0273-2289/94/4403—0271$04.00

Fermentation Metabolism and Kinetics in the Production of Organic Acids by *Propionibacterium acidipropionici*

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Received March 6, 1993; Accepted November 1, 1993

ABSTRACT

The genus *Propionibacterium acidipropionici* was grown under pH-controlled batch fermentation conditions for the production of acetic and propionic acids using 19.1 g/L glucose as a carbon source. The optimum pH range was found to be between 5.5 and 6.5. Bacterial metabolism and fermentation pathways were altered at pH values outside this range. Lactic acid was produced as a key intermediate, with the final acetic and propionic acid production entirely dependent on the cell's ability to metabolize the lactic acid. Most of the glucose in the medium was consumed in less than 20 h of fermentation and converted to lactic acid. Batch fermentation at pH 6 showed that lactic acid was completely utilized to produce 8.5 g/L propionic acid and 5.7 g/L acetic acid. However, the bacteria were unable to metabolize lactic acid at pH 7, resulting in 0.7 g/L propionic acid and 7.0 g/L acetic acid in the fermenter. A kinetic study of batch fermentation at pH 6 showed two distinct growth phases during the fermentation. Most of the cell growth was achieved in the exponential growth stage when glucose was consumed as a main substrate. A nonexponential growth stage was observed when lactic acid was utilized as a carbon source, producing propionic and acetic acids as secondary metabolites.

Index Entries: *Propionibacterium acidipropionici*; glucose; fermentation metabolism; kinetics; acetic, propionic, and lactic acids.

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Nomenclature: P, concentration of primary product (g/L); S, substrate concentration (g/L); X, cell concentration (g/L); $Y_{p/s}$, yield coefficient of products (g product/g substrate); $Y_{x/s}$, yield coefficient of cells (g cell/g substrate); m, maintenance coefficient (g substrate/g cell/h); q_p, specific production rate (g product/g cell/h); q_s, specific substrate utilization rate (g substrate/g cell/h); t, fermentation time (h); β, proportionality constant (g product/g cell/h); μ, specific growth rate (h^{-1}).

INTRODUCTION

Anaerobic bacterial fermentations with high theoretical substrate-to-product conversion yields for the production of organic acids are of great importance to industry. Culture selection is essential, considering the variety of metabolic pathways leading to the end product and the corresponding overall yield in fermentation (*1*). Propionic acid bacteria have been utilized in some industrial processes for production of vitamin B_{12} and some enzymes (*2–3*). Advantages of this bacteria include: (1) decreased contamination owing to the bacteriostatic and fungistatic properties of propionate, and (2) low energy requirements since the fermentation is run predominantly under anoxic conditions for cell growth (*2*).

Theoretically, *Propionibacterium acidipropionici* produces 2 mol of propionic acid and 1 mol of acetic acid (2.47:1 mass ratio) if 1.5 mol of glucose is utilized (*4–5*). However, wide variations in the ratios of organic acid products are observed in glucose fermentations, with propionic-to-acetic acid mass ratios of 1.5–1.8. The yields of propionic acid vary from 0.44–0.61 g propionic acid/g glucose (*6*). In studying propionic acid production with *P. acidipropionici* and *P. freudenreichii*, *P. acidipropionici* produced nearly twice as much propionic acid as did *P. freudenreichii* under the same conditions (*5*). Overall yields of 0.48–0.5 g propionic acid and 0.13–0.17 g acetic acid/g glucose were observed for *P. acidipropionici* in batch fermentation when the pH was not controlled (*2,5*).

Propionic acid bacteria produce an exceptionally high yield of cells from glucose under anoxic conditions (*7–8*). This may be due to the fact that they utilize inorganic pyrophosphate as well as adenosine-5-triphosphate (ATP) (*9–10*). In addition, they may use inorganic polyphosphates (*11*) and may produce high-energy phosphate compounds during the conversion of fumarate to succinate (*8–9,12*).

The generalized pathways from glucose to the major fermentation products formed are shown in Fig. 1. Formation of propionic acid is usually accompanied by the formation of acetic acid. This occurs for stoichiometric reasons and for maintaining hydrogen and redox balance (*13*). In addition, product ratios are controlled for thermodynamic reasons, such as

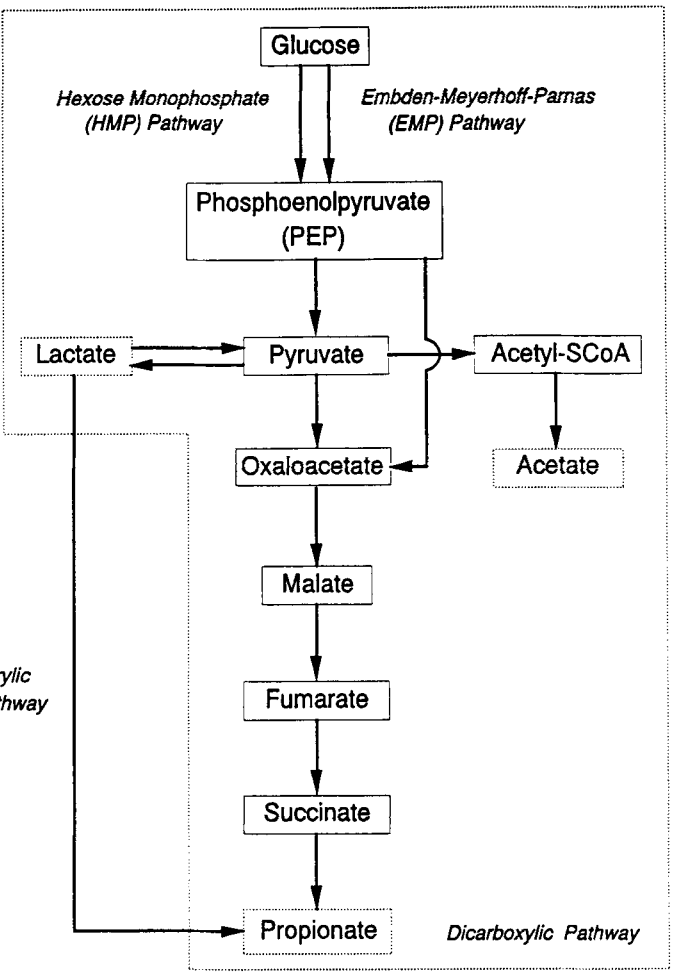

Fig. 1. Biochemical pathways of glucose fermentation by *Propionibacterium* species.

the management of ATP production and entropy generation (*14*). The dicarboxylic acid pathway is the most common for the formation of propionic acid. A preferred substrate for propionic acid-forming bacteria is lactic acid, so that these organisms can grow with the major end product of the lactic acid fermentation. In the dicarboxylic acid pathway, lactic acid is converted to propionic acid via pyruvate and succinate. Lactic acid is also reduced stepwise to propionic acid in the acrylic acid pathway. The route taken depends on the bacterial species (*13,15*). The acrylic pathway is known to occur only in a few species of bacteria such as *Clostridium propionicum, Megasphera elsdenii* (*15*), and *Bacteroides ruminicola* (*16*).

Propionibacteria possess most or some of the enzymes of the hexose monophosphate (HMP) pathway in the conversion of glucose to pyruvate (*6*), and are thus expected to catabolize glucose by a combination of the

Embden-Meyerhof-Parnas (EMP) and HMP pathways. However, the degree of involvement of each pathway is not known (*17*). Pyruvate formation from glucose through EMP pathway can be represented as follows (*18–19*):

$$\text{glucose} \rightarrow 2\,\text{pyruvate} + 2\,NADH_2 + 2\,\text{ATP} \quad (1)$$

When the HMP pathway is employed, the reaction can be represented as follows (*18*):

$$3\,\text{glucose} \rightarrow 5\,\text{pyruvate} + 3\,CO_2 + 11\,NADH_2 + 5\,\text{ATP} \quad (2)$$

The resulting equation for the dicarboxylic acid pathway when the EMP path is considered is:

$$3\,\text{glucose} \rightarrow 6\,\text{pyruvate} \rightarrow 4\,\text{propionic acid} + 2\,\text{acetic acid} + 2\,CO_2 + 2\,H_20 \quad (3)$$

The equation for the same pathway via HMP path is:

$$3.6\,\text{glucose} \rightarrow 6\,\text{pyruvate} \rightarrow 4\,\text{propionic acid} + 2\,\text{acetic acid} + 2\,CO_2 + 2\,H_2O \quad (4)$$

Therefore, the validity of the fermentation equation for *Propionibacterium* depends on the extent of involvement of the HMP pathway. When the EMP pathway is involved, more acid production can be expected with the same amount of glucose. However, the HMP pathway can liberate a larger amount of energy because of high $NADH_2$ production, which can be used to generate ATP. This ATP production is related to bacterial cell yield.

Theoretical maximum yields from sugar are 54.8% (w/w) as propionic acid and 77% (w/w) as total acid (*13*). The HMP pathway involvement seems to be quite variable even among different fermentations using the same strain. Glucose fermentations by *P. arabinosum*, *P. pentosaseum*, and *P. shermanii* have indicated that the HMP pathway is responsible for 24–60% of the glucose catabolism (*17*).

MATERIALS AND METHODS

Microorganism

The microorganism employed in the study was *Propionibacterium acidipropionici* (ATCC 4875). A freeze-dried culture stored in a double-vial was obtained from American Type Culture Collection (ATCC), Rockville, MD.

Media

Trypticase (25.0 g), yeast extract (7.0 g), KH_2PO_4 (0.5 g), and K_2HPO_4 (0.5 g) were dissolved in 1 L of distilled water under vigorous stirring and boiled for 20 min. The solution was placed in the ice bath while CO_2 was

bubbled through the medium until the pH of the solution reached approximately 6.5. Glucose (20.0 g), Na_2CO_3 (4.0 g), and cysteine HCl (0.5 g) were added to the solution and mixed well. Carbon dioxide bubbling was continued until the pH of the solution decreased to around 7.5. The medium was adjusted to pH 7 with 5 N HCl and autoclaved at 121°C for 20 min. The final concentration of glucose prior to fermentation was determined to be 19.1 g/L.

Inoculation and Incubation

The freeze-dried bacteria were rehydrated by aseptically adding a few drops of sterilized fresh medium and inoculated to the liquid medium in a roll tube. A VPI (Virginia Polytechnic Institute) Anaerobic Culture System (Bellco, Vineland, NJ) was used to supply CO_2 gas in the tube. The cultures were transferred twice as described below before being used as an inoculum.

Oxygen-free medium was placed in the roll tubes (about 10 mL each) under a CO_2 environment. The tubes were autoclaved at 121°C for 15 min and placed in the VPI Anaerobic Culture System while CO_2 gas was supplied through the tube. The first inoculation was performed by transferring 2 drops of rehydrated bacteria to the roll tube with a pasteur pipet. The cultures were incubated in a shaker incubator at 30°C for 48 h. The second inoculation was done by transferring the culture from the first inoculation using the same method and condition described in the first inoculation. The bacteria were allowed to grow for another 48 h in a shaker incubator. The tubes were centrifuged at 2000 rpm for 5 min using a TJ-6R Tabletop centrifuge (Beckman, Palo Alto, CA) and the supernatant was discarded. About 4 mL of sterilized fresh medium was then added to the aggregated bacteria and mixed well. The cultures were kept in a refrigerator at 4°C for short-term storage. The starting inocula were replaced by fresh ones every 3 wk. For long-term storage of cultures, a 20% (w/w) glycerol stock solution was prepared. About 0.5 mL of the glycerol stock solutions was placed in 2-mL vials and autoclaved at 121°C for 15 min. An equal volume of the bacterial cultures in fresh medium was aseptically transferred to the vials and stored in the freezer at −80°C. The final cultures contained 10% (w/w) glycerol. The bacterial growth was not reduced after 1 y of storage under these conditions.

Fermentation Equipment and Procedures

The optimum composition of the medium was determined in 10-mL roll tubes in the shaker incubator at 37°C. The initial pH was set at 7.5 and the pH was not controlled during fermentation. A 500-mL Erlenmeyer flask was used for comparative study on pH variations. The flasks were fixed in a shaker water bath and nitrogen gas was supplied through a two-holed rubber stopper. The bath temperature was set at 30°C and 5 N NaOH or HCl was used to control pH. A Virtis Omni-Culture Bench-Top

Fermenter (Virtis, Gardiner, NY) equipped with a 1-L Pyrex glass vessel was used in all other batch experiments. The impeller agitation speed was set at 175 rpm. The fermentation temperature was set at 30°C. Anaerobic grade nitrogen was supplied through a double-stage regulator. Dissolved oxygen (DO) in the medium was detected by a dissolved oxygen indicator module and kept at near zero for all experiments. A pH electrode (Ingold, Wilmington, MA) inserted into the medium was connected to a microprocessor-controlled pH meter (Orion, Boston, MA). The medium pH was adjusted by adding 5 N NaOH or HCl through the feed port.

Inoculation was done by directly transferring an inoculum (about 1 ml) through a feed port. A 10-mL sample was periodically withdrawn from the fermenter. From this sample, about 3 mL was used for analysis of organic acids and residual sugar concentrations, 2 mL was used for cell mass determination, and the rest was stored in the freezer at −15°C.

Analytical Methods

Cell growth was monitored using a Spectronic 710 spectrophotometer (Bausch and Lomb, Rochester, NY). Samples from the fermenter were diluted 1:50 and absorbances were detected at 540 nm. Absorbance values were converted to biomass concentration by using a standard curve based on cell mass dry-weight measurements. Dry cell mass was determined by vacuum-filtering samples through 0.45 μm nitrocellulose filters. The filters were then placed in an oven at 65°C for 72 h before weighing.

Substrate and product concentrations were assayed using high performance liquid chromatography (HPLC). The chromatographic system consisted of a Varian 5000 (Varian, Palo Alto, CA) liquid chromatograph equipped with a loop-injection device (10 μL), a Varian Model 9176 strip chart recorder, and a Varian Vari-Chrom UV-Vis detector. All separations were accomplished by an Aminex Ion-Exclusion Column (HPX-87H, 300 mm × 7.8 mm, Bio-Rad, Richmond, CA) operated at 45°C.

Three milliliters of the fermentation medium was centrifuged for 10 min at 4000 rpm using a TJ-6R Tabletop centrifuge equipped with a TJ-R Beckman refrigeration unit to control the temperature at 9°C. One milliliter of supernatant was diluted in 3 mL distilled water and filtered through a 0.5-μm Fluoropore filter (Millipore, Bedford, MA). Components were eluted with 0.02*N* aqueous sulfuric acid at a flow rate of 0.7 mL/min. The mobile phase was degassed at 45°C overnight under a vacuum of 30 torr. Detection of glucose and organic acids was accomplished by UV absorption at 193 nm. Organic acids separated in this study were acetic, propionic, lactic, formic, succinic, and pyruvic acids. Standard curves were obtained from solutions of known concentrations of glucose and organic acids. The curves were calibrated each time the HPLC was restarted.

Table 1
Factor Levels for Media Optimization

Factors	Compositions
Glucose, %	0.0, 1.0, 2.0, 3.0
Trypticase peptone, %	1.0, 1.5, 2.0, 2.5, 3.0
Yeast extract, %	0.25, 0.5, 0.7, 1.0

RESULTS AND DISCUSSION

Determination of Optimum Medium Composition

The medium composition for *Propionibacterium acidipropionici* was evaluated by a sequential examination of the factors listed in Table 1. The composition remained the same as the one described in Materials and Methods, except for the component examined. Organic acids production increased almost linearly as glucose concentration in the medium increased from 1–3%. However, maximum acid yields occurred at a glucose concentration of 2% (data not given). The tube without glucose did not show any evidence of bacterial growth. The color of the medium remained clear light yellow and no turbidity formed at any time. Cell growth conditions were mainly affected by trypticase even though no measurable change in acid production was detected within the range tested. Cell lysis started occurring after 2 d of fermentation with 1% trypticase and after 4 d with 2% trypticase. The cells remained in a healthy condition for more than 1 wk with 2.5% trypticase. The effect of yeast extract on cell growth was significant at concentrations below 0.5% and began to level off above 0.5%. Maximum cell growth was achieved with 0.7% yeast extract (data not given).

Determination of Optimum pH

Optimum ranges of pH were determined by measuring final concentrations of the organic acids and maximum cell mass. The bacteria in Erlenmeyer flasks (500 mL) containing about 300 mL of medium were grown separately in a shaker water bath with pH ranges from 5.0–7.0 at each 0.5 intervals. The fermentation period depended on the pH of the medium and took longer at lower pH. Most fermentations leveled off within 1 wk, with the exception of those at pH 5.0, which took about 9 d. Figure 2 shows the final concentrations of organic acids and maximum cell mass achieved during the fermentation. Cell mass was decreased

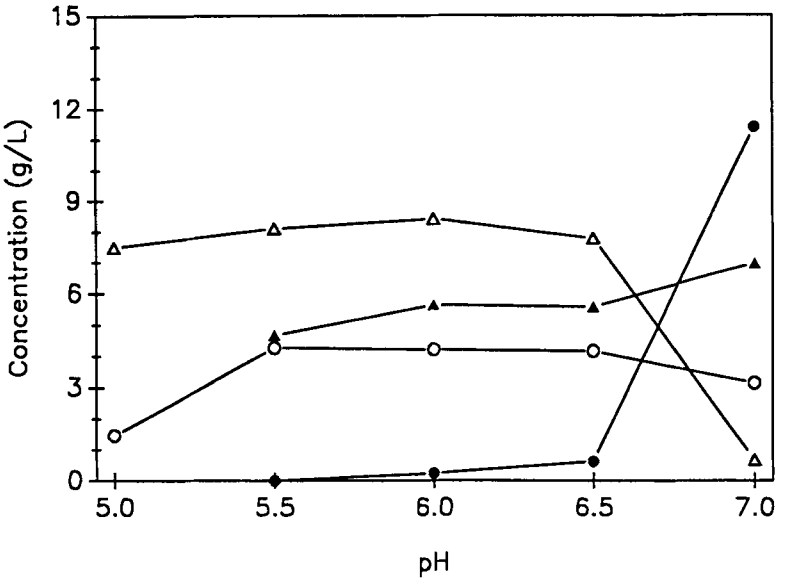

Fig. 2. Organic acids and maximum cell mass produced during the pH controlled batch fermentation by *P. acidipropionici* at 30°C using 19.1 g/L glucose as a sugar source. Symbols: △, HPr (propionic acid); ▲, HAc (acetic acid); ●, HLa (lactic acid); and ○, cell mass.

below pH 5.5 and above pH 6.5. At pH 5.5, the cell mass was highest at 4.27 g/L. Acetic acid production slowly increased as the pH increased from 5.5 to 7.0, with the maximum concentration of 6.96 g/L at pH 7.0. The concentration of lactic acid increased sharply while that of propionic acid dropped abruptly between pH 6.5 and 7.0. This phenomenon indicates that the bacterial metabolism is blocked by some factors at pH levels above 6.5. The propionic acid production remained steady between pH 5.5 and 6.5 with a concentration of 8.4 g/L at pH 6.0. The optimum pH range for the bacteria is, therefore, determined to be between 5.5 and 6.5.

Batch Fermentation with Controlled pH

Figure 3 shows the organic acid and cell mass production during batch fermentation when the pH was set at 6.0. Most of the glucose (19.1 g/L) added to the medium was utilized in 20 h of fermentation (Fig. 3A). At the same time, the lactic acid concentration increased sharply up to 15.5 g/L and then slowly decreased to 0.25 g/L for the rest of the fermentation period. The bacteria started producing propionic acid when the lactic acid concentration reached its maximum, with propionic acid production continuing until all of the lactic acid in the fermenter had been consumed (Fig. 3B). The maximum propionic acid concentration was found to be 8.5 g/L at 120 h of fermentation. Acetic acid was produced in an almost linear mode from the initial stage of fermentation. The maximum yield was

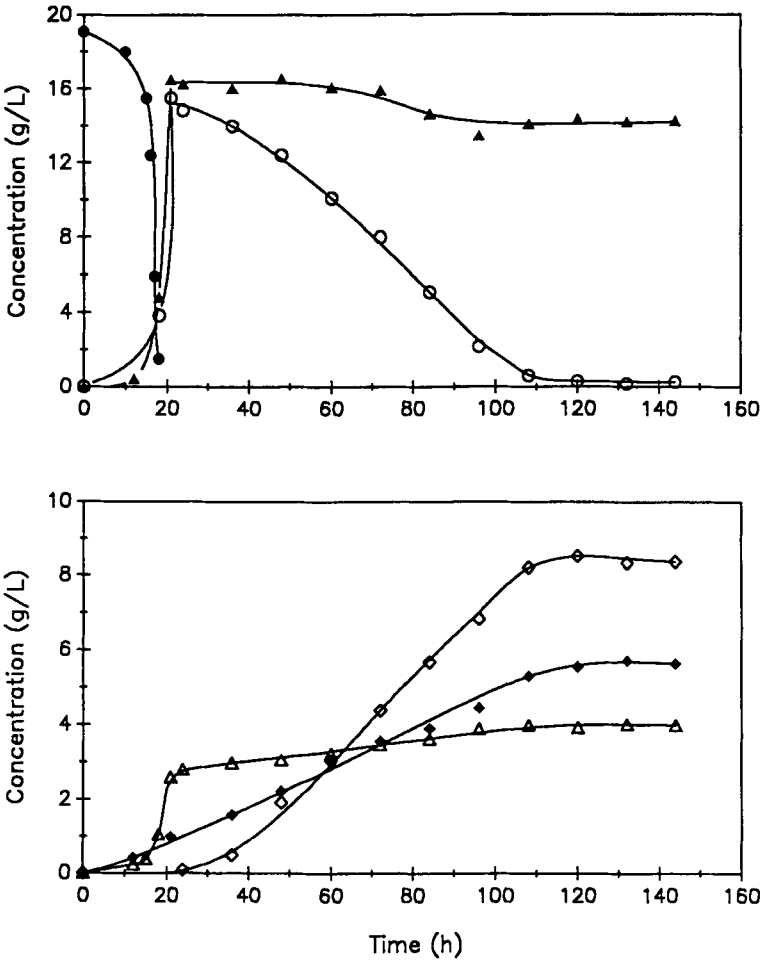

Fig. 3. Organic acids and cell mass production during the batch fermentation of *P. acidipropionici* at 30°C and pH 6 using 19.1 g/L glucose as a sugar source. Symbols: ○, HLa (lactic acid); ●, glucose; ◇, HPr (propionic acid); ◆, HAc (acetic acid); ▲, total organic acids (HPr + HAc + HLa); △, dry cell mass.

attained at about 120 h with a concentration of 5.7 g/L. Cell mass increased exponentially during glucose consumption. However, cell growth was maintained even after all the glucose was consumed. The maximum cell mass attained was 4.0 g/L at 132 h of fermentation. Based on these maximum values, the product and cell yields obtained from the fermentation were 0.45 g propionic acid/g glucose, 0.30 g acetic acid/g glucose, and 0.21 g dry cell/g glucose, respectively.

The fermentation trend at pH 7 was quite different from the one obtained at pH 6 (Fig. 4). Glucose consumption and lactic acid production rates at pH 7 were faster than at pH 6. However, from the lactic acid produced during the initial stage (15.2 g/L), only a small amount (about 3.6 g/L) was consumed by the bacteria in the next 50 h, and the fermentation

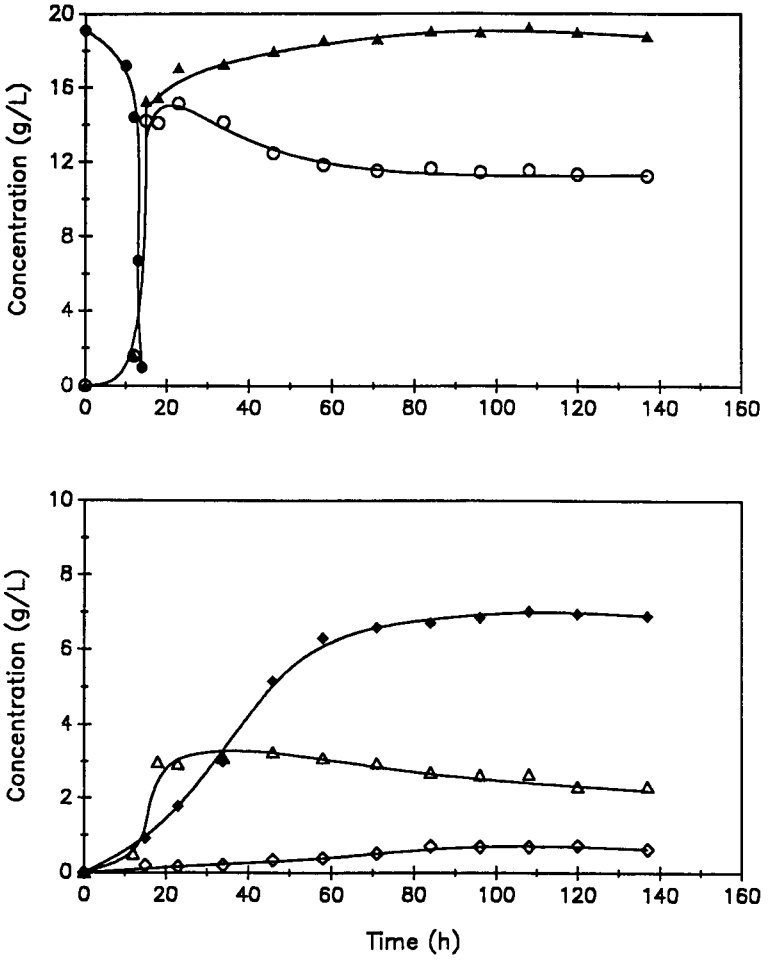

Fig. 4. Organic acids and cell mass production during the batch fermentation of *P. acidipropionici* at 30°C and pH 7 using 19.1 g/L glucose as a sugar source. Symbols: ○, HLa (lactic acid); ●, glucose; ◇, HPr (propionic acid); ◆, HAc (acetic acid); ▲, total organic acids (HPr + HAc + HLa); △, dry cell mass.

then ceased (Fig. 4A). The concentration of lactic acid which remained in the fermenter was 11.3 g/L. A minimal amount of propionic acid was produced during the fermentation (Fig. 4B). Acetic acid was continuously produced up to 70 h of fermentation, then stopped when the lactic acid was no longer utilized by the bacteria. The total acetic and propionic acids produced were 7.0 g/L and 0.7 g/L, respectively. The cell concentration increased sharply in the first 15 h of fermentation, reached its maximum after 50 h with 3.25 g/L, then dropped continuously due to cell lysis. The overall product and cell yields based on these values were 0.37 g acetic acid/g glucose, 0.04 g propionic acid/g glucose, and 0.17 g cell/g glucose. The performance comparisons of the batch fermentations at pH 6 and 7

Table 2
Performance Comparisons in the Controlled Fermentations at pH 6 and 7 Using Glucose as a Sugar Source

	pH 6	pH 7
Initial glucose, g/L	19.1	19.1
Residual lactic acid, g/L	0.25	11.32
Maximum cell mass, g/L	4.0	3.25
Maximum cell yield, g cell/g sugar	0.21	0.17
Final acid production, g/L		
Propionic acid	8.52	0.68
Acetic acid	5.71	6.96
Total product yield, g acid/g sugar (acetic + propionic Acid)	0.75	0.40
Maximum productivity, g product/L/h	0.12	0.11

are summarized in Table 2. The volumetric productivities based on the maximum yield and corresponding time were 0.12 g product/L/h and 0.11 g product/L/h at pH 6 and 7, respectively.

The batch fermentation of *P. acidipropionici* at controlled pH shows that the bacteria consume all of the glucose during the initial stage of fermentation and produce large amounts of lactic acid. Most of the cell growth has been achieved during the glucose consumption. This result indicates that cell growth energy comes mainly from the initial stage (EMP and/or HMP pathway) of the dicarboxylic pathway, which is a common route from glucose to propionic and acetic acids for this bacteria. Fermentation at pH 6 shows that the bacteria are able to produce propionic and acetic acids by utilizing lactic acid produced during the early stage of fermentation. The cells continued to remain in a healthy condition during the rest of the fermentation even though the observed growth rate was small. This result indicates that cell maintenance energy also comes from the pathway from lactic acid to propionic acid. Fermentation at pH 7 shows that the bacteria are unable to metabolize lactic acid. The propionic acid pathway during glucose metabolism is strongly inhibited by highly ionized acid at this pH. However, the continuous production of acetic acid indicates that the inhibition occurred during the late stage of the propionic acid pathway. The decreasing cell mass due to cell lysis in the latter stage of fermentation also indicates that maintenance energy can not be supplied by this pathway.

The fermentation study of *C. thermoaceticum* by Wang and Wang (*20*) indicated that undissociated acetic acid is responsible for growth inhibition at low pH, and ionized acetate ion is responsible for the inhibition at high pH. They also reported that the undissociated acetic acid was much more

inhibitory than the ionized form. Low cell mass production at pH 5 indicates that this phenomenon may also be true for the fermentation by *P. acidipropionici*. Minimal production of propionic acid at pH 7 suggests that the dissociated form of organic acids may be responsible for end product inhibition. Exact mechanisms for such effects should be further studied.

Theoretically, 4 mol of propionic acid and 2 mol of acetic acid are produced by using 3 mol of glucose in the dicarboxylic pathway when EMP pathway is considered by glucose catabolism to pyruvate. However, this theoretical ratio has rarely been observed before, owing to the complex pathway involved in the formation of propionic acid. From our fermentation data obtained at pH 6, the stoichiometric equation can be written as follows:

$$3\ C_6H_{12}O_6 \xrightarrow{\text{Medium}} \text{Cell} + 3.25\ CH_3CH_2COOH + 2.69\ CH_3COOH \quad (5)$$

The differences of coefficients compared to the theoretical value can be explained by many factors involved in the actual situation. The pH variations strongly affect the final acid production by influencing bacterial metabolism. The involvement of HMP pathway may also affect the overall yields. Papoutsakis and Meyer (*17*) reported that the degree of involvement of HMP pathway was up to 60% in some *P. arabinosum* and *P. pentosaceum* metabolisms.

Kinetics of Batch Fermentation

The rates of cell growth and product formation at the optimum pH were calculated for batch fermentation at pH 6 (Fig. 3). A sigmoidal shape of biomass accumulation shows two distinct growth phases. An exponential growth achieved in the initial stage was stopped when all the glucose in the fermenter was consumed. However, a slow growth (non-exponential growth phase) was maintained until the lactic acid in the fermenter was exhausted. Propionic acid production was initiated when the bacteria started utilizing lactic acid as a substrate, even though acetic acid was produced in a linear mode from the initial stage of fermentation. The production of both acetic and propionic acid continued until all of the lactic acid was consumed.

The rates of cell growth and acid production were calculated by a differential method. Shown in Fig. 5 are specific rates of cell growth, the sum of acetic and propionic acid production, and the utilization of lactic acid as a substrate. The specific growth rate increased quickly to a maximum, $\mu = .373\ h^{-1}$, with an average $\mu = 0.332\ h^{-1}$ in the glucose consumption stage. With an average $\mu = 0.00387\ h^{-1}$, the specific growth rate was very low during lactic acid consumption. In the case of mixed cultures of *L. plantarum* and *P. shermanii*, Lee et al. (*21*) observed that the growth of

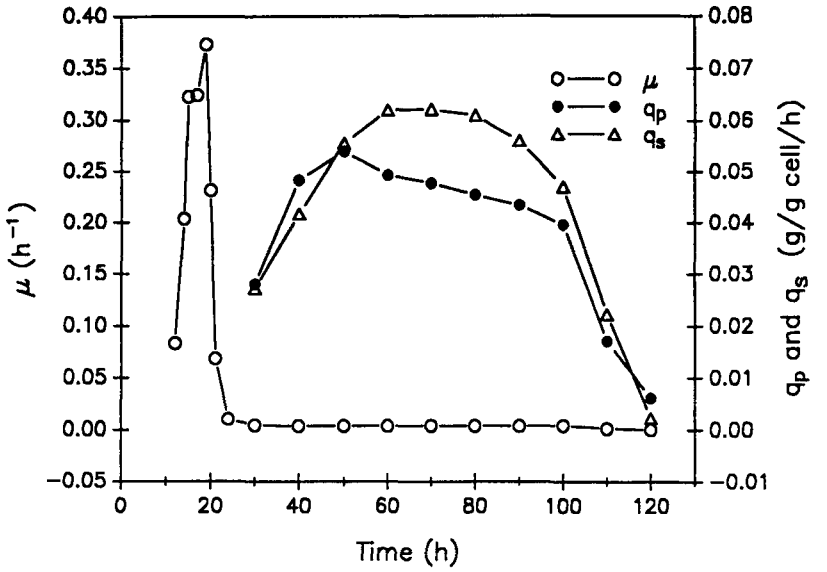

Fig. 5. Specific rates of cell growth (μ), ○; production for sum of propionic and acetic acid (q_p), ●; and lactic acid consumption (q_s), △, during the batch fermentation at pH 6.

P. shermanii ceased temporarily near the time of glucose exhaustion, with the same growth rate resumed in the presence of lactic acid. The low growth rate in the latter stage of fermentation indicates that the inhibitory effect of acetic and propionic acids is strong even with adequate amounts of substrate (lactic acid). The specific rate of product formation (q_p) started increasing after most of the cell growth was achieved. The product formation rate remained steady with a slightly decreasing mode for most of the time and decreased as specific rate of substrate consumption (q_s) decreased. This delayed product formation clearly indicates that the bacteria produce acids as secondary metabolites. The specific production rate can be approximately related to the nongrowth associated model,

$$q_p = (1 / X)(dP / dt) = \beta \tag{6}$$

During the period of linear production rate (from about 35–100 h), the experimental data were fitted to the equation (6) to give an estimated $\beta = 0.0468$.

In the latter stage of fermentation (nonexponential growth stage), the bacteria used lactic acid as the main carbon source. As shown in the fermentation at pH 6 (Fig. 3), the lactic acid consumption was closely related to the product formation. For the period when the rates of substrate utilization coincided with the rates of product formation and cell growth (from about 60–90 h of fermentation), the specific substrate consumption rate can be described using equation (7).

Table 3
The Parameters from the Kinetic Study
of the Nonexponential Growth Stage of the Batch Fermentation at pH 6

Parameter	Value
Average specific growth rate, μ	
Exponential stage	0.332, h^{-1}
Nonexponential stage	0.00387, h^{-1}
Proportionality constant, β	0.0468, g product/g cell/h
Coefficients for specific consumption rate, q_s	
Cell coefficient, $Y_{x/s}$	0.703, g cell/g lactate
Product coefficient, $Y_{p/s}$	0.878, g product/g lactate
Maintenance coefficient, m	0.003, g lactate/g cell/h

$$q_s = (1 / X)(dS / dt) = \mu / Y_{x/s} + m + q_p / Y_{p/s} \quad (7)$$

This equation was evaluated from the experimental data by multivariable regression, and the coefficients were determined to be $Y_{x/s} = 0.703$, $Y_{p/s} = 0.878$, and $m = 0.003$. In the fermentation period between 30 and 60 h, the equation may not be applicable due to slow metabolism for the production of propionic acid in the cell. The specific production rate is higher than the specific consumption rate in this period, indicating that part of organic acids are produced from glucose catabolism without involving lactic acid pathway. In the fermentation period between 100 and 120 h, cell lysis starts increasing due to substrate (lactic acid) limitation. Therefore, the terms in equation (7) cannot properly represent the situation in this fermentation period. The various parameters obtained from the nonexponential growth stage of the batch fermentation at pH 6 are summarized in Table 3.

CONCLUSIONS

The batch fermentation at pH 6 shows that *Propionibacterium acidipropionici* produced a high cell yield (0.21 g cell/g glucose) and over 75% substrate-to-product conversion yields. Two distinct growth phases are observed during the entire fermentation. In the first exponential growth stage, cell growth energy mainly comes from the initial stage of glucose catabolism. Lactic acid is slowly converted to acetic and propionic acids growth phase is maintained in the second nonexponential growth stage where cell maintenance energy comes from the latter stage of glucose catabolism. Lactic acid is slowly converted to acetic and propionic acid during this stage. However, with an optimum pH range between 5.5 and 6.5, the bacteria are quite sensitive to pH changes during fermentation.

The bacterial metabolism and fermentation pathway are altered at pH values outside this optimum range. Low cell mass production at pH 5 indicates that undissociated forms of organic acids are responsible for cell growth inhibition. In batch fermentation at pH 7, only a minimal amount of propionic acid is produced while acetic acid is produced at the normal pace, suggesting that the inhibitory effect occurs at the late stage of the propionic acid pathway. This end-product inhibition is probably caused by the dissociated form of the organic acids. However, exact mechanisms of such effects need to be studied further.

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