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Optimization and the Effect of pH Adjustment for Trehalose Production by *Propionibacterium acidipropionici* DSM 20273

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ABSTRACT

Trehalose is a non-reducing disaccharide, which is used in the food, cosmetics and pharmaceutical industries. Microorganisms produce trehalose to protect their cells from stress conditions. The optimum conditions were determined for trehalose production by *Propionibacterium acidipropionici* DSM 20273 using the Taguchi method with four parameters: lactose concentration, initial pH, temperature and agitation rate. The trehalose production was optimum at a lactose concentration of 20 $g.L^{-1}$, an initial of pH 7, a temperature of 30°C and an agitation rate of 100 revolutions per minute. Under these conditions, the highest yield of trehalose (595 $mg.\cdot L^{-1}$)was produced. Of the four parameters tested, the trehalose production was most affected by the temperature. To investigate the effect of pH adjustment on trehalose production, the pH was adjusted to four levels: no control of pH, control of pH at 5.5, part 1, control of pH at 7 and control of pH at 5.5, part 2. The study found that the pH adjustment increased the accumulation of trehalose. The highest volumetric production rate and yield of trehalose were found when controlling the pH at 7, during the period 114.30–192 hr (0.0113 $g.L^{-1}.hr^{-1}$ and 0.346 $g.g^{-1}$, respectively). The concentration of trehalose reached 0.925 $g.L^{-1}$ at the end of the fermentation. Furthermore, the efficiency of the lactose consumption was around 100 ± 10%.

Keywords: trehalose, *Proponibacterium acidipropionici*, optimization, Taguchi method

INTRODUCTION

Trehalose is a non-reducing disaccharide consisting of two glucose molecules linked by an α,α-1,1 bond and is colorless, odorless, has mild sweetness, exhibits no Maillard reaction and is highly stable (Higashiyama, 2002; Richards *et al*., 2002). Due to these advantageous properties, trehalose is used in many industries. In the food industry, trehalose can be used in the preparation of dried food, frozen food and concentrated food, in order to preserve aromas, flavor and organoleptic properties (Jittinandana *et al*., 2006; Aktas *et al*., 2007; Kopjar *et al*., 2008). In the cosmetic industry, trehalose can be used as a moisturizer, liposome stabilizer and an additive for deodorants (Higashiyama, 2002; Schiraldi *et al*., 2002; Chang *et al*., 2010). In medicine, trehalose can be used to stabilize antibodies, adjuvants, enzymes and to protect mammalian cells during freeze-drying

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(Dráber *et al*., 1995; Ma *et al*., 2005; Christensen *et al*., 2007; Zhang *et al*., 2007).

Trehalose is found in various organisms, including microorganisms, invertebrates and plants (Goddijn and van Dun, 1999; Zhang *et al*., 2010). It is capable of protecting these organisms against environmental stresses, such as thermal, alcohol, osmotic and oxidative stress (Penna, 2003; Li *et al*., 2009; Erdei *et al*., 2011). Propionibacteria are capable of producing trehalose accumulation within cells where it functions as a reserve compound and as a stress-response metabolite and trehalose accumulation increased many fold when propionibacteria were cultured under stressful conditions; for instance, under acid, oxidative and osmotic stress (Hugenholtz *et al*., 2002). With acid stress, trehalose accumulation increased 2.3-fold when the pH was adjusted from 7 to 5 (Cardoso *et al*., 2004; Cardoso *et al*., 2007) which indicated that the pH adjustment during fermentation stimulates the change in trehalose accumulation and pH control is an easy technique to adopt in a fermenter. In addition, fermentation by propionibacteria also produces many useful metabolites, such as propionic acid, acetic acid, vitamin B12 and porphyrins as well as biomass (Vorobjeva, 1999).

The present study was undertaken to determine the optimum conditions for maximum trehalose production and to investigate the effect of pH adjustment on trehalose production by *Propionibacterium acidipropionici* DSM 20273.

MATERIALS AND METHODS

Microorganism and medium

The *P. acidipropionici* DSM 20273 used in this study was cultured in a complete medium. The medium contained (per liter): 10 g yeast extract, 5 g pancreatic digest of casein, 0.25 g K_2HPO_4, 0.05 g $MnSO_4$ and 20 g lactose and the pH of the medium was adjusted to 6.5 before autoclaving with 2 N HCl solution (Suwannakham, 2005).

For inoculum preparation, 0.4 mL of stock culture was transferred to 3.6 mL of the medium in a test tube and incubated at 30 °C for 36 hr. This culture was transferred to 36 mL of the medium in a 125 mL Erlenmeyer flask and incubated at 30 °C for 36 hr and this culture was transferred to 360 mL of the medium in a 500 mL Erlenmeyer flask and incubated at 30 °C for 36 hr.

Optimization of trehalose production

The Taguchi technique was used to design and to analyze the experiments using the Qualitek-4 software (Version 7.5.0, Nutek, Inc, MI, USA). The Taguchi technique applies fractional factorial experimental designs, called orthogonal arrays, to reduce the number of experiments whilst obtaining statistically meaningful results, with the selection of a suitable orthogonal array depending on the number of factors and their levels (Mousavi *et al*., 2007). In this study, four factors at three levels were investigated (Table 1). An L9 array was selected to determine the effect of the four 3–level factors on trehalose production. Based on the L9 Taguchi design, the number of experiments required can be considerably reduced to nine (Table 2). In the full-factorial experimental design procedures, at least 64 experiments would be necessary to reach the same conclusions as those obtain from applying Taguchi's array method using these factors (Roy, 2001; Zarei *et al*., 2010).

All experiments were performed according to their respective L9 conditions in a 500 mL Erlenmeyer flask containing 360 mL of complete medium. The medium was inoculated with 10% (volume per volume; v/v) of inoculum and incubated in a incubator shaker. All experiments were performed in duplicate.

Effect of pH adjustment on trehalose production

For this study, the optimum conditions from the above studies (lactose concentration 20 $g.L^{-1}$, initial pH 7.0 and temperature 30 °C) were used for the investigation of the effect of

pH adjustment on trehalose production. The fermentation was performed in a 3 L fermenter (Model BioFlo 110, New Brunswick Scientific Co. Inc.; Edison, NJ, USA) containing 1,620 mL of complete medium. All compositions of medium were autoclaved in the fermenter except the lactose, which was autoclaved separately. Lactose solution (concentration 60 $g.L^{-1}$) was added aseptically. To establish anaerobic conditions, the medium was aseptically gassed with nitrogen until the dissolved oxygen level was 0% of air saturation. The 10% (v/v) inocula were added aseptically. An agitation rate of 80 revolutions per minute (rpm) was used to keep the fermentation broth homogeneous without aeration (Cardoso *et al*., 2004). The trehalose fermentation could be divided into four steps: In the first step, the fermentation was performed with no control of the pH until the pH was almost constant. In the second step, the fermentation was performed by controlling the pH at 5.5 with the addition of 5 N NaOH until the fermentation broth was around 60% of the initial volume. In the third step, the lactose solution (concentration 20 $g.L^{-1}$) was added to the final volume of 1,800 mL and then the fermentation was performed by controlling the pH at 7 with the addition of 5 N NaOH until the lactose concentration was almost exhausted (around 5 $g.L^{-1}$). In the final step, the fermentation was performed by controlling the pH at 5.5 with the addition of 5 N NaOH until the end of fermentation.

Intracellular trehalose extraction and determination

The cells were harvested using centrifugation at 7,000 rpm for 10 min and washed twice with 50 mM potassium phosphate buffer (pH 7.0). The cell pellets were stored at –20 °C until use. The frozen cells were suspended in 1 mL of 0.5 M trichloroacetic acid, mixed vigorously, and incubated on a shaker at 250 rpm and room temperature for 1 hr. Subsequently, the supernatant harvested by centrifugation was transferred to new tubes and the cell debris was used for re-extraction. The supernatants from the two extractions were mixed and used for the trehalose determination.

Table 1 Four factors and their levels for the experiments.

Factor	Level 1	Level 2	Level 3
A Lactose concentration ($g.L^{-1}$)	10	20	30
B Initial pH	5	6	7
C Temperature (˚C)	30	35	40
D Agitation rate (revolutions per minute)	0	100	150

Table 2 L9 orthogonal array and the result of each experiment.

Experiment	A	B	C	D	Trehalose ($mg.L^{-1}$)[a]
1	1	1	1	1	437.2
2	1	2	2	2	344.0
3	1	3	3	3	55.2
4	2	1	2	3	423.6
5	2	2	3	1	31.0
6	2	3	1	2	595.0
7	3	1	3	2	0.0
8	3	2	1	3	353.0
9	3	3	2	1	306.0

[a] = Results obtained from the maximum trehalose concentration of each experiment.

The trehalose concentration was determined using high performance liquid chromatography (LC-20AD Model; Shimadzu; Kyoto, Japan) using an APS–2 Hypersil column (5 µm, 4.6 × 250 mm; Thermo Electron Corporation; Fitchburg, WI, USA) and a refractive index (RI) detector (RID–10A; Shimadzu; Kyoto, Japan). Acetonitrile (75%, v/v) was used as the mobile phase with a flow rate of 1.0 mL.min^{-1}. The column temperature was maintained at 30 °C and the injection volume was 20 µL. The trehalose concentration was calculated by comparing the peak areas of external standards.

Lactose and organic acid determination

Lactose, propionic acid, acetic acid and succinic acid in the fermented medium were determined using high performance liquid chromatography with an Aminex HPX-87H ion exclusion column (9 µm, 7.8 × 350 mm, Bio-Rad Laboratories Inc.; Hercules, CA, USA). The RI detector was used for lactose determination and an ultraviolet detector (SPD–20A, Shimadzu; Kyoto, Japan) at wavelength 210 nm was used for organic acid determination. H_2SO_4 (5 mM) was used as the mobile phase with flow rate of 0.6 mL.min^{-1}. The column temperature was maintained at 45 °C and the injection volume was 20 µL. Lactose and organic acid concentrations were calculated by comparing the peak areas with those of external standards.

Biomass and pH determination

The optical density (OD) of the cells was determined in a spectrophotometer (Spectronic Helios Gamma, Thermo Electron Corporation; Ficksburg, WI, USA).) at 660 nm. A standard curve was plotted between the dry cell weight and absorbance and was used to determine the dry cell weight in the samples from the absorbance values.

The pH of the portion sample was determined with a pH meter (FiveEasy™ pH, Mettler-Toledo International Inc.; Beaumont Leys, UK).), which was calibrated with two standard points before the measurement.

Carbon recovery determination

To study the effect of the pH adjustment, lactose was consumed for bacterial growth and production of trehalose, organic acids (propionic acid, acetic acid and succinic acid) and carbon dioxide. Therefore, these variables are related to the carbon recovery calculation. The carbon recovery was calculated from the amount of carbon and the specific rate of each variable using Equation 1:

$$R_C = \frac{\alpha_2 \quad _R + \alpha_3 q_{Tre} + \alpha_4 q_{Pro} + \alpha_5 q_{Ace} + \alpha_6 q_{Suc} + \alpha_7 q_{CO_2}}{\alpha_1 q_{Lac}} \times 100$$

where α_1, α_2, α_3, α_4, α_5, α_6 and α_7 are the amounts carbon of lactose, residual cells, trehalose, propionic acid, acetic acid, succinic acid and carbon dioxide, respectively, μ_R is the specific growth rate of the residual cells, q_{Lac}, q_{Tre}, q_{Pro}, q_{Ace}, q_{Suc} and q_{CO_2} are the specific rates of lactose consumption and the specific rates of trehalose, propionic acid, acetic acid, succinic acid and carbon dioxide production, respectively.

The amount of carbon for each variable was calculated from the molecular weight and the number of carbon atoms in a molecule. To calculate the amount of carbon in the form of the residual cell, the average biomass composition of $CH_{1.8}O_{0.5}N_{0.2}$ was used in the molecular weight calculation (Duboc *et al*., 1995). The calculation of the amount of carbon in the form of CO_2 assumed that CO_2 was generated in acetic acid production and was used in succinic acid production. Thus, 1 mol of CO_2 was produced from the production of 1 mol of acetic acid and was used for the production of 1 mol of succinic acid (Suwannakham, 2005).

RESULTS AND DISCUSSION

Optimization for trehalose production

The results of the experimental design using the Taguchi method are shown in Table 2.

These results were used to determine the optimum conditions for trehalose production using the Qualitek 4 software. Analysis of variance was used to analyze the results and to determine the influence of each factor to trehalose production. The percentage contributions of each factor are shown in Table 3. The percentage contribution of temperature was the largest (88.32%) followed by the lactose concentration (7.27%), the initial pH (2.44%) and the agitation rate (1.26%), respectively (Table 3). Therefore, the temperature was the most influential factor in these results.

The main effect at each level of each factor was plotted to investigate the changes in the factor levels of trehalose production. The peak points in these plots correspond to the optimum conditions. The lactose concentration at level 2 (A2), initial pH at level 3 (B3), temperature at level 1 (C1) and agitation rate at level 2 (D2) were the highest levels of factors (Figure 1).

The optimum conditions for maximum trehalose production and the expected result under the optimum conditions are shown in Table 4. The optimum conditions for trehalose production were lactose concentration 20 $g.L^{-1}$, initial pH 7, temperature 30 °C and agitation rate 100 rpm. In addition, the expected trehalose concentration under the optimum conditions was 595 $mg.L^{-1}$. Generally, trehalose is produced to protect a microorganism from stress conditions (Attfield, 1987; Storm and Kaasen, 1993). The results showed that *P. acidipropionici* DSM 20273

Table 3 Analysis of variance of trehalose production in shake flask.

Factor	DOF	SS	MS	F	S'	Contribution (%)
Lactose ($g.L^{-1}$)	2	50998.58	25499.29	89.18	50426.74	7.27
Initial pH	2	17513.89	8756.94	30.63	16942.05	2.44
Temperature (˚C)	2	613211.90	306605.95	1072.34	612640.06	88.32
Agitation rate (rpm)	2	9326.47	4663.23	16.31	8754.62	1.26
Other error	9	2573.29	285.92	-	-	0.70
Total	17	693624.13	-	-	-	100.00

DOF = Degree of freedom; SS = Sum of squares; *MS* = Mean sum of squares; *F* = *F*-test statistic; *S'* = Pure sum of squares; rpm = Revolutions per minute.

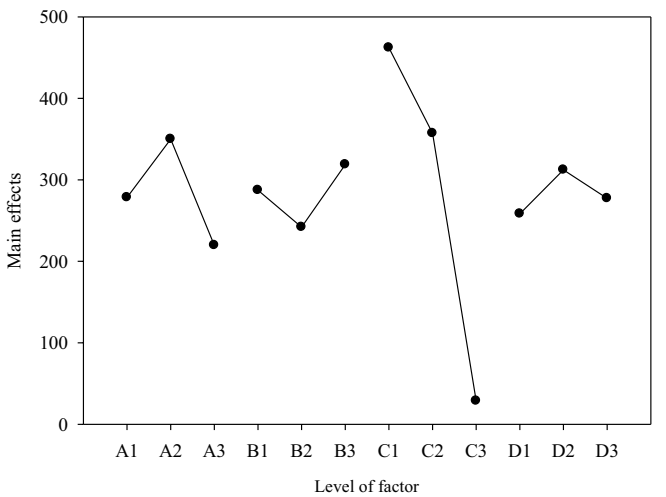

Figure 1 Main effects of each factor (A–D, Table 1) and level (1–3, Table 1) for trehalose production in a shake flask.

can consume lactose for trehalose production, in agreement with previous reports for dairy propionibacteria and *P. acidipropionici* (Deborde *et al*., 1996; Cardoso *et al*., 2004). During fermentation, the lactose consumption requires the enzyme for the hydrolysis of lactose. The β-galactosidase is the only enzyme involved in the lactose metabolism in *P. acidipropionici*. The optimum pH and temperature of β-galactosidase were 7.0 and 50 °C, respectively (Zárate *et al*., 2002).

From the experimental results, initial pH 7 and temperature 30 °C were the optimum conditions for trehalose production, which is the same optimum pH as for enzymatic activity. However, the experiments were performed with no control of the pH. At the beginning of fermentation, there was no the accumulation of trehalose. The trehalose accumulation increased when pH decreased. It has been reported that the activity of this enzyme decreased when the pH decreased from 7.0 to 3.8 and furthermore, an excess of lactose also repressed the enzyme synthesis (Zárate *et al*., 2002). Therefore, the lactose consumption of bacteria was arduous. Bacteria were encouraged to accumulate trehalose. The agitation rate is related to the mixing and aeration of broth and the results showed that the optimum agitation rate was 100 rpm. This agitation rate is sufficient for the fermentation of *P. acidipropionici* DSM20273 because this bacteria is anaerobic, unlike the study reported by Ruhal and Choudhury (2012),

Table 4 Optimum conditions and performance**.**

Factor	Level description	Level	Contribution
Lactose concentration ($g.L^{-1}$)	20	2	67
Initial pH	7	3	36
Temperature (˚C)	30	1	179
Agitation (revolutions per minute)	100	2	30
Total contribution from all factors	-	-	312
Current grand average of performance	-	-	283
Expected result at optimum condition	-	-	595

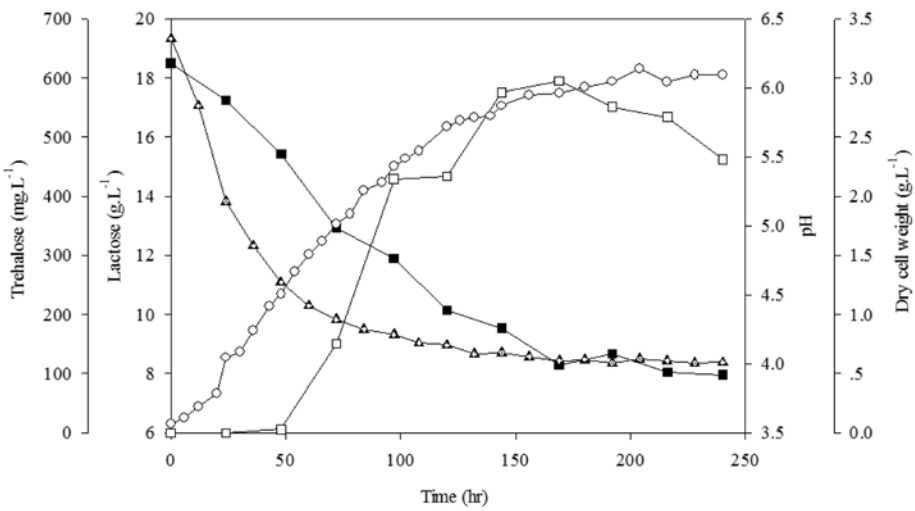

Figure 2 Fermentation to determine optimum conditions in complete medium. Concentration of dry cell weight (○), trehalose (□), lactose (■) and pH (△).

where *Propionibacterium freudenreichii* subsp. *shermanii* was grown under aerobic conditions (200 rpm) and produced a trehalose concentration of 361 mg.L^{-1} from crude glycerol. Furthermore, the optimum conditions for trehalose production were also the growth conditions for these bacteria (Lewis and Yang, 1992; Suwannakham, 2005; Coral *et al*., 2008). Under these conditions, salts of organic acid (propionate and acetate) were continuously produced, which inhibited the cell growth (Furuichi *et al*., 2006). Hence, trehalose was accumulated by the bacteria to protect their cells.

Confirmation of optimum conditions

The final step of the optimization by the Taguchi method is the experimental confirmation of the optimum conditions. In this study, the optimum conditions were the same as for experimental condition 6. The profiles of the fermentation under the optimum conditions are shown in Figure 2. Lactose was continuously consumed for the bacterial growth and trehalose production until 168 hr and then slightly decreased. During the first 48 hr, the number of bacterial cells continuously increased. However, the trehalose accumulation slightly increased. After 48 hr, the bacterial cell and the trehalose accumulation rapidly increased. The trehalose accumulation reached the maximum level (595 mg.L^{-1}) at 168 hr and then decreased. The number of bacterial cells continuously increased until 192 hr (2.969 g.L^{-1}) and then the process entered the stationary phase. The pH continuously decreased until 132 hr and then remained constant (around pH 4).

Effect of pH adjustment

The effect of pH adjustment on trehalose production was determined under the controlled conditions of temperature 30 °C, agitation rate at 80 rpm and anaerobic conditions. The initial concentration of lactose was 20 g.L^{-1}. The profiles for the fermentation are shown in Figure 3.

Trehalose was slightly accumulated under the conditions of no control of pH (0.005 g.L^{-1}) and the control of pH 5.5, part 1 (0.0093 g.L^{-1}) (fermentation during 0–114 hr, periods A and B in Figure 3). However, the trehalose rapidly accumulated (0.845 g·L^{-1}) when the pH was controlled at 7, part 1 (fermentation during 114.30–192 hr, period C1 in Figure 3). This might be explained by the kinetic parameters of the fermentation (Table 5) and the trend of some kinetic parameters in the different parts of the fermentation: no pH control, control of pH at 5.5, part 1 and control of pH at 7, part 1 (Figure 4). Microorganisms produce trehalose to protect themselves from stress conditions and therefore, the specific growth rate decreases under stress conditions (Kuenzi and Fiechter, 1972; Jorge *et al*., 1997). In the current results, the specific growth rate with no control of the pH was 0.043 hr^{-1}, which then continuously decreased under control of the pH at 5.5, part 1 (0.014 hr^{-1}) and control of the pH at 7, part 1 (0.004 hr^{-1}) as shown in Table 5 and Figure 4.

In contrast, the specific production rate of trehalose under those conditions continuously increased. The specific production rates of trehalose with no control of pH, control of pH at 5.5, part 1 and control of pH at 7, part 1 were 0.0002, 0.0009 and 0.0042 $g.g^{-1}.hr^{-1}$, respectively (Table 5 and Figure 4). In addition, the volumetric production rate of trehalose under those conditions continuously increased. The volumetric production rates of trehalose were 0.0001, 0.0012 and 0.0113 $g.L^{-1}.hr^{-1}$, respectively (Table 5 and Figure 4), unlike the volumetric production rate of cell mass, which increased from 0.016 $g.L^{-1}.hr^{-1}$ (no control of pH) to 0.19 $g.L^{-1}.hr^{-1}$ (control of pH at 5.5, part 1). The volumetric production rate of cell mass decreased with the control of pH at 7, part 1 (0.017 $g.L^{-1}.hr^{-1}$ as shown in Table 5 and Figure 4).

With the control of the pH at 7, part 2 (fermentation during 192–216 hr, shown at C2 in Figure 3), the trehalose accumulation decreased. The trehalose accumulation surged again when the bacteria were fermented with control of the pH at

5.5, part 2 (fermentation during 216.45–258 hr, shown at D in Figure 3). The trehalose maximum accumulation (0.925 $g.L^{-1}$) occurred at 222 hr and then decreased.

These results indicated that the cell growth was interfered with by the pH adjustment. This effect has been previously reported by Cardoso *et al*. (2007), as when *P. freudenreichii* was stimulated to acid stress, the level of trehalose increased 2.3 fold.

Carbon recovery

Carbon recovery was used to investigate the efficiency of the lactose consumption and was calculated using Equation 1. The amounts of carbon in lactose (α_1), residual cells (α_2), trehalose (α_3), propionic acid (α_4), acetic acid (α_5), succinic acid (α_6) and carbon dioxide (α_7) in Equation 1 were 0.42, 0.49, 0.42, 0.49, 0.40, 0.41 and 0.27 $g.g^{-1}$, respectively. The carbon recovery in each part of the fermentation is shown in Table 5. The carbon recovery with no control of pH, control of pH at 5.5, part 1, control of pH at 7, part 1 and control of pH at 5.5, part 2 were 111.2, 91.3, 98.8 and 90.7%, respectively. Therefore, the efficiency of the lactose consumption was around 100 ± 10%.

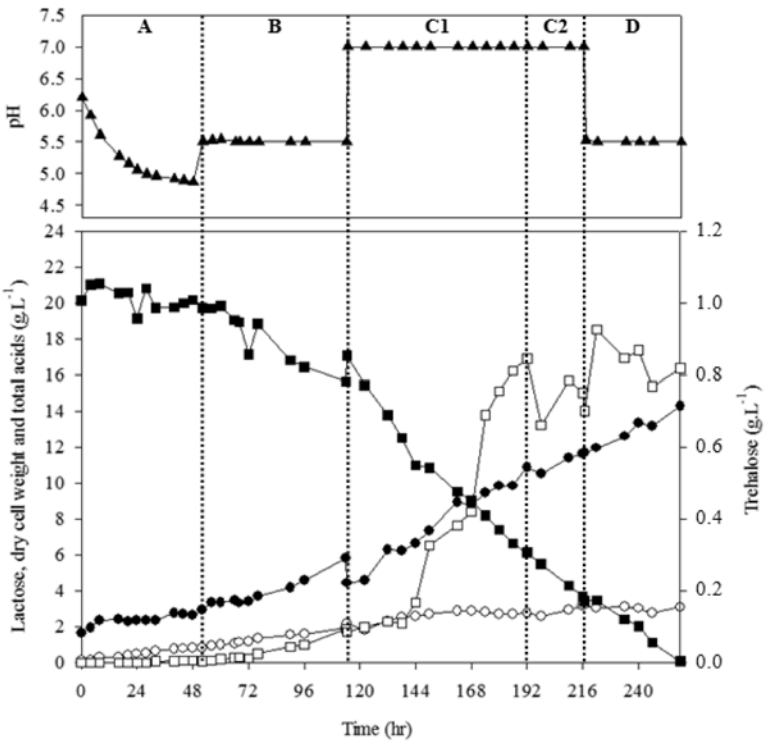

Figure 3 Fermentation with pH adjustment. A shows fermentation period 0–48 hr (no pH control); B shows fermentation period 52–114 hr (control of pH at 5.5, part 1); C1 shows fermentation period 114.30–192 hr (control of pH at 7, part 1); C2 shows fermentation period 192–216 hr (control of pH at 7, part 2); D shows fermentation period 216.45–258 hr (control of pH at 5.5, part 2). Concentration of dry cell weight (○), trehalose (□), lactose (■), total acids (●) and pH (△).

CONCLUSION

The optimum conditions for trehalose production by *P. acidipropionici* DSM 20273 using the Taguchi method were a lactose concentration of 20 $g.L^{-1}$, an initial pH of 7, a temperature of 30 °C and an agitation rate at 100 rpm. Under these conditions, the maximum concentration of trehalose reached 595 $mg.L^{-1}$. Furthermore, the pH adjustment stimulated the accumulation of trehalose. The specific production rate of trehalose was at a maximum with control of pH at 7, part 1 (fermentation during 114.30–192 hr).

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Table 5 Kinetic parameters and carbon recovery of fermentation by pH adjustment.

Parameter[a]	Part of fermentation				
	A	B	C1	C2	D
C_R[b] ($g.L^{-1}$)	0.825	1.937	2.725	3.159	3.072
C_{Tre}[c] ($g.L^{-1}$)	0.005	0.093	0.845	0.784	0.925
μ_R (hr^{-1})	0.043	0.014	0.004	ND	ND
q_{Lac} ($g.g^{-1}.hr^{-1}$)	0.212	0.084	0.057	ND	0.030
q_{Tre} ($g.g^{-1}.hr^{-1}$)	0.0002	0.0009	0.0042	ND	ND
q_{Pro} ($g.g^{-1}.hr^{-1}$)	0.138	0.031	0.021	ND	0.011
q_{Ace} ($g.g^{-1}.hr^{-1}$)	0.012	0.014	0.015	ND	0.009
q_{Suc} ($g.g^{-1}.hr^{-1}$)	ND	0.003	0.001	ND	0.002
q_{Ta} ($g.g^{-1}.hr^{-1}$)	0.272	0.036	0.032	ND	0.019
q_{CO_2}[d] ($g.g^{-1}.hr^{-1}$)	0.020	0.011	0.012	ND	0.006
Q_{Lac} ($g.L^{-1}.hr^{-1}$)	0.086	0.099	0.141	ND	0.089
Q_R ($g.L^{-1}.hr^{-1}$)	0.016	0.019	0.017	ND	ND
Q_{Tre} ($g.L^{-1}.hr^{-1}$)	0.0001	0.0012	0.0113	ND	ND
Q_{Ta} ($g.L^{-1}.hr^{-1}$)	0.040	0.045	0.079	ND	0.057
$Y_{R/Lac}$ ($g.g^{-1}$)	0.248	0.638	0.116	ND	ND
$Y_{Tre/Lac}$ ($g.g^{-1}$)	0.001	0.042	0.094	ND	ND
$Y_{Tre/R}$ ($g.g^{-1}$)	0.007	0.066	0.346	ND	ND
R_C (%)	111.2	91.3	98.8	ND	90.7

α_1, α_2, α_3, α_4, α_5, α_6 and α_7 = Amounts carbon of lactose, residual cells, trehalose, propionic acid, acetic acid, succinic acid and carbon dioxide, respectively;

μ_R = Specific growth rate of the residual cells; q_{Lac}, q_{Tre}, q_{Pro}, q_{Ace}, q_{Suc} and q_{co_2} = Specific rates of lactose consumption and the specific rates of trehalose, propionic acid, acetic acid, succinic acid and carbon dioxide production, respectively; Q_{Lac} Q_R Q_{Tre} and Q_{Ta} = Volumetric rates of lactose consumption and the volumetric rates of cell mass, trehalose and total acid production, respectively.

[a] = All data calculated using positive values; [b] = Final concentration; [c] = Maximum concentration; [d] = Derived from calculation; A = Fermentation during 0–48 hr (no control of pH); B = Fermentation during 52–114 hr (control of pH at 5.5, part 1); C1 = Fermentation during 114.30–192 hr (control of pH at 7, part 1); C2 = Fermentation during192–216 hr (control of pH at 7, part 2); D = Fermentation during 216.45–258 hr (control of pH at 5.5, part 2); ND = Not detected.

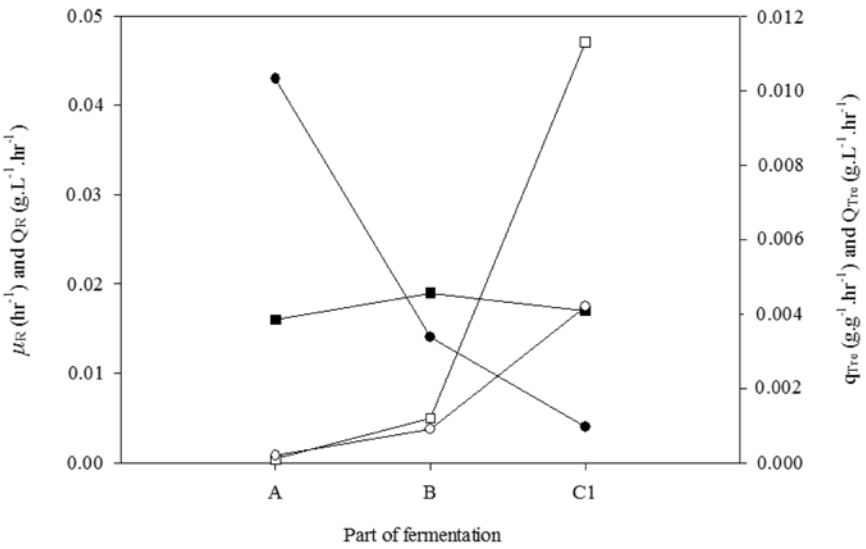

Figure 4 Effect of the pH adjustment on specific growth rate, μ_R (●), specific rate of trehalose production, q_{Tre} (○),volumetric production rate of cell mass, Q_R (■) and volumetric production rate of trehalose, q_{Tre} (□). A = Part of fermentation with no control of pH; B = control of pH at 5.5, part 1; C1 = control of pH at 7 part 1.

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