

Diauxic Growth of *Propionibacterium shermanii*

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Propionibacterium shermanii has been anaerobically propagated in batch and continuous culture with glucose and/or lactate as energy source. Specific growth rate on lactate was observed to be the same as that on glucose. In terms of cell density, the yield on glucose is higher than the yield on lactate. But the molar ratio of yield on glucose to that on lactate, 8.35, is in good agreement with the theoretical value of 8. In a mixture of glucose and lactate, *P. shermanii* showed diauxic growth. It used lactate before glucose utilization began. Neither temporary growth cessation nor two distinct growth phases were observed. A mathematical model is proposed to describe the diauxic growth.

The gram-positive, nonmotile, catalase positive, non-sporeforming, facultative anaerobic bacteria of the genus *Propionibacterium* were first isolated from Swiss (Emmentaler) cheese. Orla-Jensen (9) studied the formation of "eyes" in Swiss cheese. Later extensive studies, especially in relation to cheese manufacture, were carried out by several investigators (5, 12, 13).

On the basis of morphological, cultural, and biochemical differences, van Niel (Ph.D. thesis, Technische Hoogeschool, Delft, Netherlands, 1928) listed eight main species; eleven species are presently recognized. Their complete characterizations are as described (2).

These microorganisms play a very important role in several industrial processes. The fermentation of lactic acid to propionic acid and carbon dioxide results in the characteristic sharp flavor and the unique "eyes" of Swiss cheese; these microbes play an important role in the ripening of Swiss cheese. In addition, since they synthesize relatively large amount of vitamin B₁₂, they can be utilized for commercial production of this vitamin.

The nutritional requirements of the propionic acid bacteria are complex; they usually grow rather slowly. The nutritional requirements and metabolism of propionic acid bacteria have been extensively studied by several workers and they have been comprehensively reviewed by Hettinga and Reinbold (6). However, little quantitative information on growth dynamics are available. An attempt is made in this paper to fill this void.

MATERIALS AND METHODS

Organism. All experiments were made with *Propionibacterium shermanii*, kindly given to us by the

Department of Food Science and Nutrition, University of Minnesota, St. Paul. It was maintained as stab culture in tryptone-glucose-yeast extract agar.

Medium. The culture medium used for the growth experiments was of the composition shown in Table 1. It was similar to the microbial vitamin assay medium for *Lactobacillus casei* reported by Roberts and Snell (11). The medium was adjusted to pH 6.5 with NaOH and filtered through membrane filters (Millipore Filter Corp., type RA 1.2- μ m pore size); it was then autoclaved for 30 min. Glucose was separately autoclaved and added aseptically.

Growth conditions. All batch and continuous culture experiments were carried out in 50-ml or 100-ml chemostats similar to the original design of Novick and Szilard (8). To provide anaerobic condition and mixing, 2% CO₂ and 98% N₂ gas was sparged into chemostat. Nutrient supply for the continuous culture was regulated by a capillary feed system (10); use was made of a length (4 feet [121.96 cm]) of 21-gauge stainless-steel hypodermic tube. Temperature was kept at 27 C with a water jacket. The nutrient carboy, the capillary feed, and the chemostat were kept at 25 C.

Analytical methods. (i) **Microbial count.** Bacterial counts were taken with a model Z_B Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.) with a 30- μ m aperture tube. Samples were diluted in saline solution (0.6% NaCl; 0.02% disodium ethylenediaminetetraacetate · 2H₂O filtered through a 0.2 μ m membrane filter).

(ii) **Chemical analysis.** Glucose determination was carried out by the glucostat method (Worthington Biochemicals).

RESULTS

Batch culture. Figure 1 shows a batch growth curve of *P. shermanii* grown on glucose as the carbon source. The culture had an initial pH of 6.5 and an initial glucose concentration of 0.41 g/liter.

TABLE 1. Composition of medium

Medium	Amount
Vitamin-free casein hydrolyzate	10 g/liter
Sodium acetate	1 g/liter
KH ₂ PO ₄	0.5 g/liter
K ₂ HPO ₄	0.5 g/liter
L-Cysteine	0.5 g/liter
DL-Tryptophane	80 mg/liter
Adenine sulfate	40 mg/liter
Guanine hydrochloride	10 mg/liter
Uracil	10 mg/liter
Thiamine	10 mg/liter
Ca-pantothenate	0.5 mg/liter
Nicotinamide	0.5 mg/liter
Riboflavin	0.5 mg/liter
p-Aminobenzoic acid	0.1 mg/liter
Biotin	4 mg/liter
MgSO ₄ ·7H ₂ O	100 mg/liter
NaCl	10 mg/liter
MnSO ₄ ·4H ₂ O	10 mg/liter
Fe ethylenediaminetetraacetate	0.002 M

where *B* and *S* are concentrations of bacteria and substrate, respectively.

The constants forming the basis of Monod model, maximal specific growth rate (μ_m), apparent Michaelis constant or saturation constant (K_s), and stoichiometric coefficient for glucose consumption (a_s) can be determined from the batch growth curve. The slope of the exponential phase, μ_m , was 0.141 per h. This corresponds to a doubling time of 4.9 h.

If batch yield is defined as

$$Y_{BAT} = (B_{final} - B_{initial}) / (S_{initial} + S_{final}) \quad (4)$$

and in the Monod model as

$$Y_{BAT} = 1/a_s \quad (5)$$

then

$$Y_{BAT} = 3.03 \times 10^{12} \text{ (cells per g of glucose).}$$

A value of K_s , the Michaelis constant, was calculated by the Megee iterative method (C. B. van Niel, Ph.D. thesis, Univ. of Minnesota, Minneapolis, 1971) to be 1.02×10^{-2} g/liter.

Figure 2 is the same batch growth curve of *P. shermanii* on L-lactate as carbon source. Comparison of batch growth on glucose and L-lactate is summarized in Table 2. The solid lines in Fig. 1 and 2 are predictions of the Monod model using the growth parameters listed on Table 2.

The phase-plane relationship between cell

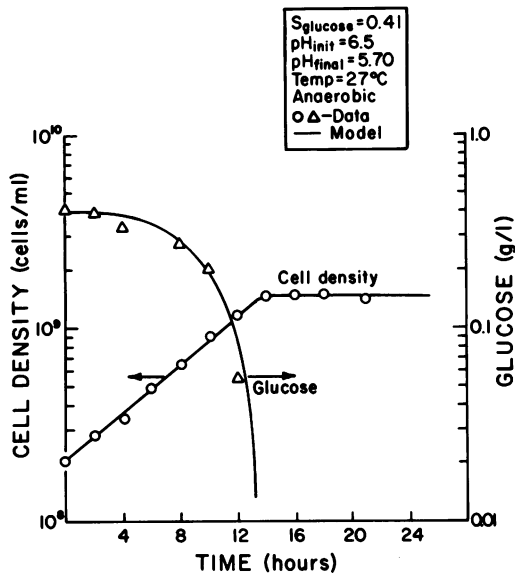
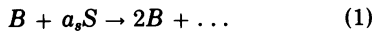


FIG. 1. Batch growth curve of *P. shermanii* on glucose.

The growth of *P. shermanii* may be represented by the Monod model as:



The equations for batch growth are:

$$dB/dt = \mu_m BS / (K_s + S) \quad (2)$$

and,

$$dS/dt = -a_s \mu_m BS / (K_s + S) \quad (3)$$

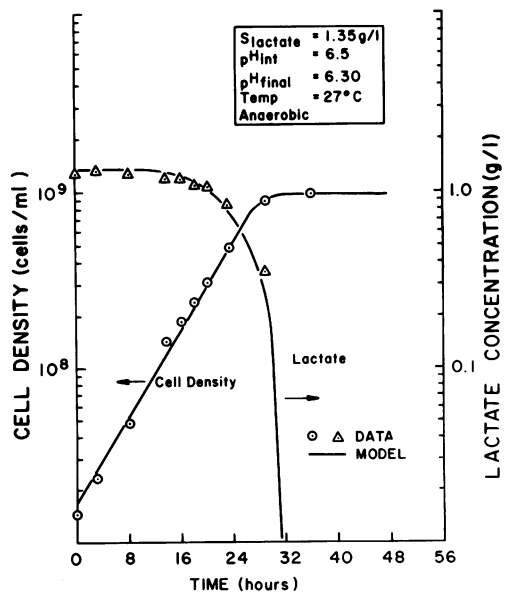


FIG. 2. Batch growth curve of *P. shermanii* on lactate.

density and glucose concentration was obtained by substituting equation 2 into equation 3;

$$dB = -a_s dS. \quad (6)$$

The solution to the above equation is

$$B = B_o + 1/a_s (S_o - S) \quad (7)$$

where subscript *o* denotes initial values. Figures 3 and 4 show the phase-plane relation of bacterial cell density and glucose, or lactic acid concentration, respectively. The solid lines in figures were predicted by the Monod model.

Continuous culture. The steady-state concentrations of cell density of *P. shermanii* and glucose are plotted as a function of holding time (the reciprocal of the dilution rate) on Fig. 5. The solid lines are predictions of the Monod model using the same growth parameters listed on Table 1 except for the Michaelis constant. The Michaelis constant obtained from batch experiments was too small to fit the continuous culture data. It was reevaluated from $K_s = 1.02 \times 10^{-2}$ g/liter by the least squares method to give $K_s = 1.40 \times 10^{-1}$ g/liter.

Diauxic growth. In general, if two or more substances, each capable of serving as energy source, are present in the medium, growth may occur on the one substrate preferentially metabolized, or growth may occur on both simultaneously. In the case of the former, it was sometimes almost completely used before the utilization of the second substrate was initiated. There may be a temporary growth cessation before growth is resumed on the other substrate. This sequential substrate utilization as shown by a distinct growth phase with temporary growth cessation in the batch curve was referred to as diauxic growth by Monod (7).

TABLE 2. Batch growth of *P. shermanii* on glucose and L-lactate

Determinants	Carbon source	
	Glucose	L-Lactate
Maximal growth rate (μ_m)	0.141 (per h)	0.142 (per h)
Stoichiometric coefficient (a_s)	3.28×10^{-13} (g/cells)	1.37×10^{-12} (g/cells)
Yield (Y_{bat})	3.03×10^{12} (cells per g of glucose)	7.27×10^{11} (cells per g of lactate)
Michaelis, constant (K_s)	1.05×10^{-2} (g/liter)	3.41×10^{-2} (g/liter)
Final pH ^a	5.45	6.30

^a Based on the initial pH of 6.5 and the initial substrate concentration of 1.0 g/liter.

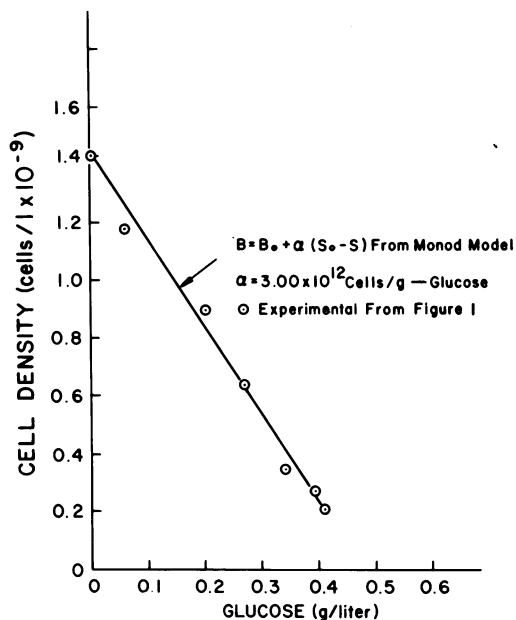


FIG. 3. Phase plane plot of cell density of *P. shermanii* and glucose concentration.

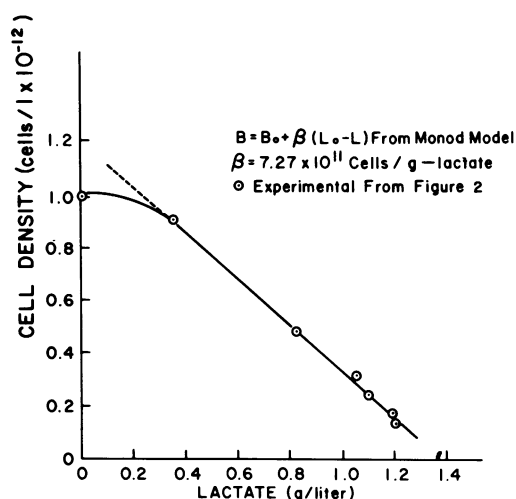


FIG. 4. Phase plane plot of cell density of *P. shermanii* and lactate concentration.

As shown in Fig. 6, when *P. shermanii* grew on a mixture of glucose and lactate as energy sources, diauxic growth could be observed. *P. shermanii* used lactate completely first and then glucose, but neither temporary growth cessation nor two distinct growth phases could be observed. Standing et al. (15) have also noted that it is possible to get diauxic growth without an obvious intermediate lag period. They noted that there is no apparent lag period

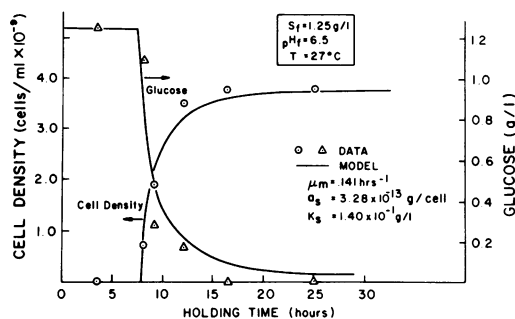


FIG. 5. Steady-state concentrations of *P. shermanii* and glucose in continuous culture versus holding time.

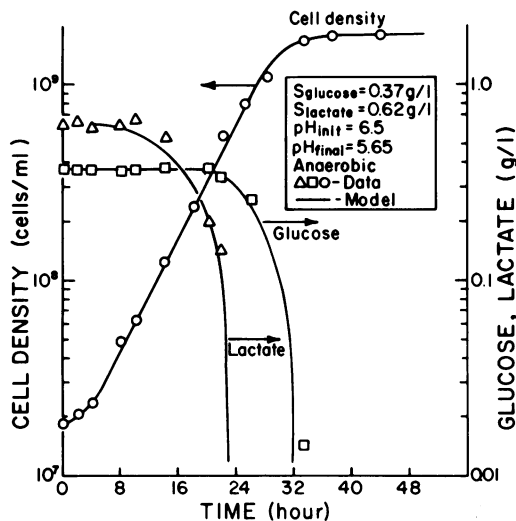
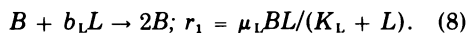


FIG. 6. Diauxic growth of *P. shermanii* on glucose and lactate.

after depletion of glucose, although utilization of galactose is sequential.

It is of interest to note that the specific growth rate on a mixture of glucose and lactate was the same as that on each substrate.

A simple unstructured model for diauxic growth is proposed for growth of *P. shermanii*. For this model, one may have the following reactions, where the Monod equation is assumed for growth rate. (i) Bacteria (*B*) grow on lactate:



(ii) Bacteria (*B*) grow on glucose, but are inhibited by the presence of lactate:



$$r_2 = \mu_2BS/(K_s + S)(K_m + L). \quad (9)$$

Then, the differential equations for batch growth are:

$$dB/dt = \mu_1BL/(K_L + L) + \mu_2BS/(K_s + S)(K_m + L) \quad (10)$$

$$dL/dt = -b_1\mu_1BL/(K_L + L) \quad (11)$$

and

$$dS/dt = -a_s\mu_2BS/(K_s + S)(K_m + L) \quad (12)$$

Using the growth parameters listed on Table 3, the model is numerically integrated. This is shown in solid lines on Fig. 6.

DISCUSSION

The growth of *P. shermanii* in batch and continuous culture can be readily described by the simple Monod model.

It is noteworthy that *P. shermanii* preferentially metabolizes lactate which is, in general, an end product of fermentation for many bacteria.

In nature, *P. shermanii* usually coexists with lactic acid bacteria. However, due to their low growth rate, they cannot survive in competition for the common substrate with lactic acid bacteria. This seems to make them prefer lactate to glucose.

It is also interesting that specific growth rates on lactate and glucose are the same. The mechanism of lactic acid fermentation by propionic acid is not clear. Wood et al. (16) proposed that lactic acid is the intermetabolite from pyruvate to propionate. On the other hand, one can postulate that lactate is converted to pyru-

TABLE 3. Growth parameters of *P. shermanii* for diauxic growth

Determinant	Definition	Growth
μ_m	Maximal growth rate on lactate	0.142 per h
μ/K_m	Maximal growth rate on glucose	0.142 per h
K_L	Michaelis constant for lactate	2.06×10^{-2} g/liter
K_s	Michaelis constant for glucose	1.02×10^{-2} g/liter
K_m	Michaelis constant for inhibition of glucose utilization by lactate	1.0×10^{-3} g/liter
a_s	Stoichiometric coefficient for glucose consumption	3.28×10^{13} cells per g of glucose
b_L	Stoichiometric coefficient for lactate consumption	1.37×10^{12} cells per g of lactate

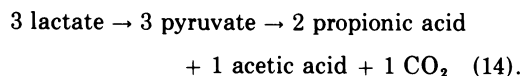
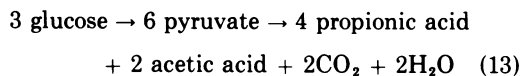
vate and then pyruvate is converted to propionate following the metabolic pathway of glucose (3). Thus, lactate or an intermetabolite may repress the conversion of glucose to pyruvate by inhibiting some enzymes involved in the Embden-Meyerhof pathway. Furthermore, one can postulate that in propionic acid fermentation, the conversion of pyruvate to propionate is the limiting step for growth. The conversion rate of glucose to pyruvate may be much faster and not affect the overall growth rate.

The high yield of glucose is due to more adenosine 5'-triphosphate (ATP) produced in the Embden-Meyerhof pathway. Batch yields of *P. shermanii* on glucose and lactate are:

$$Y_{\text{glucose}} = 3.03 \times 10^{12} \text{ cells per g of glucose} \\ = 5.454 \times 10^{14} \text{ cells per mol of glucose;}$$

$$Y_{\text{lactate}} = 7.27 \times 10^{11} \text{ cells per g of lactate} \\ = 6.543 \times 10^{13} \text{ cells per mol of lactate.}$$

Since the average cell volume of bacteria grown on glucose and lactate, as measured by size distribution curves (14), are the same, the molar ratio of yields is $R = 8.35$. The ideal stoichiometries for the propionic acid bacteria fermentations of glucose and lactate have been suggested by several workers (1, 3, 4, 17) to be



As mentioned above, the evidence indicates that glucose is converted to pyruvate by the Embden-Meyerhof pathway, yielding 2 ATP/mol of glucose. Therefore, a total of 8 ATP are produced by equation 13. However, equation 14, representing the process of extracting energy from lactate, yields only 1 ATP per 3 mol of lactate.

Although de Vries et al. (18) suggested that the ATP yields from glucose and lactate might be twice as high, the theoretical molar ratio of ATP yields is 8.

Experiment is therefore in good agreement with theory based on ideal stoichiometries.

Bauchop and Elsdén (1) reported that the molar yield ratio of *P. pentosaceum* on glucose and DL-lactate is 4.93. If one takes into account the fact that *Propionibacterium* can utilize only L-lactate, it should be greater than the theoretical value, 8.

However, de Vries et al. (18) reported that the molar yield ratios of *P. freudenreichii* on glucose and lactate in complex medium and synthetic medium are 8.04 and 8.02, respectively.

Experimentally, it is concluded that *P. shermanii* preferentially metabolizes lactate to glucose, but the molar yield ratio of glucose to lactate is 8.

ACKNOWLEDGMENTS

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