Biocatalysis and Agricultural Biotechnology 17 (2019) 613–619

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

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

Kinetic models for production of propionic acid by *Propionibacter freudenrechii* subsp. *shermanii* and *Propionibacterium freudenreichii* subsp. *freudenreichii* in date syrup during sonication treatments

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ARTICLE INFO

Keywords:
Baranyi model
Gaussian curve fitting
Propionic acid
P. freudenrechii subsp. *shermanii*
P. freudenreichii subsp. *freudenreichii*

ABSTRACT

The effect of different sonication amplitudes (30 kHz, 100 W; 25%, 50% and 75%) as well as the exposure time (5, 10 and 15 min) on the microbial growth and propionic acid production by *Propionibacter freudenrechii* subsp. *shermanii* PTCC 1661 and *Propionibacterium freudenreichii* subsp. *freudenreichii* PTCC 1674 in the matrix of date syrup was investigated. Results showed the ultrasonication significantly caused reductions around 37% and 47% of lag time (λ) for *P. freudenrechii* subsp. *shermanii* and 47% and 52% for *P. freudenreichii* subsp. *freudenreichii* at 25% and 50% amplitudes, respectively. However, an increase around 120% in λ was observed at 75% amplitude for 15 min. The growth rate (μ_{max}) had an increase rate with respect to the increase in sonication strength and exposure time except of 75% amplitude for 15 min. For 25% and 50% amplitudes, increment in the exposure time to 15 min resulted in increasing in propionic acid about 25.8% and 41.7% by *P. freudenrechii* subsp. *shermanii* and about 22.3–38.7% by *P. freudenreichii* subsp. *freudenreichii* while compared with the control, respectively. However, increasing in the exposure time to 15 min at 75% amplitude significantly decreased propionic acid production about 55.5% and 39.1% by *P. freudenrechii* subsp. *shermanii* and *P. freudenreichii* subsp. *freudenreichii*, respectively. The maximum propionic acid was produced using *P. freudenrechii* subsp. *shermanii* (6.43 g/L) at 75% amplitude for 10 min. However, there is no significant different ($p < 0.05$) in propionic acid production by both microorganisms at 75% amplitude for 10 min while compared with 50% amplitude for 15 min. The findings of this study indicated that proper sonication treatments could be used as an important factor in improving production of propionic acid from date syrup during fermentation.

1. Introduction

Propionic acid is the main product from the succinate pathway and is able to inhibit fungi in foods and feeds (Eş et al., 2017). Moreover, this organic acid is used for the production of thermoplastics, flavors, solvents, perfumes and herbicides (Coral et al., 2008; Zhang and Yang, 2009; Eş et al., 2017). Commercial manufacturing of propionic acid is mainly performed by chemical synthesis from petroleum resources (Eş et al., 2017). However, concerns regarding the unsure supply sources and the final reduction of world petroleum reserves have promoted investigations into substitute ways to manufacture petrochemical derivatives such as propionic acid (Ozadali et al., 1996). In this regard, the further processes of propionic acid production by fermentation of low-cost agricultural wastes could be point of interest as an efficient and competitive as well as decreasing technique in the mass of agricultural wastes (Zhang and Yang, 2009; Piwowarek et al., 2018).

Phoenix dactylifera var. *Kabkab* as a plant can be found in dry and semi-dry regions (Homayouni et al., 2015). *P. dactylifera* var. *Kabkab* syrup is one of the date by-products can offers a cheap source of minerals, dietary fibers, vitamins and carbohydrates both for human consumption and in microbial fermentations (Al-Farsi et al., 2007; Jridi et al., 2015; Jalali et al., 2014).

The ultrasound as a promising technique has demonstrated some valuable effects on the metabolic performance of live systems as well as fermentation (Nguyen et al., 2012; Hashemi et al., 2018). It has been carried out to help lactic acid fermentation by lactobacilli (Hashemi et al., 2018). Additionally, it was also used to promote milk fermentation by *Lactobacillus* strains (Wu, Hulbert, and Mount, 2000; Gholamhosseinpour and Hashemi, 2018). In this regard, some increases in both qualitative and quantitative performance of fermentation can be achieved due to releasing of enzymes and further increases in the mass transfer between the cells and the environment (Hashemi et al., 2018).

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https://doi.org/10.1016/j.bcab.2019.01.027
Received 25 September 2018; Received in revised form 4 January 2019; Accepted 14 January 2019
Available online 18 January 2019
1878-8181/

The assessment of microbial growth kinetic models is crucial to examine the effects of various environmental parameters and also to optimize the process. Up to now, several models were presented to estimate the growth patterns of various microbes (Nielsen and Villadsen, 1992). However, considering the fact that the appropriate mathematical modeling should cover the main three phases of microbes' growth (i.e. lag, exponential and stationary phases), some theoretical models failed to accurately predict the experimental measurements (Swinnen et al., 2004). Therefore, in the present study the effect of ultrasonic parameters (i.e. exposing time duration and amplitude on the microbial growth curves, as well as the propionic acid production) were determined. To do so, the experimental and mathematical cases were defined based on 5, 10 and 15 min of exposing duration at sonication amplitudes of 25%, 50% and 75%, in addition to the control case. The *Baranyi* model for the microbial growth and the Gaussian curve for propionic acid production were implemented. The model parameters such as maximum growth rates and lag time were obtained for all of the examined cases and were compared to the control case to determine the effect of sonication process.

2. Materials and methods

2.1. Date syrup and chemicals

Fifteen Kg of date syrup were obtained (November 2017) from a market in Jahrom city (Fars province, Iran). The analysis of date syrup's content was performed according to the National Standard (ISIRI 5075, 2013) (Table 1). After centrifugation (EBA21, Hettich, Germany) of date syrup at 3500 g for 20 min, the samples were autoclaved (121 °C, 15 min). All chemicals in analytical grade were purchased from Merck Co. (Darmstadt, Germany).

2.2. Bacterial strains and cultures

Propionibacter freudenrechii subsp. *shermanii* PTCC 1661 and *Propionibacterium freudenreichii* subsp. *freudenreichii* PTCC 1674 were obtained from Iranian Research Organization for Science and Technology, Tehran, Iran. They were grown in a culture medium containing (per liter): 10 g casein peptone, 5 g yeast extract, 10 g sodium lactate and 15 g agar under anaerobic condition at 30 °C for 48 h.

2.3. Fermentation process

Fermentations were carried out under anaerobic conditions using 250 mL glass containing 200 mL of the sterilized date syrup supplemented with 5 g/L yeast extract. Medium was gassed with sterile nitrogen gas to eliminate the oxygen. The inoculations were performed with 4.3 and 4.4 log CFU/mL of *P. freudenrechii* subsp. *shermanii* and *P. freudenreichii* subsp. *freudenreichii*, respectively. The temperature was kept at 30 °C and the medium was stirred by magnetic agitation at 200 rpm. The pH was controlled at 6.5–7.0 by addition of the required concentrations of 10 M NaOH. For sonication of samples, a Hielscher Ultrasonic apparatus (UP100H model, Germany; 100 W, 30 kHz) was used before fermentation. The ultrasound was regulated at 25%, 50% and 75% amplitude of waves for 5, 10 and 15 min.

Table 1
Date syrupproximate composition Mean ± SD.

Content	Amount (g/L)
Water	849.1 ± 5.31
Glucose	69.42 ± 1.15
Fructose	53.13 ± 1.12
Ash	15.24 ± 0.23
Protein	10.16 ± 0.05

2.4. Enumeration of microbial cells

For enumeration of microbial cells, serial dilutions of date syrup containing *P. freudenrechii* subsp. *shermanii* or *P. freudenreichii* subsp. *freudenreichii* were carried out and plated in *Propionibacterium* agar plates followed by incubated at 30 °C for 48 h under anaerobic condition.

2.5. Quantitative determinations of propionic acid

After centrifugation at 4000 g for 15 min, the supernatants containing propionic acid were quantified by a High-performance liquid chromatography (HPLC) (Knauer, Azura, Germany). The separation column was an Ultrasep ES-FS special (250 × 30 mm, 5 µm, Knauer column). H_2SO_4 0.01 M in ultrapure water at 0.4 mL/min was used out as eluent and temperature of column was set at 35 °C (Hashemi et al., 2017).

2.6. Mathematical modeling

The obtained growth curves of *P. freudenrechii* subsp. *shermanii* and *P. freudenreichii* subsp. *freudenreichii* were plotted using the Baranyi model (Baranyi and Roberts, 1994). The Baranyi model is appropriate for cases with both growth and stationary phases. The variations of microbes' population as a function of time can be predicted using Eqs. ((1)–(3)).

$$\ln(N(t)) = \ln(N_0) + \mu_{max}F(t) - \ln\left[1 + \frac{e^{\mu_{max}F(t)} - 1}{e^{(N_{max} - N_0)}}\right] \quad (1)$$

$$A(t) = t + \frac{1}{\mu_{max}}\ln\left[\frac{e^{(-\mu_{max}t)} + q_0}{1 + q_0}\right] \quad (2)$$

$$\lambda = \frac{\ln\left(1 + \frac{1}{q_0}\right)}{\mu_{max}} \quad (3)$$

where, *ln (N(t))* is the logarithm of cell concentration at any specific time *t* [h] (CFU/g), *ln (N_0)* is the cell concentration at the initial time (CFU/g) and *ln (N_{max})* is the maximum cell concentration (CFU/g)for which the stationary behavior is observed. Moreover, μ_{max} is the maximum of exponential growth rate log (CFU/g/h)), q_0 is used to express the cells physiological state at $t = t_0$ and λ is the lag time [h].

Moreover, to predict the production of propionic acid for the examined cases, the Gaussian function was applied to the experimental data as:

$$[p] = a \times exp\left(\frac{-(t - b)^2}{2c^2}\right) \quad (4)$$

where, [*p*] is the propionic acid concentration (g/L), *a* is the height of the curve's peak, *b* is the position of the center of the peak and *c* (the standard deviation, also called the Gaussian RMS width) controls the width of the curve.

2.7. Statistical analysis

The parameters of Baranyi model (i.e. μ_{max} and λ) as well as the Gaussian function (i.e. *a*, *b* and *c*) were determined using nonlinear curve fitting to the available experimental data. The assessment of the models accuracy was performed using both the adjusted coefficient of determination (adj-R^2) and the root mean square error (RMSE). Moreover, one-factor analysis of variance (ANOVA) followed by the Duncan's test was implemented to check for the significant statistical differences ($p \leq 0.05$). All the processes and analyses were performed in triplicate.

Table 2
Growth kinetic parameters (μ_{max} (log CFU/g/h) and λ (h)) for *P. freudenrechii* subsp. *shermanii* and *P. freudenreichii* subsp. *freudenreichii* at various operating sonication conditions.

	Amplitude (%)	Time (min)	Generation time	Growth parameters		Statistical parameters	
			g (*h*)	μ_{max}	λ	Adj-R^2	RMSE
P. freudenrechii* subsp. *shermanii	Control	–	3.90	0.1776^{d}	27.64432^{b}	0.9978	0.08329
	25	5	3.67	0.1888^{b}	24.64606^{c}	0.9989	0.06072
		10	3.55	0.1953^{a}	21.77209^{d}	0.9968	0.1103
		15	3.83	0.1808^{c}	17.27465^{e}	0.9968	0.1123
	50	5	3.77	0.1839^{b}	20.82687^{d}	0.9963	0.1189
		10	3.99	0.1738^{d}	16.16057^{e}	0.9968	0.1113
		15	3.84	0.1807^{c}	13.9133^{f}	0.9967	0.1162
	75	5	4.11	0.1685^{d}	15.16365^{e}	0.997	0.1084
		10	4.01	0.1728^{d}	14.57785^{e}	0.9976	0.1011
		15	4.92	0.141^{e}	33.4024^{a}	0.9963	0.1001
P. freudenreichii* subsp. *freudenreichii	Control	–	4.79	0.1447^{d}	31.36801^{b}	0.998	0.07712
	25	5	3.55	0.1951^{a}	23.85074^{c}	0.9984	0.07782
		10	3.60	0.1926^{a}	20.3437^{d}	0.9975	0.09998
		15	3.87	0.179^{c}	16.469^{e}	0.9962	0.1242
	50	5	3.78	0.1836^{b}	19.22126^{d}	0.9978	0.09514
		10	3.90	0.1778^{c}	15.71934^{e}	0.9963	0.1252
		15	3.95	0.1754^{c}	14.85909^{e}	0.9961	0.9961
	75	5	3.81	0.182^{b}	15.64803^{e}	0.9972	0.1104
		10	3.94	0.1759^{c}	14.14226^{e}	0.9974	0.108
		15	4.69	0.1479^{d}	35.1168^{a}	0.9961	0.102

For each microbe, Means within a column with the same superscript lowercase letters are not significantly different at $P < 0.05$.

3. Results and discussion

The growth kinetic parameters of *P. freudenrechii* subsp. *shermanii* and *P. freudenreichii* subsp. *freudenreichii* were presented in Table 2. The initial population of *P. freudenrechii* subsp. *shermanii* and *P. freudenreichii* subsp. *freudenreichii* were measured as 10^4–10^5 CFU/g. The adj-R^2 parameter was estimated in the range of 0.9961–0.9989, which emphasis the accuracy of the Baranyi model to predict the microbes' growth behavior. The lag time parameter (λ) had an overall decreasing trend as the time duration of the ultrasonic imposing increased from 5 to 15 min with the except of 75% amplitude at 15 min duration time case for both *P. freudenrechii* subsp. *shermanii* and *P. freudenreichii* subsp. *freudenreichii*.

Increasing in the exposure time from 5 to 15 min for *P. freudenrechii* subsp. *shermanii* reduced the lag time about 29% for both amplitudes of 25% and 50%, while 120% increase of λ was observed at the highest experimented amplitude (75% amplitude). Almost the similar trends were noted for *P. freudenreichii* subsp. *freudenreichii* growth behavior by increasing in exposure time (i.e. 31% and 22% reduction of λ for 25% and 50% amplitudes in contrary to 124% increasing for 75% amplitude). Moreover, in comparison with the control, the ultrasonication significantly reduced the λ (37% and 47% reduction for *P. freudenrechii* subsp. *shermanii* and 47% and 52% for *P. freudenreichii* subsp. *freudenreichii* at 25% and 50% amplitudes, respectively). The observed and predicted microbes' growth data were illustrated in Figs. 1 and 2 for *P. freudenrechii* subsp. *shermanii* and *P. freudenreichii* subsp. *freudenreichii*, respectively. In this regard, the initiation, growth and stationary phases in the microbes' growth curves were obvious. Additionally, the growth of *P. freudenrechii* subsp. *shermanii* was more pronounced in 50% amplitude while compared with 25% amplitude, however, by increasing in the amplitude to 75% especially for the longest exposure time (15 min), a rapid reduction in the growth was observed. Almost the same trends were observed for *P. freudenreichii* subsp. *freudenreichii*. It should be noted that the mentioned case with 75% amplitude and exposure time of 15 min was the only case in which the ultrasonication had harmful effects on the microbial growth (the growth curves were lower than those of the control case without sonication).

The effect of ultrasonication on the maximum growth rate (μ_{max}) and λ were depicted in Figs. 3 and 4. Generally, increasing in the amplitude and the time duration of the sonication process reduced μ_{max} (despite some minor deviation from the stated behavior). Although μ_{max} was not correlated with the number of final population (see Figs. 1 and 2). Moreover, the μ_{max} parameter for the control were also revealed in Figs. 3 and 4, which demonstrate a higher sonication effectiveness for *P. freudenreichii* subsp. *freudenreichii*'s growth rate in comparison to *P. freudenrechii* subsp. *shermanii* (e.g. 5 min of sonication at 25% amplitude increased the μ_{max} parameter for *P. freudenrechii* subsp. *shermanii* by 6% while the same treatment for *P. freudenreichii* subsp. *freudenreichii* resulted in a 31% increase in the μ_{max} parameter). On the other hand, the λ had a decreasing order with respect to the increase of sonication strength and duration time with except of 75% amplitude for 15 min.

The curve fitting parameters for propionic acid was illustrated in Table 3. The value of the adj-R^2 was calculated as 0.9901–0.9946, which demonstrated the model accuracy. The parameter *a*, which determines the peak height of the curves was the highest with the value of 6.429 at 75% amplitude and exposure time of 10 min for *P. freudenrechi* subsp. *shermanii* and 6.494 at the same test conditions for *P. freudenreichii* subsp. *freudenreichii*. Also, the parameter *b*, which determines the required time to reach to peak value of curves was between 3 and 5 min as higher value for *P. freudenrechii* subsp. *shermanii* in comparison to *P. freudenreichii* subsp. *freudenreichii*, however, the width of the curves (parameter *c*) were almost the same for all of the examined bacteria.

It was reported data syrup is as a good culture medium for fermentation contains fermentable sugars and essential elements (Moosavi-Nasab et al., 2010, Jalali et al., 2014). Hashemi et al. (2018) reported *L. helveticus* PTCC 1332 and *L. acidophilus* PTCC 1643 can grow on date syrup and reached cell populations of 9.2 and 9.6 log (CFU mL^{-1}) after 14 fermentation, respectively. According to Dahroud et al. (2016), the low intensity ultrasound (60% amplitude; 15 s) increased specific growth rate (from 4.286 to 5.357 (h^{-1})) and logarithmic phase duration (from 10 to 12 (h)) of *Lactobacillus casei* subsp.*casei* ATTC 39392 during fermentation. It was reported sonication (28 kHz, 140 W/L, 1 h) increased 127.03% biomass of *Saccharomyces cerevisiae* during fermentation (Dai et al., 2017). The cavitation bubble releases shock waves in constant oscillatory movement, however, the amplitude of these waves is extreme decreased, consequently not to cause any physical damage to the microbial cells. The micro-streaming stimulated by the sonication wave produces strong convection, which can have

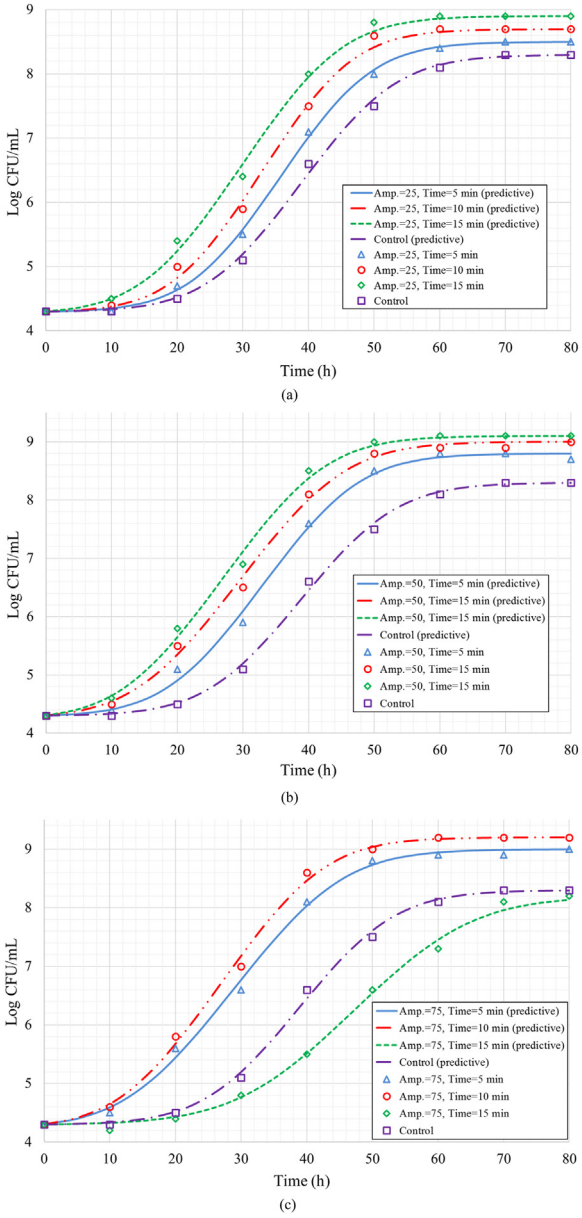

Fig. 1. The effect of ultrasonication time duration on *P. freudenrechii* subsp. *shermanii* growth curve at a) 25% amplitude b) 50% amplitude c) 75% amplitude.

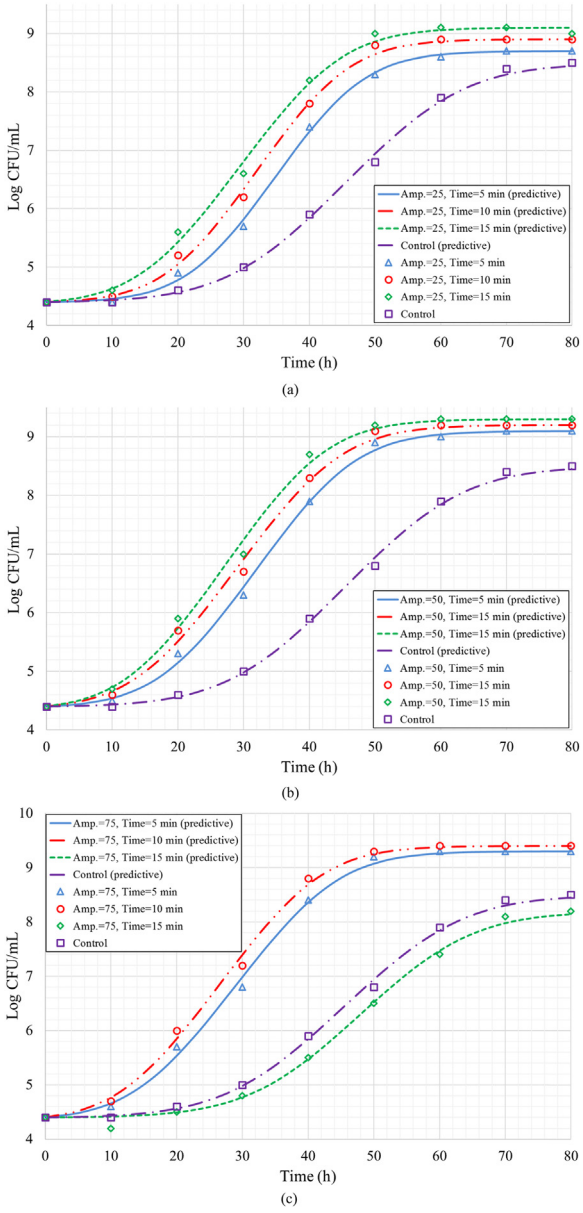

Fig. 2. The effect of ultrasonication time duration on *P. freudenreichii* subsp. *freudenreichii* growth curve at a) 25% amplitude, b) 50% amplitude, c) 75% amplitude.

helpful results for overcoming the limitations of mass transfer in the process (Khanna et al., 2013).

The concentrations of produced propionic acid as a function of sonication amplitude and time duration were depicted in Figs. 5 and 6 for *P. freudenrechii* subsp. *shermanii* and *P. freudenreichii* subsp. *freudenreichii*, respectively. For both bacteria, as the amplitude was increased, the magnitude of the produced propionic acid was increased while compared to the control except of 75% amplitude for 15 min. The magnitude of the propionic acid at early stages of sonication was low while its production rate increased at the middle stage to reach a nearly stationary magnitude at the final time. However, in contrary to the growth rate curves (which had constant microbe's population at the end stage), the propionic acid curves had slight increasing trend at $t = 80$ [h] (Fig. 7).

For amplitudes of 25% and 50%, increasing the sonication time duration to 15 min, enhanced the production of propionic acid from 4.62 to 5.65 g/L and from 4.62 to 6.41 g/L for *P. freudenrechii* subsp. *shermanii* and from 4.38 to 5.51 g/L and from 4.38 to 6.21 g/L for *P. freudenreichii* subsp. *freudenreichii*, respectively. For the case of 75% amplitude similar to the growth curves behavior, an increase in the production of propionic acid from 4.62 to 6.43 g/L and from 4.38 to 6.22 g/L for *P. freudenrechii* subsp. *shermanii* and *P. freudenreichii* subsp. *freudenreichii* was observed by up to 10 min utilization of sonication. However, increasing the time duration of exposure beyond 10 min, significantly decreased the amount of propionic acid (i.e. 55.5% and 39.1% reduction for *P. freudenrechii* subsp. *shermanii* and *P. freudenreichii* subsp. *freudenreichii* with respect to the 10 min utilization of sonication).

Both microorganisms used in this study were able to produce propionic acid from data syrup as a substrate during 80 h fermentation. In our study, the productivity of propionic acid was 4.03–6.43 g/L. Yang et al. (2018) reported *Propionibacterium acidipropionici* ATCC 4875 can proficiently use soy oligosaccharides in soy molasses for growth and production of propionic acid. Feng et al. (2011) reported *Propionibacterium freudenreichii* CCTCC M207015 can produce 12.69 ± 0.40 g/

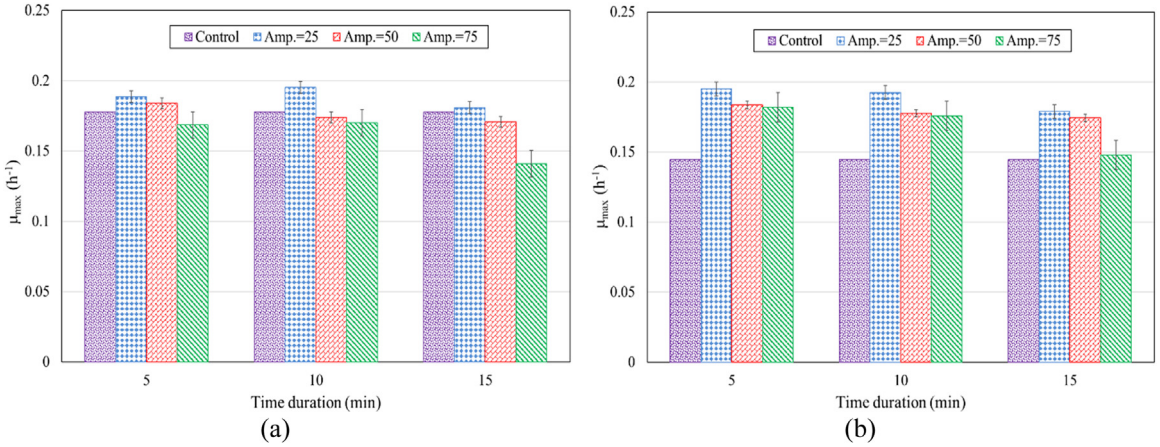

Fig. 3. The effect of ultrasonication time duration and amplitude on μ_{max} for a) *P. freudenrechi* subsp. *shermanii* and b) *P. freudenreichii* subsp. *freudenreichii*.

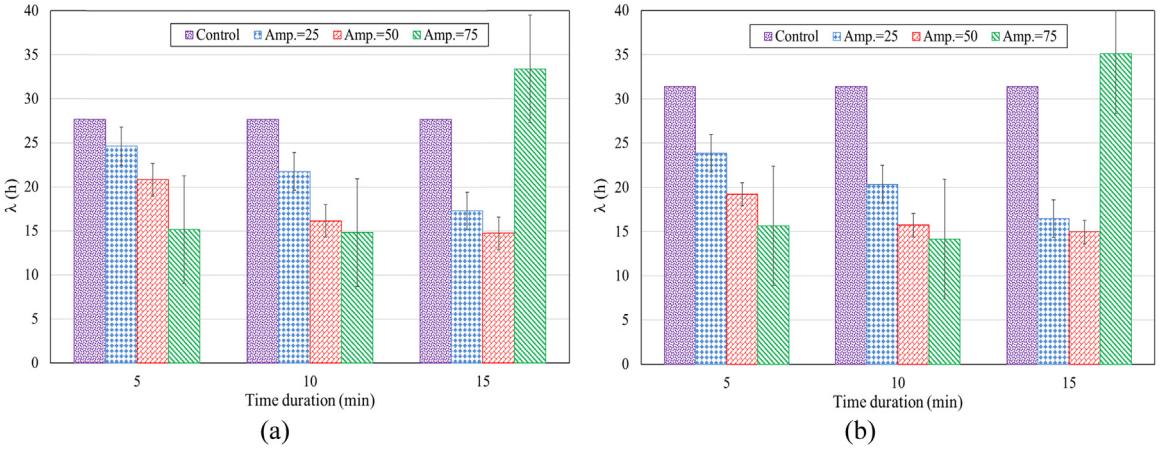

Fig. 4. The effect of ultrasonication time duration and amplitude on λ for a) *P. freudenrechi*i subsp. *shermanii* and b) *P. freudenreichii* subsp. *freudenreichii*.

Table 3
Gaussian curve fitting parameters for propionic acid (g/L) at various operating ultrasound conditions.

	Amplitude (%)	Time (min)	Gaussian parameters			Statistical parameters	
			A	b	c	Adj-R^2	RMSE
P. freudenrechi*i subsp. *shermanii	Control	–	4.515^{f}	84.59^{f}	43.47^{f}	0.9931	0.1384
	25	5	4.8^{d}	83.64^{d}	45.34^{d}	0.9933	0.06072
		10	5.297^{c}	85.65^{c}	47.13^{c}	0.9934	0.1103
		15	5.617^{b}	85.45^{b}	47.51^{b}	0.9935	0.1123
	50	5	5.325^{c}	85.9^{c}	47.42^{c}	0.9933	0.1189
		10	5.638^{b}	85.45^{b}	47.64^{b}	0.9931	0.1113
		15	6.421^{a}	87.83^{a}	48.76^{a}	0.9925	0.1162
	75	5	5.664^{b}	85.74^{b}	47.95^{b}	0.993	0.1084
		10	6.429^{a}	87.66^{a}	48.75^{a}	0.9924	0.1011
		15	4.154^{g}	84.96^{f}	42.66^{g}	0.9932	0.1001
P. freudenreichii* subsp. *freudenreichii	Control	–	4.754^{f}	83.24^{c}	42.44^{e}	0.9914	0.1648
	25	5	5.074^{d}	80.52^{g}	43.27^{d}	0.9946	0.142
		10	5.37^{c}	81.19^{f}	44.78^{c}	0.9925	0.1755
		15	5.665^{b}	81.55^{e}	46.08^{b}	0.9934	0.1724
	50	5	5.378^{c}	80.91^{g}	44.51^{c}	0.9924	0.1772
		10	5.67^{b}	81^{f}	45.82^{b}	0.9928	0.1808
		15	6.493^{a}	85.23^{a}	48.78^{a}	0.9924	0.2048
	75	5	5.689^{b}	81.09^{f}	45.91^{b}	0.9927	0.1821
		10	6.494^{a}	84.35^{b}	47.33^{a}	0.9901	0.2373
		15	4.205^{g}	82.56^{d}	41.57^{f}	0.9941	0.1213

For each microbe, Means within a column with the same superscript lowercase letters are not significantly different at $P < 0.05$.

L propionic acid from molasses after 120 h fermentation. Wang and Yang (2013) found production of propionic acid by *Propionibacterium freudenreichii* subsp. *shermanii* DSM4902 from glucose and glycerol reached ~0.39 g/g and ~0.65 g/g, respectively. Piwowarek et al. (2016) found *Propionibacterium freudenreichii* T82 utilized apple pomaces as a substrate of fermentation. Propionic acid reached

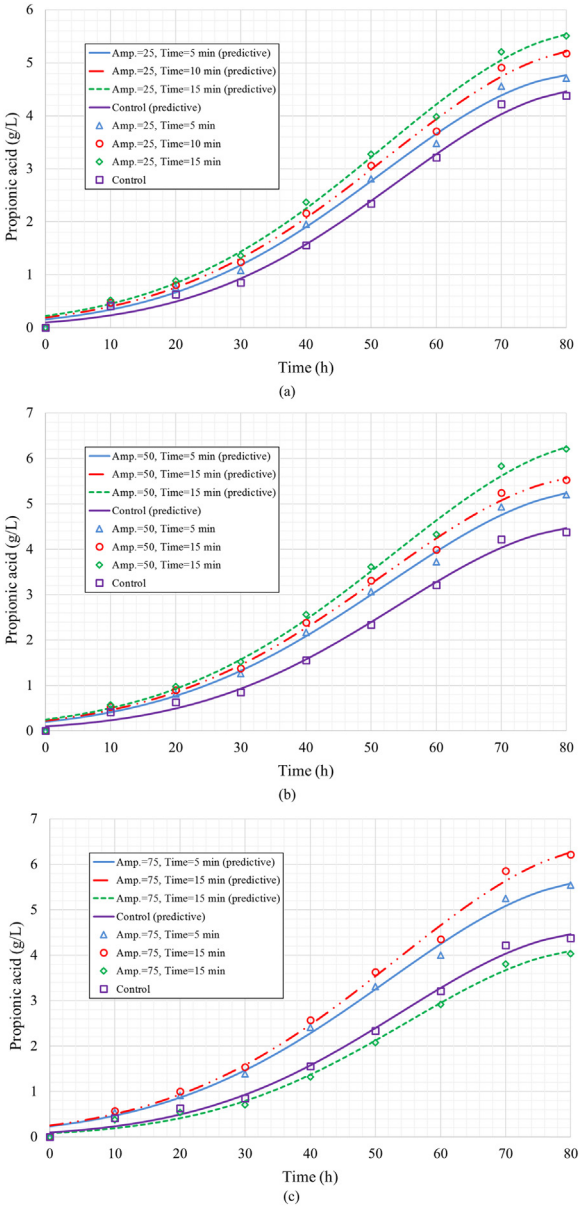

Fig. 5. The effect of ultrasonication time duration on propionic acid (g/L) for *P. freudenrechi* subsp. *shermanii* at: a) 25% amplitude, b) 50% amplitude, c) 75% amplitude.

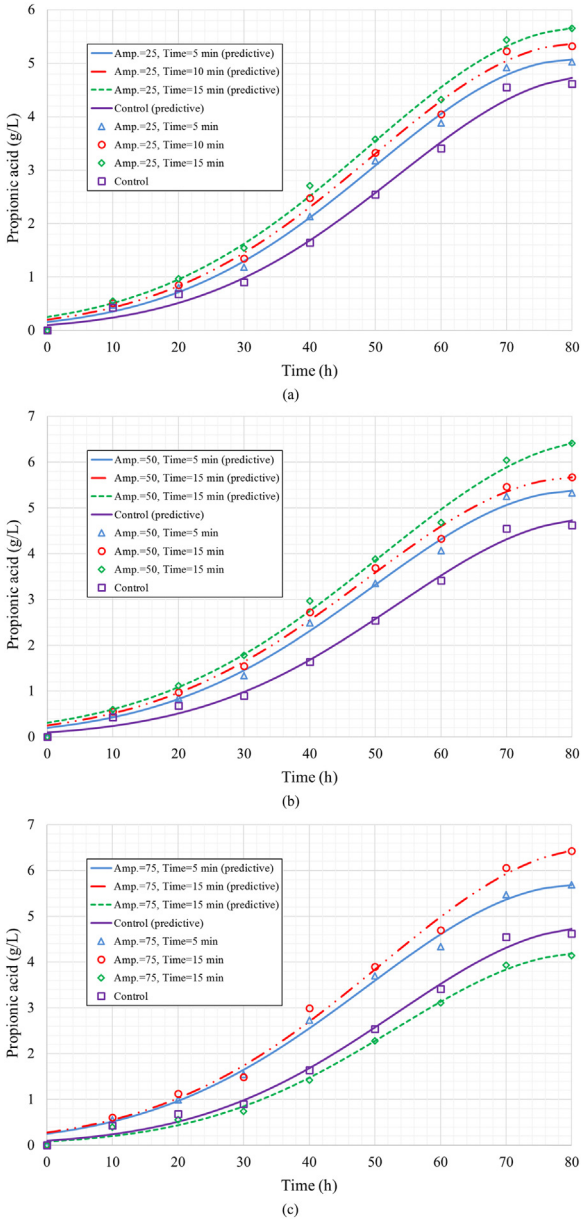

Fig. 6. The effect of ultrasonication time duration on propionic acid (g/L) for *P. freudenreichii* subsp. *freudenreichii* at: a) 25% amplitude, b) 50% amplitude, c) 75% amplitude.

production of 1.771 g/L after 120 h fermentation. Coral et al. (2008) studied propionic acid production by *P. acidipropionici* ATCC 4965 using sugarcane molasses, lactate or glycerol at 30 and 36 °C. It was found biomass and propionic acid production were higher at 30 °C than at 36 °C. Lactate or glycerol was the best productivity (0.113 g/L h), even though the yielding of propionic acid was higher when using glycerol as carbon source (0.724 g/g). Himmi et al. (2000) reported *P. acidipropionici* ATCC 25562 had higher efficiency in propionic acid production using glycerol as carbon source with a faster substrate consumption (0.64 g/L h) and a higher propionic acid production (0.42 g/L h) compared to *P. freudenreichii* ssp. *shermanii* ATCC 9614. Zhang and Yang (2009) found metabolically engineered *P. acidipropionici* ACK-Tet can use glycerol for its growth and converted glycerol to propionic acid at a high yield of 0.54–0.71 g/g, which was much higher compared to glucose as carbon source (0.35 g/g). Wang et al. (2015) developed a high cell density fermentation process for propionic acid production from glucose with an acid tolerant *P. acidipropionici* strain. They found the yield of propionic acid was 0.44 g/g at pH 6.5. Differences in propionic acid production may be due to various factors including differences in bacterial strains, differences in fermentation conditions and the use of different substrates (Wang et al., 2015).

For both microorganisms, sonication treatments of 25% and 50% amplitudes for 5, 10 and 15 min increased the propionic acid production while compared to the control samples, which suggests that these sonication treatments stimulated fermentation by these microorganisms in date syrup. It was reported sonication can improve the mass transfer of products through the cellular wall and membrane and modify cellular metabolism, thus, increasing the fermentation efficiency (Ojha et al., 2017). In this study, application of a 75%-sonication treatment for 15 min caused a decrease in propionic acid production compared to the control may be due to the higher loss of cell ingredients that formed cell organization or function (Ojha et al., 2017). Hashemi et al. (2018) found that application of ultrasound (30 kHz, 100 W) at 10 and 20 min increased lactic acid production in *Lactobacillus helveticus* and *Lactobacillus acidophilus*. However, the treatment extension to 30 min

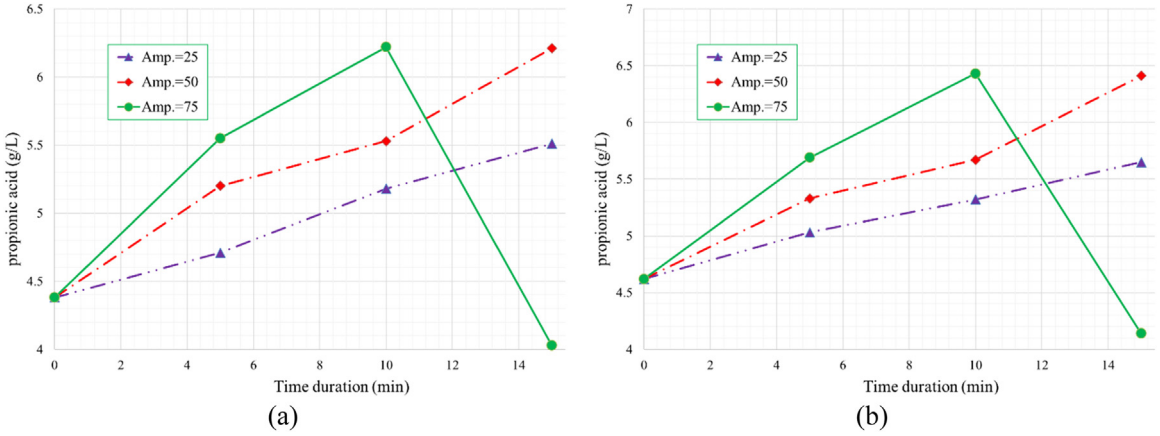

Fig. 7. The produced propionic acid (g/L) at various amplitude and time durations for: a) *P. freudenrechi* subsp. *shermanii* and b) *P. freudenreichii* subsp. *freudenreichii*.

negatively affected lactic acid production. Sulaiman et al. (2011) reported ultrasound (20 kHz; duty cycles of ≤20%; 11.8 W cm^{-2}) stimulated biomass and ethanol production of *K. marxianus*. However, enhancing the duty cycle to 40% had an obvious adverse effect on this microorganism. Therefore, the effects of sonication on the production of propionic acid by *P. freudenrechii* subsp. *shermanii* and *P. freudenreichii* subsp. *freudenreichii* were found to be dependent on the ultrasound amplitude and duration of the treatments.

4. Conclusion

P. freudenrechii subsp. *shermanii* and *P. freudenreichii* subsp. *freudenreichii* were able to grow in date syrup to produce propionic acid. The effects of sonication on cell growth and fermentation were dependent on the amplitude and exposure time, with the shorter amplitudes (25% and 50%) and shorter exposure time (5 and 10 min at 75% amplitude) positively affecting the cell growth and fermentation process. However, when the sonication treatment was increased to 30 min at 75% amplitude, fermentation and cell growth were negatively affected in comparison to the control samples.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bcab.2019.01.027.

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