A Kinetic Study of the Lactic Acid Fermentation. Batch Process at Controlled pH*

Robert Luedeking† and Edgar L. Piret

Department of Chemical Engineering, University of Minnesota, Minneapolis 14, Minnesota

Abstract: Kinetic data are needed to develop basic understanding of fermentation processes and to permit rational design of continuous fermentation processes.

The kinetics of the fermentation of glucose to lactic acid have been studied at six constant pH levels between 4.5 and 6.0 by measuring the instantaneous rates of bacterial growth and of lactic acid formation throughout each fermentation.

It was found that the instantaneous rate of acid formation d*P*/d*t,* should be related to the instantaneous rate of bacterial growth d*N*/d*t,* and to the bacterial density *N,* throughout a fermentation at a given pH, by the expression

$$\frac{dP}{dt} = \alpha \frac{dN}{dt} + \beta N$$

when the constants α and β are determined by the pH of the fermentation.

INTRODUCTION

Largely because of products such as antibiotics, vitamins, feed supplements, and blood plasma expanders, fermentation as an industrial method for making speciality chemicals is attracting wide attention. Consequently, a better understanding of the kinetics of fermentation is becoming increasingly important and, although the advantages and economies of continuous fermentation have been widely reported,[7,10,22,29] instantaneous rate measurements which are needed to predict continuous operation from batch data are not available.[6,7]

Because its rate processes are relatively simple, the lactic acid fermentation, particularly one using a homofermentative thermophilic organism such as *Lactobacillus delbrueckii* is well suited for basic fermentation studies. Inasmuch as a single material (glucose) is converted to a single product (lactic acid) with negligible amounts of intermediate and side products, attention may be focused on three primary processes—rates of nutrient disappearance, product formation, and bacterial growth. Also, sterility demands are not high, pH is easily controlled, and high aeration is not needed. Moreover, there is a need for continued research into the lactic acid fermentation in view of the attractive potential of lactic acid as a versatile intermediate, especially in plastics.[4,25,27]

The data reported here show the changes in nutrient concentration, product concentration, and bacterial density which occur during fermentation at constant temperature and controlled pH levels. Graphical differentiation of these curves provides the instantaneous rate data leading to the kinetic relationships developed. The kinetic equations relate quantitatively the rates of acid production to the rates of bacterial growth and to the bacterial population. The constants of the equation are a function of the pH of the fermentation. The application of these rate data to the design of continuous fermentation processes have been reported.[21]

This study of the batch process represents a continuation of research on the lactic acid fermentation previously conducted in this laboratory,[2,3,16] in which the extreme influence of pH upon yields, degree of completion, and fermentation rate was established for lactic acid fermentations with the pH continuously controlled. The present work differs from the earlier studies in that here it is the *instantaneous* rate of acid formation which is measured and followed throughout the fermentation rather than obtaining an average rate for the whole fermentation[16] or for a part of it.[2,3] Moreover, instantaneous rates of bacterial growth are also determined in the present study, but were not in the earlier work.

Longsworth[17] and Longsworth and MacInnes[18–20] had earlier noted that a *Lactobacillus acidophilus* fermentation proceeded far more rapidly when the pH was controlled than when it was not controlled. They also established methods for measuring instantaneous rates. Gillies[9] measured the instantaneous rate of acid formation during a lactic acid fermentation at a controlled pH of 5.4 and found that the rate was in part a function of the nutrilite (corn-steep liquour) concentration.

EXPERIMENTAL EQUIPMENT AND PROCEDURES

The equipment and methods used by Kempe[16] and Finn[3] have been modified for these studies of instantaneous rates. The experimental apparatus, Figs. 1–3, consisted basically of a fermentor plus its attendant services, including temperature control, pH control, introduction of carbon dioxide, and means for measurement of bacterial growth and acid

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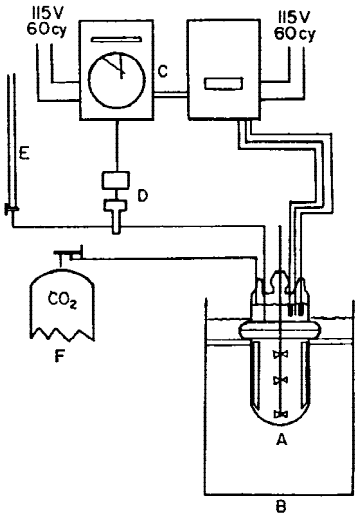

Figure 1. Sketch of equipment for fermentation and pH control.

A. Fermentor with stirrer, baffles, pH electrodes and ground, inlets for carbon dioxide and sodium carbonate solution
B. Constant temperature water batch (45°C)
C. pH meter and pH Recorder–Controller
D. Pump for carbonate solution
E. Measuring reservoir (burette) for carbonate solution
F. Carbon dioxide tank

production. The two-litre fermentor, also designed for continuous fermentation studies, was a two-piece, borosilicate-glass reaction flask fitted with baffles and stirrer. Of the six top openings of standard taper ground glass grouped about

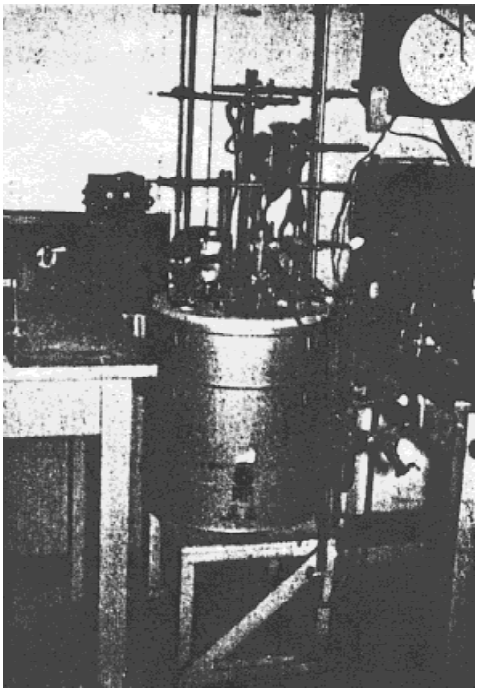

Figure 2. General view of equipment for rate studies in batch fermentation.

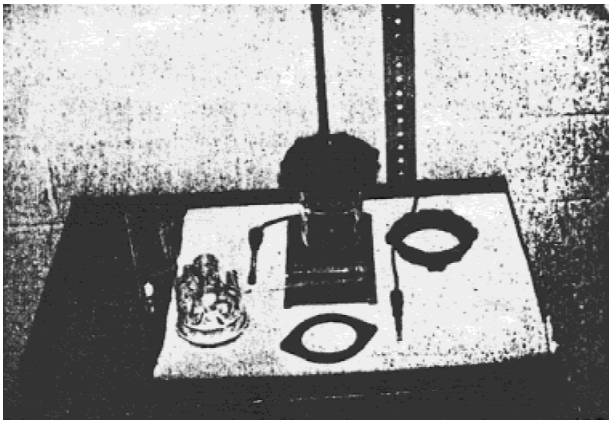

Figure 3. Dismantled fermentor with agitator and pH electrodes.

the central agitator entry of the fermentor, three were used for the pH meter electrodes and ground, and the remaining three were used for sampling, for introduction of alkali, and for introduction of carbon dioxide.

A Beckman Model RX pH meter and Bristol Pyromaster Potentiometer continuously recorded the pH and controlled it to any desired level between pH 3.0 and pH 10.0 by the on–off operation of a small proportioning pump, which injected a 2·5 N solution of sodium carbonate into the fermentation mash as required. Control was within ±0.05 pH units. The equipment was grounded to the soil to prevent erratic operation of the meters.[3,16] The pH electrodes were equipped with standard taper ground glass sleeves to fit into the top openings of the fermentor.

All fermentations were conducted at 45°C using the homofermentative *L. delbrueckii* NRRL–B445 obtained originally from the United States Department of Agriculture for earlier studies.[3,16] The species has been described elsewhere,[1] as have this strain and the culture techniques.[2]

In order to help exclude atmospheric oxygen, the surface of the medium was blanketed with carbon dioxide by slowly bleeding the gas into the reactor during the fermentations.[2,3] Since the fermentor was closed, no evaporation losses occurred.

The basal medium was an aqueous solution containing 5 per cent anhydrous dextrose, 3 per cent dehydrated yeast extract, and added mineral salts.[3] The yeast extract–glucose solution and the fermentor assembly were essentially sterilized by bubbling steam into the medium in the fermentor for two hours while holding it at 100°C. In order to prevent the formation of a troublesome flocculent precipitate, the mineral salts solutions were sterilized separately and added to the cooled medium just before inoculation. The volume was then adjusted with sterile water.

After calibration against buffer solutions, the pH electrodes were sterilized by immersing them in a dilute qua-

ternary ammonium solution and rinsing them twice in sterile water before inserting them into the fermentor.

Prior to inoculation, the optical density of the 18 to 24-h starter culture was measured, and the volume of the inoculum was chosen such that it contained 180 units of optical density (to be defined below). The volume of inoculum was usually between 50 and 80 ml for an initial charge of 1600 ml.

During each fermentation the calibration of the pH recorder controller was checked several times by measuring the pH of samples of the fermentation broth on a second meter. Once the fermentation had started, no special precautions were taken to maintain sterile conditions since the high temperature and low pH of the medium, and the heavy inoculum and rapid rate of fermentation, all tended to discourage the growth of chance contaminants. There was no evidence of contamination in any of the batch fermentations conducted during this study.

MEASUREMENTS

For the rate studies the two processes, bacterial growth and lactic acid synthesis, were closely measured during the entire fermentation.

The lactic acid was determined as it was formed by measuring the addition to the fermentation mash of an alkali solution (2.5) sodium carbonate with 5 g hexametaphosphate added per litre to compensate for depletion of phosphate during the fermentation. The alkali was pumped from a burette and the volume of alkali was noted at frequent intervals, preferably at the instant the pump was activated. Each millilitre of alkali is theoretically equivalent to 0.2252 g of lactic acid, but, because of the low pH levels, it was necessary to correct for the undissociated acid. The validity of measuring the lactic acid formed by measuring the volume of carbonate added, was confirmed by analyzing the final fermentation broth specifically for lactic acid.

Since the growth measuring techniques used in this study are somewhat novel, the reasons for their selection and the actual procedures used are described.

For this study indirect schemes such as measuring carbon dioxide production, nutrient consumption or other results of growth were unsuitable for measuring the bacterial growth rates since they do not necessarily remain proportional to growth. Cumbersome direct weighing schemes were also excluded because they do not permit making the frequent measurements necessary for rate studies.

For measuring bacterial growth, optical density, which measures bacterial density, is preferred to plate counting and direct counting methods which measure cell concentration.[11,13,23] Cell concentration is defined[23] as the number of cells per unit volume of solution, while bacterial density is defined as the dry weight of bacteria per unit volume of solution. Because the average size of the bacterial cells changes during fermentation, bacterial density is more closely related to the quantity of bacterial protoplasm, and therefore, more closely related to enzyme quantity.

Optical density techniques are preferred for measuring bacterial density[11,23] since they are the most convenient, and because the optical density can be expected to remain proportional to the bacterial density during the positive phases of growth cycle.

Since correlations of optical densities with plate counts were erratic, primarily because the plate counts failed to distinguish between single cells and groups or chains of cells, all results are reported in terms of optical densities.

Optical densities for this study were measured on an Industrial Model LCZ Leitz Rouy-Photrometer, using the 610 filter (maximum transmission at 610μ) with a 10 mm × 10 mm absorption cell of 9 ml capacity furnished by the manufacturer. The instrument dial reads in per cent transmission, which may be converted to optical density by the equation

$$D = \log_{10} I_0 - \log_{10} I$$

where D is the optical density, I is the instrument reading of the sample in per cent transmission, and I_0 is the instrument reading for the blank in per cent transmission. The instrument was adjusted to $I_0 = 100$ for a distilled water blank, and the correction was made later for the optical density of the sterile medium.

For convenience, and in order to represent the optical densities as the concentrations which they actually are, bacterial densities are reported as 'units of optical density per millilitre' or U.O.D./ml, which is defined as follows: the bacterial suspension is diluted with distilled water until the dilution reads 75 per cent transmission on the Leitz Rouy-Photrometer (75 per cent transmission is equal to an optical density of 0.125); if r is the number of times which the sample is diluted, the bacterial density of the original sample in U.O.D./ml is given as $N = 0.125r$.

In order that all samples would not have to be diluted to exactly 75 per cent transmission, a chart was prepared which converted a per cent transmission reading of the photrometer directly to an optical density value corrected for experimental deviations from the Beer–Lambert Law. For the *L. delbrueckii* used in the study, one unit of optical density corresponded to between 150,000,000 and 450,000,000 cells as determined by plate count.

Instantaneous rates of growth and of acid formation were determined by graphical differentiation of curves of values computed from the original measurements of alkali addition and of optical density versus time after inoculation. The specific rate of growth and the specific rate of acid formation were determined by dividing the instantaneous rates by N, the bacterial density at that time. Included in the calculations are procedures originally outlined by Longsworth and MacInnes,[19] to correct for the continual dilution of the medium by alkali solution, and for the withdrawal of

samples. Errors from each of these sources, if neglected, can be as high as 15–20 per cent.

For each run a sample of the final fermentation broth was frozen and analyzed later for glucose, using the Hodge and Davis modification of the Somogyi semimicro method for reducing sugars.[15] The Friedemann–Graeser method[5] was used to analyze for lactic acid. Results of the lactic analyses were erratic, however, until two modifications recommended by Smith[28] were incorporated into the analysis: specifically, that the time of the Van Slyke pretreatment was limited to 1 min, and that a 0.01 N solution of potassium permanganate was used as the oxidizing agent in place of colloidal manganese dioxide. With these two modifications, recoveries of lactic acid ran consistently above 96 per cent and often at about 99 per cent for prepared samples.

DISCUSSION

The experimental data and the curves presented here are derived from six fermentations, each of which was continuously controlled at a different pH level (6.0, 5.6, 5.4, 5.2, 4.8 and 4.5). All fermentations were conducted at the same temperature (45°C), with the same organism (*L. delbrueckii* NRRL–B445), and on the same medium (an aqueous solution of 5 per cent glucose fortified with 3 per cent yeast extract and additional mineral salts). As an example, the rate information for the fermentation at pH 6.0 is tabulated in Tables I and II, and the curves for the fermentations at pH 6.0, 5.4 and 4.5 are shown in Figures 4–7.

The two important processes, growth and acid formation, are shown in three different ways in Figs. 4–6. Firstly, both are plotted against fermentation time in Fig. 4 with the bacterial density N, expressed as units of optical density per millilitre and the product or lactic acid concentration P, expressed as milligrams of acid per millilitre of solution. Bacterial density curves are also presented in the semilogarithmic plots of Fig. 5 where growth at the several pH levels is compared. Secondly in Fig. 5 the two rates are plotted against fermentation time. The rate of growth is shown as the rate of change of bacterial density with respect to time (dN/dt), and the rate of acid synthesis is shown as the rate of change of lactic acid concentration with time (dP/dt). These curves were obtained by graphical differentiation of plots similar to Fig. 4. Finally, specific rates, defined as the rates per unit of bacterial quantity, are plotted against time in Fig. 6. Specific growth rate is computed as $(1/N)(dN/dt)$ whose units are reciprocal hours. Specific growth rate is also called the logarithmic growth coefficient k, or the Naperian growth rate.[23,24] The specific rate of acid synthesis is given as $(1/N)(dP/dt)$ and has the units milligrams of acid per hour per unit of optical density.

The growth rates as functions of bacterial density are compared at the six pH levels in Fig. 8, and in Fig. 9 the specific growth rates are similarly compared. Plots such as Figs. 8 and 9 where bacterial density is the parameter are most useful in detecting trends, and are also important in continuous fermentation studies.[21]

Table I. Bacterial density during batch fermentation.*

Time, h	N, U.O.D./ml	Time, h	N, U.O.D./ml
0.10	0.11	8.30	3.10
1.00	0.12	8.80	3.97
1.50	0.14	9.30	4.94
2.00	0.17	9.35	5.02
3.20	0.30	9.55	5.53
3.70	0.39	9.80	5.99
4.20	0.49	9.85	6.10
4.70	0.65	10.10	6.49
4.95	0.73	10.35	6.86
5.20	0.80	10.55	7.05
5.45	0.89	10.80	7.35
5.50	0.90	11.05	7.70
5.70	1.01	11.10	7.73
5.75	1.01	11.30	8.01
5.90	1.11	11.35	8.05
6.00	1.13	11.80	8.39
6.20	1.25	12.10	8.62
6.50	1.37	12.35	8.75
6.75	1.56	12.85	9.03
7.00	1.76	13.00	9.47
7.25	1.93		
7.50	2.22		
7.75	2.48		
8.00	2.79		
8.25	2.95		

*At 45°C, pH controlled at 6.0, on a 5 per cent glucose medium fortified with 3 per cent yeast extract and added mineral salts, *L. delbrueckii.*

Growth as a Function of pH

The curves of Figs. 4 and 7 confirm earlier observations[3,18,19,26,27] that fermentation time is lengthened markedly when the pH of the fermentation is lowered from 6.0 to 4.5. However, the effect of pH on fermentation kinetics is complex and new information is gained from the curves of Figs. 4–9 by studying its effect on some individual aspects of growth.

For example, as the pH at which a fermentation is controlled is lowered from 6.0 to 4.5, the following effects may be observed from the curves: (1) the specific growth rate for the logarithmic growth phase k, i.e., the logarithmic growth constant, decreases markedly, (2) the length of the logarithmic growth phase steadily decreases from 8 h at pH 6.0 to an unmeasurably short time at pH 4.5, (3) furthermore, the logarithmic phase ends at successively lower bacterial density levels, (4) the overall fermentation time is lengthened, (5) the fermentation attains its maximum growth rate ($dN/$

Table II. Growth and lactic acid synthesis during batch fermentation.†

Time, h	N* U.O.D./ml	P mg/ml	dN/dt U.O.D./ml h	dP/dt mg/ml h	k h^{-1}	1 dP/N dt mg/U.O.D.
1.00	0.12		0.04		0.34	
1.50	0.14		0.05			
2.00	0.17		0.06		0.36	
2.50	0.22	0.8	0.09	0.4	0.40	1.8
3.00	0.28		0.13		0.46	
3.50	0.35	1.25	0.16	0.56	0.47	1.60
4.00	0.45	1.6	0.21	0.74	0.478	1.66
4.50	0.57	1.95	0.27	0.92	0.48	1.61
5.00	0.72	2.5	0.35	1.20	0.48	1.66
5.50	0.91	3.2	0.44	1.51	0.48	1.66
6.00	1.15	4.0	0.55	1.81	0.48	1.58
6.50	1.39	5.0	0.67	2.10	0.48	1.51
7.00	1.76	6.1	0.85	2.54	0.48	1.45
7.50	2.21	7.6	1.06	3.48	0.478	1.58
8.00	2.79	9.5	1.33	4.37	0.475	1.57
8.50	3.46	12.0	1.63	5.58	0.470	1.62
9.00	4.31	14.9	1.99	6.53	0.460	1.51
9.50	5.35	18.2	2.09	7.05	0.390	1.32
10.00	6.30	21.6	1.70	7.34	0.270	1.16
10.50	7.00	25.1	1.30	7.19	0.186	1.03
11.00	7.60	28.6	1.06	7.05	0.140	0.93
11.50	8.14	31.8	0.89	6.44	0.109	0.79
12.00	8.51	34.9	0.74	6.24	0.087	0.72
12.50	8.84	37.9	0.62	5.86	0.070	0.66
13.00	9.13	40.7	0.54	5.57	0.059	0.61
13.50	9.40	43.3	0.44	5.00	0.047	0.53
14.00		44.9				

*Interpolated.

†At 45°C, pH controlled at 6.0, on a 5 per cent glucose medium fortified with 3 per cent yeast extract and added mineral salts, *L. delbrueckii*.

dt), successively later in the growth cycle, (6) the greatest bacterial density is attained by fermentation at pH 5.4, and (7) a change in the shapes of the curves for growth rate, for specific growth rate, and for rate of acid synthesis also occurs at pH 5.4.

In addition to the above changes at pH 5.4, it might also be noted here that this same pH 5.4 appears later in this study as a point of inflection for α (Fig. 10), and as the pH for maximum yield for lactic acid.

For batch fermentations there is little practical difference between pH 5.4 and pH 6.0. In both cases the total fermentation times are about the same (Fig. 7), approximately the same level of bacterial density is reached (Fig. 7), and conversion to lactic acid is nearly the same at each pH. From the standpoint of continuous fermentation, however, the differences between the two pH levels are significant. This can be seen from Fig. 8 by noting the difference in the positions of the growth rate curves at these two pH's. To explain briefly, at pH 6.0 a continued fermentation may be conducted at a very high flow rate (which means that the nominal holding time is short) but at a relatively low bacterial density, below 5.0 U.O.D./ml. This is because the maximum growth rate occurs at a lower bacterial density that with pH 5.4. At this lower pH, however, one can operate a continuous fermentation at a higher bacterial density for a given flow rate than would be possible at pH 6.0. The differences are discussed more fully elsewhere.[21]

Relation Between Acid Synthesis and Growth

During the fermentation, lactic acid production appears to parallel the growth of the organisms (Fig. 4). Like the growth rate, the rate of acid production rises to a maximum value in the latter part of each fermentation and then declines sharply from it (Fig. 5). The sharpness and short duration of these maxima, and the shortness of any other periods where the rate is approximately constant, demonstrate that the 'phase of constant rate of acid production' reported earlier[3] is a period of vigorous acid production where the 'constant rate' is in reality the average of rates that vary widely over the entire period.

The specific rates, both of growth and of acid production, are in a sense measures of the metabolic activity of the individual cells. It is to be expected, therefore, that if the lag phases are ignored, the specific rates are high in the early part of the fermentations, and that they decline steadily as the fermentation proceeds due to the disappearance of nutrients and the accumulation of toxic products.[14] The curves of Fig. 6 do follow this pattern.

It is desirable, both for the subsequent studies of continuous fermentation and for a better insight into the batch fermentation, to establish the relationship between the rate of acid synthesis and the rate of growth.

An examination of the data and curves bears out neither of the two assumptions commonly made concerning this relationship. If, as is often assumed, the rate of acid synthesis is proportional to the number of bacteria present, then the bacteria should maintain the same level of metabolic activity and $(1/N)(\mathrm{d}P/\mathrm{d}t)$ should be constant throughout the fermentation. It is immediately clear from Fig. 6 that $(1/N)(\mathrm{d}P/\mathrm{d}t)$ does not remain constant in any of the fermentations except briefly during the phase of logarithmic growth, and that this first assumption is therefore not valid here. The second assumption, that the rate of acid synthesis is proportional to the rate of growth, is also inadequate as may be seen from Fig. 5. After attaining their maxima, the two rate curves diverge so widely that a sample proportionality obviously does not express the relationship between the two.

A reasonably close correlation can be obtained if it be assumed that the rate of acid synthesis is related *both* to the rate of growth and to the quantity of bacteria present, using the simplest possible relationship,

$$\frac{\mathrm{d}P}{\mathrm{d}t} = \alpha \frac{\mathrm{d}N}{\mathrm{d}t} + \beta N \qquad (2)$$

where α and β are constants of proportionality.

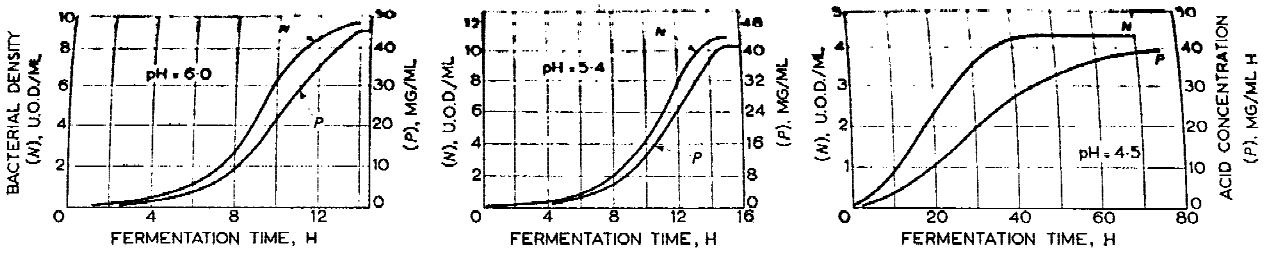

Figure 4. Bacterial growth and lactic acid synthesis during batch fermentations at controlled pH levels.

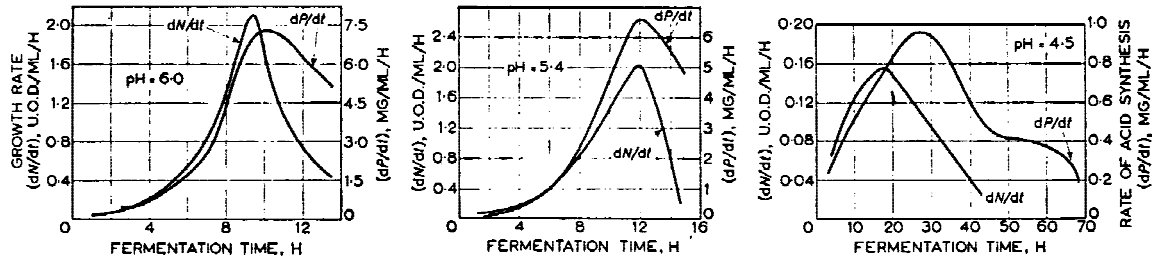

Figure 5. Bacterial growth rate and lactic acid synthesis rate versus time during batch fermentations at controlled pH levels.

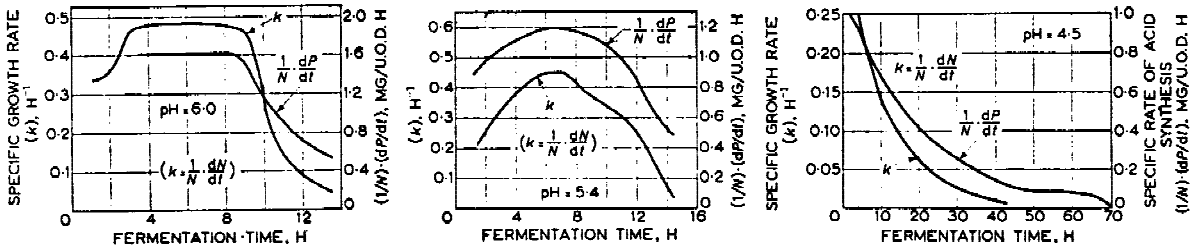

Figure 6. Specific rate of bacterial growth and of lactic acid synthesis versus time during batch fermentations at controlled pH levels.

It is easier to verify this assumed relationship and to evaluation the constants if the equation be modified by dividing by N to give

$$\frac{1}{N}\frac{dP}{dt} = \frac{\alpha}{N}\frac{dN}{dt} + \beta$$

Since, by definition, $k = (1/N)(dN/dt)$, the equation finally simplifies to

$$\frac{1}{N}\frac{dP}{dt} = \alpha k + \beta \qquad (3)$$

When the experimental values of $(1/N)(dP/dt)$ are plotted against k in Fig. 11, the points fall close to a straight line, thus tending to confirm the validity of equations 3 and 2. Thus the rate of lactic acid synthesis is not a function of bacterial density alone, nor of growth rate alone, but it is a function of the bacterial density and of the rate of bacterial growth together.

The constants α and β are determined from the plots of Fig. 11 where α is equal to the slope of the straight line and β is equal to the $(1/N)(dP/dt)$ intercept. The manner in which α and β vary with pH can be seen from Table III and from Fig. 10.

Equations 2 and 3 suggest that the production of lactic acid is related to two life processes. One can speculate that the cell dissimilates glucose to lactic acid in order to obtain the energy required to form new bacterial protoplasm, and at the same time it does so as a normal metabolic activity irrespective of growth. The rate of acid production per cell for the first process is represented by αk and is therefore proportional to the specific growth rate. For the second process the rate of acid production per cell is a constant at a given pH level. In the early phases of a normal fermentation when the specific growth rate is high, the first term of equation 3 is the important one, while towards the end of the fermentation the second term becomes more important. For 'resting' cells where there is supposedly no growth occurring, the first term should be zero and all acid is produced in accordance with the second term of the equation, the constant β.

It is also noteworthy that during the phase of logarithmic growth the two common assumptions mentioned above for relating rate of acid production to growth are both valid.

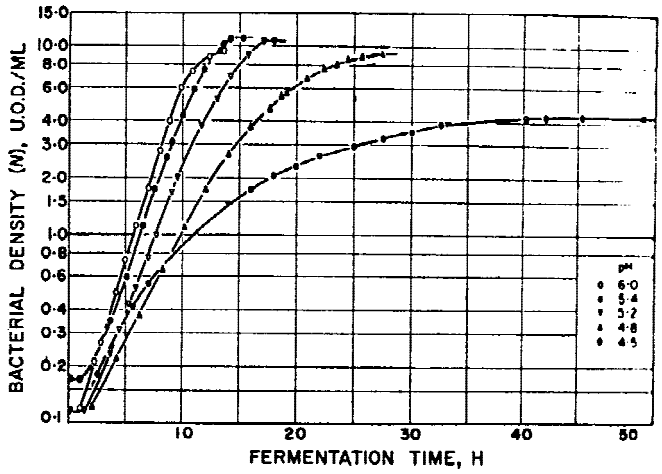

Figure 7. Semi-logarithmic plot showing increase of bacterial density during batch fermentations at controlled pH levels.

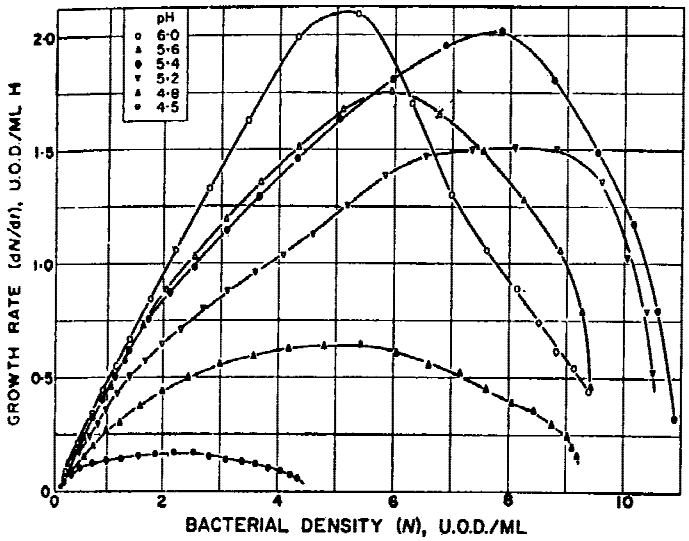

Figure 8. Bacterial growth rates as functions of the bacterial density during batch fermentations at controlled pH levels.

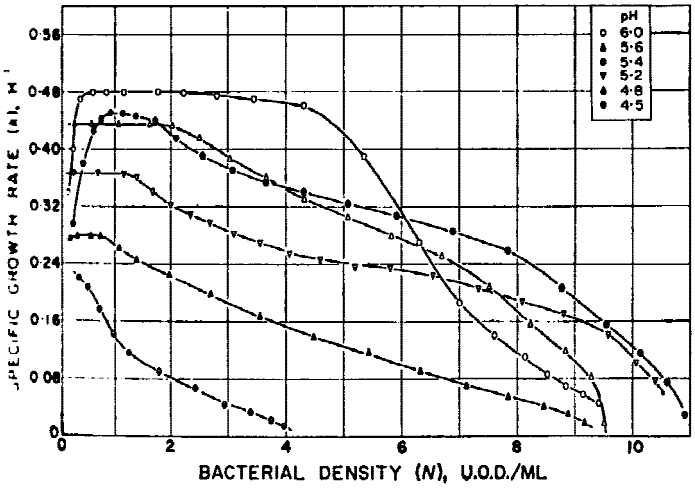

Figure 9. Specific bacterial growth rates as functions of the bacterial density during batch fermentations at controlled pH levels.

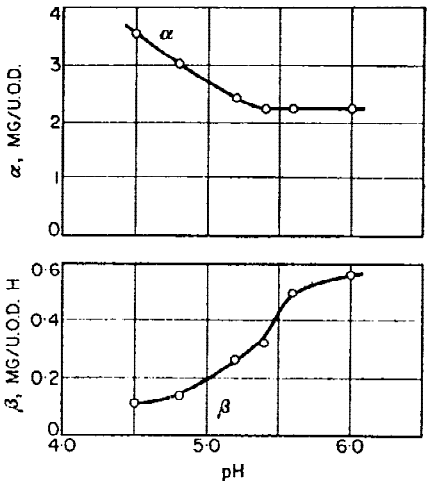

Figure 10. The effect of pH on the coefficients α and β in the equation $(1/N)(dP/dt) = \alpha k + \beta$

This is true because $(dN/dt) = kN$, and during the logarithmic phase k is a constant, k_c. Thus one can state with equal validity that during the logarithmic phase the rate of acid production is proportional to the growth rate of the bacteria, or that during this phase the rate of acid production is proportional to the quantity of bacteria present. Neither statement was found to hold true outside of the period of logarithmic growth, while equation 3, of course, was found to apply throughout the entire fermentation cycle.

NOMENCLATURE

D	Optical density of a diluted sample
I	Leitz Rouy-Photrometer instrument reading of diluted sample, per cent transmission
I_n	Instrument reading of blank, per cent transmission
k	Specific (Naperian) growth rate ($k = 1\ dN/N\ dt$), h^{-1}
k_c	Logarithmic growth constant (constant value of k during phase of logarithmic growth), h^{-1}
$\log_{10}$	Logarithm to the base 10
N	Bacterial density, units of optical density (U.O.D.) per ml
P	Lactic acid or product concentration, mg/ml
r	Volume ratio of diluted sample to original sample for optical density measurements
dN/dt	Rate of bacterial growth, U.O.D./ml h
dP/dt	Rate of lactic acid synthesis, mg/ml h
$1\ dP/N\ dt$	Specific rate of lactic acid synthesis, mg/U.O.D. h
α, β	Constants

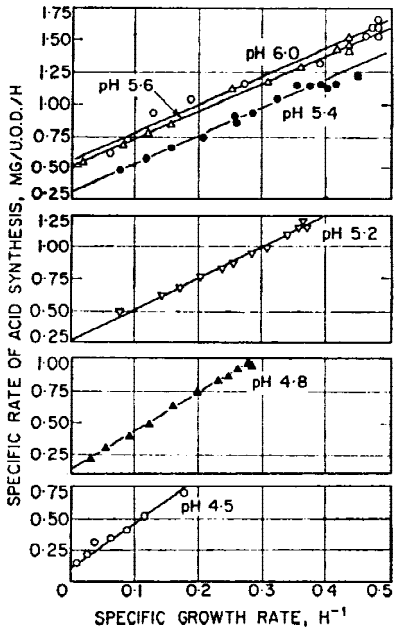

Figure 11. Specific rate of lactic acid synthesis as a function of the specific rate of bacterial growth during batch fermentations at controlled pH levels

Table III. Experimental constants α and β of equations 3 and 2 for each pH level

pH	α	β
6.0	2.2	0.55
5.6	2.2	0.49
5.4	2.2	0.32
5.2	2.45	0.26
4.8	3.0	0.14
4.5	3.55	0.11

α has the units mg/lactic/U.O.D.
β has the units mg lactic/U.O.D./h

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References

[1] Bergey, David H. *Bergey's Manual of Determinative Bacteriology*, 6th ed., p. 359 (1948). Baltimore: Williams and Wilkins Co.
[2] Finn, R. K. Ph.D. Thesis, Dept. of Chemical Engineering, University of Minnesota (1949)
[3] Finn, R. K., Halvorson, H. O. and Piret, E. L. *Industr. Engng. Chem.* (*Industr.*), 42 (1950), 1857
[4] Fisher, C. H., and Filachione, E. M. U.S. Dept. of Agriculture, Bur. Agric. Industr. Chem., AIC–178 (1948); see also AIC–279 (1950)
[5] Friedemann, T. E. and Graeser, J. B. *J. Biol. Chem.*, 145 (1932), 131
[6] Gaden, E. L., Jr. *Chem. & Ind.* (*Rev.*) (1955), 154
[7] Gaden, E. L., Jr. *Chem. Engng. Progr.*, 51 (1955), 540
[8] Gaden, E. L., Jr. *Chem. Engng.*, 63, No. 4 (1956), 159
[9] Gillies, R. A. Ph.D. Thesis, Dept. of Bacteriology, University of Michigan (1955)
[10] Golle, H. A. *J. Agr. Food Chem.*, 1 (1953), 789
[11] Gunsalus, I. C. *Bacterial Physiology*, Chap. IV. (1951). Ed., Werkman, C. H. and Wilson, P. W. New York: Academic Press.
[12] Hamer, W. J. and Acree, S. F. *J. Res. Nat. Bur. Stand.*, 32 (1944), 215
[13] Henrici, A. T. *J. Infect. Dis.*, 38 (1926), 54
[14] Hinshelwood, C. N. *The Chemical Kinetics of the Bacterial Cell.* (1946) London: Oxford University Press

[15] Hodge, J. E. and Davis, H. A. U.S. Dept. of Agriculture, Bur. Agr. Industr. Chem., AIC–333 (1952)
[16] Kempe, L. L., Halvorson, H. O. and Piret, E. L. *Industr. Engng. Chem.* (*Industr.*), 42 (1950), 1852
[17] Longsworth, L. G. *J. Bact.*, 32 (1936), 307
[18] Longsworth, L. G. and MacInnes, D. A. *J. Bact.*, 29 (1935), 595
[19] Longsworth, L. G. and MacInnes, D. A. *J. Bact.*, 31 (1936), 287
[20] Longsworth, L. G. and MacInnes, D. A. *J. Bact.*, 32 (1936), 567
[21] Luedeking, R. and Piret, E. L. This Journal, 1 (1959), 431
[22] Maxon, W. D. *Appl. Microbiol.*, 3 (1955), 110
[23] Monod, J. *Annu. Rev. Microbiol.*, 3 (1949), 371
[24] Monod, J. *Ann. Inst. Pasteur*, 79 (1950), 390
[25] Needle, H. C. and Aries, R. S. *Sugar*, 44, No. 12 (1949), 32
[26] Porter, J. R. *Bacterial Chemistry and Physiology*. (1946). New York: John Wiley and Sons, Inc.
[27] Schopmeyer, H. H. *Industrial Fermentations*, Vol. 1, Chap. XII. Ed., Underkofler, L. A. and Hickey, R. J. (1954). New York: Chemical Publishing Co., Inc.
[28] Smith, R. J. *Analyt. Chem.*, 25 (1953), 505
[29] Underkofler, L. A. and Hickey, R. J., ed. *Industrial Fermentations*, Vol. 1. (1954). New York: Chemical Publishing Co., Inc.
[30] Wright, B. E. *J. Bact.*, 66 (1953), 407