implantation," *J. Ind. Microbiol. Biotechnol.* 39(3), 401-407. DOI: 10.1007/s10295-011-1017-5

Hendriks, A., and Zeeman, G. (2009). "Pretreatments to enhance the digestibility of lignocellulosic biomass," *Bioresour. Technol.* 100(1), 10-18. DOI: 10.1016/j.biortech.2008.05.027

Huang, J., Cai, J., Wang, J., Zhu, X., Huang, L., Yang, S.-T., and Xu, Z. (2011). "Efficient production of butyric acid from Jerusalem artichoke by immobilized *Clostridium tyrobutyricum* in a fibrous-bed bioreactor," *Bioresour. Technol.* 102(4), 3923-3926. DOI: 10.1016/j.biortech.2010.11.112

Ibraheem, O., and Ndimba, B. K. (2013). "Molecular adaptation mechanisms employed by ethanologenic bacteria in response to lignocellulose-derived inhibitory compounds," *Int. J. Biol. Sci.* 9(6), 598-612. DOI: 10.7150/ijbs.6091

Jiang, L., Cai, J., Wang, J., Liang, S., Xu, Z., and Yang, S. -T. (2010a). "Phosphoenolpyruvate-dependent phosphorylation of sucrose by *Clostridium tyrobutyricum* ZJU 8235: Evidence for the phosphotransferase transport system," *Bioresour. Technol.* 101(1), 304-309. DOI: 10.1016/j.biortech.2009.08.024

Jiang, L., Wang, J., Liang, S., Wang, X., Cen, P., and Xu, Z. (2010b). "Production of butyric acid from glucose and xylose with immobilized cells of *Clostridium tyrobutyricum* in a fibrous-bed bioreactor," *Appl. Biochem. Biotechnol.* 160(2), 350-359. DOI: 10.1007/s12010-008-8305-1

Kelly, C., Jones, O., Barnhart, C., and Lajoie, C. (2008). "Effect of furfural, vanillin and syringaldehyde on *Candida guilliermondii* growth and xylitol biosynthesis," *Appl. Biochem. Biotechnol.* 148(1-3), 97-108. DOI: 10.1007/s12010-007-8103-1

Klinke, H. B., Thomsen, A., and Ahring, B. K. (2004). "Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass," *Appl. Microbiol. Biotechnol.* 66(1), 10-26. DOI: 10.1007/s00253-004-1642-2

Larios, A., García, H. S., Oliart, R. M., and Valerio-Alfaro, G. (2004). "Synthesis of flavor and fragrance esters using *Candida antarctica* lipase," *Appl. Microbiol. Biotechnol.* 65(4), 373-376. DOI: 10.1007/s00253-004-1602-x

Lazarova, D. L., Chiaro, C., and Bordonaro, M. (2014). "Butyrate induced changes in Wnt-signaling specific gene expression in colorectal cancer cells," *BMC Res. Notes* 7(1), 226. DOI: 10.1186/1756-0500-7-226

Li, J., Shi, S., Adhikari, S., and Tu, M. (2017). "Inhibition effect of aromatic aldehydes on butanol fermentation by *Clostridium acetobutylicum*," *RSC Adv.* 7(3), 1241-1250. DOI: 10.1039/C6RA25706B

Li, L., Ai, H. X., Zhang, S. X., Li, S., Liang, Z. X., Wu, Z. Q., Yang, S. T., and Wang, J. F. (2013). "Enhanced butanol production by coculture of *Clostridium beijerinckii* and *Clostridium tyrobutyricum*," *Bioresour. Technol.* 143, 397-404. DOI: 10.1016/j.biortech.2013.06.023

Liu, X., Zhu, Y., and Yang, S.-T. (2006). "Butyric acid and hydrogen production by *Clostridium tyrobutyricum* ATCC 25755 and mutants," *Enzyme Microb. Technol.* 38(3–4), 521-528. DOI: 10.1016/j.enzmictec.2005.07.008

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

Ma, C., Ou, J., Miller, M., McFann, S., and Liu, X. (2015). "High production of butyric acid by *Clostridium tyrobutyricum* mutant," *Front. Chem. Sci. Eng.* 9(3), 369-375. DOI: 10.1007/s11705-015-1525-3

Mills, T. Y., Sandoval, N. R., and Gill, R. T. (2009). "Cellulosic hydrolysate toxicity and tolerance mechanisms in *Escherichia coli*," *Biotechnol. Biofuels* 2(1), 26. DOI: 10.1186/1754-6834-2-26

Monlau, F., Sambusiti, C., Barakat, A., Quéméneur, M., Trably, E., Steyer, J.-P., and Carrère, H. (2014). "Do furanic and phenolic compounds of lignocellulosic and algae biomass hydrolyzate inhibit anaerobic mixed cultures? A comprehensive review," *Biotechnol. Adv.* 32(5), 934-951. DOI: 10.1016/j.biotechadv.2014.04.007

Öhgren, K., Rudolf, A., Galbe, M., and Zacchi, G. (2006). "Fuel ethanol production from steam-pretreated corn stover using SSF at higher dry matter content," *Biomass Bioenerg.* 30(10), 863-869. DOI: 10.1016/j.biombioe.2006.02.002

Oliva, J. M., Sáez, F., Ballesteros, I., González, A., Negro, M. J., Manzanares, P., and Ballesteros, M. (2003). "Effect of lignocellulosic degradation compounds from steam explosion pretreatment on ethanol fermentation by thermotolerant yeast *Kluyveromyces marxianus*," *Appl. Biochem. Biotechnol.* 105(1-3), 141-153. DOI: 10.1385/ABAB:105:1-3:141

Palmqvist, E., and Hahn-Hägerdal, B. (2000). "Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition," *Bioresour. Technol.* 74(1), 25-33. DOI: 10.1016/S0960-8524(99)00161-3

Ravindran, R., and Jaiswal, A. K. (2016). "A comprehensive review on pre-treatment strategy for lignocellulosic food industry waste: Challenges and opportunities," *Bioresour. Technol.* 199, 92-102. DOI: 10.1016/j.biortech.2015.07.106

Zhang, L., Li, X., Yong, Q., Yang, S. -T., Ouyang, J., and Yu, S. (2016). "Impacts of lignocellulose-derived inhibitors on l-lactic acid fermentation by *Rhizopus oryzae*," *Bioresour. Technol.* 203, 173-180. DOI: 10.1016/j.biortech.2015.12.014

Zhu, Y., and Yang, S. T. (2003). "Adaptation of *Clostridium tyrobutyricum* for enhanced tolerance to butyric acid in a fibrous-bed bioreactor," *Biotechnol. Prog.* 19(2), 365-372. DOI: 10.1021/bp025647x

Article submitted: March 8, 2018; Peer review completed: May 31, 2018; Revised version received and accepted: June 3, 2018; Published: June 12, 2018.
DOI: 10.15376/biores.13.3.5850-5861