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ACID PRODUCTION BY HOMOFERMENTATIVE LACTOBACILLI AT CONTROLLED pH AS A TOOL FOR STUDYING THE UNIT PROCESS OF FERMENTATION

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PREFACE

Lactic acid has attractive possibilities as an intermediate in the production of plastics. Its present uses are limited to rather minor acidification applications in the food industry. However, if the cost of pure lactic acid could be reduced to about the same value as that of phenol on a pound basis, it would undoubtedly be acceptable in the plastics industry.

Also, the lactic acid fermentation is adaptable for study of certain fundamental concepts of fermentation. For these reasons we are continuing studies directed towards reducing the cost of lactic acid as an industrial product. At the same time we are using this fermentation to explore the effects of various chemical and physical agents on the overall process of fermentation.

NOTATIONS

A = activation energy, cal/gm mol,

k = fermentation rate constant, 1/hr,

k_1 = fermentation rate constant at T_1, 1/hr,

k_2 = fermentation rate constant at $(T_1 + 10)$, 1/hr,

K = rate of lactic acid formation, gm mols/hr/liter,

K' = rate of sugar utilization, gm mols/hr/liter,

N = normality of alkali,

R = gas constant, 1.987 cal/gm mol/^{o}K,

S = rate of addition of alkali in milliliters per hour,

T = absolute temperature, ^{o}K,

V = volume of media fermented, liters.

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I. INTRODUCTION

Studies of environmental factors controlling the yields and rates of fermentation product formation usually involve extensive analytical work. Fortunately, it is possible to conduct lactic acid fermentations in such a way that these data are automatically determined and recorded while the fermentations are in progress. This method has been used to observe the effect of pH on the rate of lactic acid production (1, 5) and to assess the utility of gamma radiation for sterilizing fermentation media (2). An extension of the procedure will facilitate collection of information concerning the effects of temperature, pH, osmotic pressure, etc. on batch fermentations and will simultaneously provide basic data for the study of continuous fermentations. This paper is concerned with the utilization of rate studies to determine the temperature coefficient of lactic acid production and the activation energy for this fermentation.

II. EXPERIMENTAL PROCEDURE

A diagram of the equipment used in this study is shown in Fig. 1. It was similar to that previously described by Finn (1), but, modifications were included that permitted both sterilization of the fermentor and the introduction of sterile nutrilite solutions concurrently with a neutralizing solution.

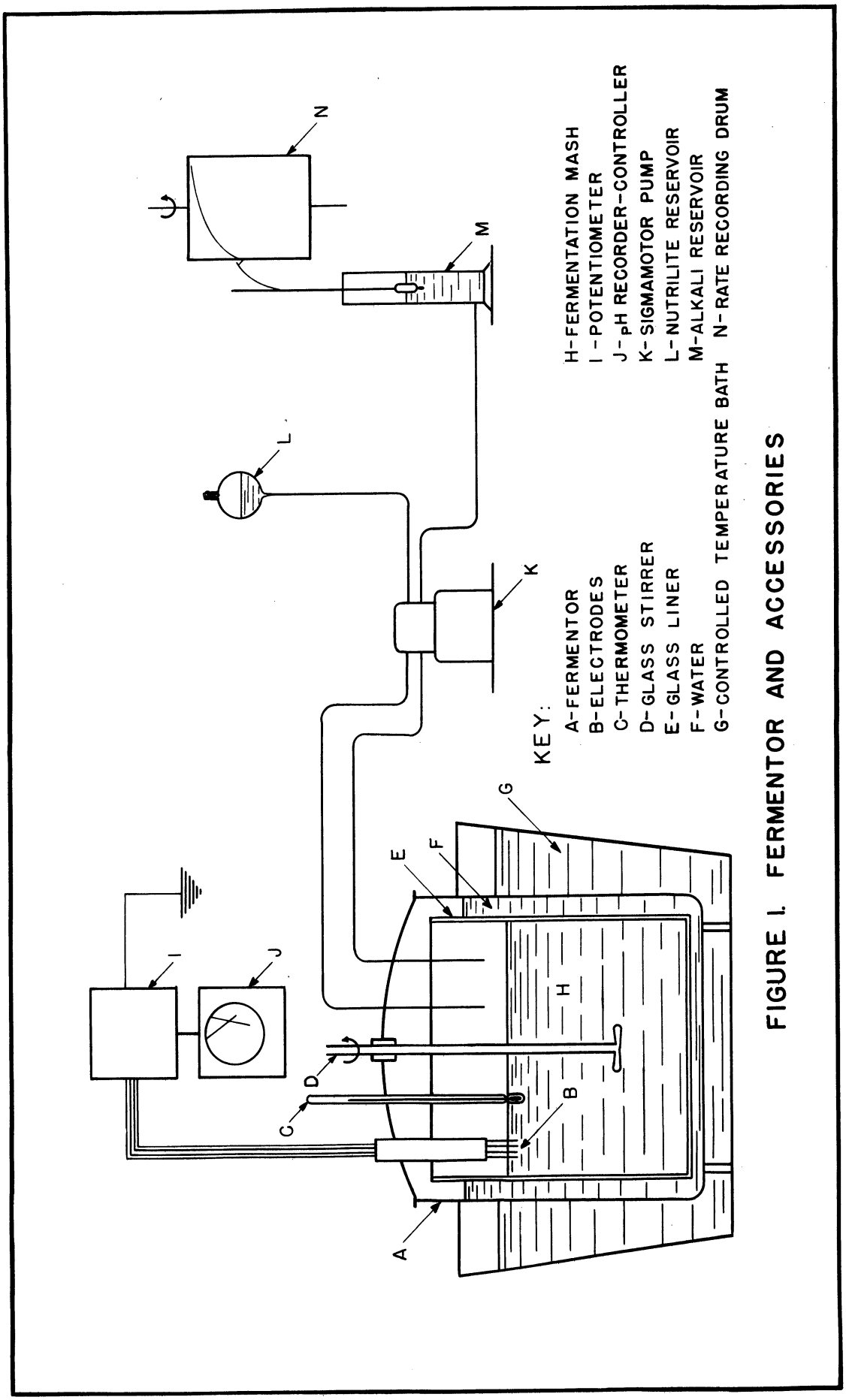

FIGURE I. FERMENTOR AND ACCESSORIES

Preparation for a fermentation involved placing 60 ml of nutrient salt solutions (5), 200 grams of dextrose, 3930 ml of distilled water and appropriate amounts of corn steep liquor concentrate in the pyrex glass jar that served as a liner for the fermentor. All openings were then closed with cotton plugs or paper wrappings and the fermentor containing the mash was autoclaved at 15 psig steam pressure for 45 minutes. After cooling, the pH of the mash was aseptically adjusted to a value slightly above 5.5. The pH electrodes were sterilized independently in a calcium hypochlorite solution and then were aseptically inserted into the cooled mash.

Sixty ml of a culture of Lactobacillus delbrueckii NRRL 445 A, growing in the exponential phase, were next aseptically injected into the mash to initiate fermentation. When the pure culture grew and produced acid, the automatic pH regulating system caused 3.4 normal alkali to be pumped into the fermentor. As the alkali level dropped in the reservoir, a curve was produced on the kymograph chart. This curve showed the instantaneous rate of acid production by the fermentation. At pH 5.5 the amount of undissociated lactic acid was negligible and approximately 97 percent of the total acid produced was lactic acid. Therefore the record of alkali level represented the total amount of lactic acid produced. Graphical differentiation of this curve yielded the instantaneous rate of lactic acid formation at any time during the fermentation.

Provision was also made for the introduction of sterile corn steep liquor during the fermentation when desired. This was accomplished by threading a sterile rubber tube from a nutrilite reservoir through the Sigmamotor pump that already carried a similar tube for pumping alkali into the mash.

Comparisons of many lactic acid fermentations, conducted with constant pH and temperature conditions, showed two distinct patterns for changes in the instantaneous rate of lactic acid formation as a function of time. These patterns depended upon the manner of nutrilite addition. When all the mash components were present initially, the rate first increased to a maximum

and then gradually decreased throughout the fermentation until the sugar was exhausted. This represented unsteady-state conditions. However, a different curve resulted when a portion of the corn steep liquor was incorporated in the original mash and the remainder was added concurrently with the alkali. Here, the rate first increased to a maximum as before and then decreased slightly. After this, the acid production rate stabilized at a constant value that was a function of the conditions of fermentation. This represented steady-state conditions. Table I and Fig. 2 show experimental data for runs 20 and 19 which demonstrate unsteady and steady-state lactic-acid fermentations respectively. In run 20, three percent of corn steep liquor was incorporated into the initial mash while in run 19, one percent was present at the beginning and two percent were added during the fermentation. Thus, runs 20 and 19 were identical except for the manner of nutrilite addition.

As shown in Fig. 3, the rate of lactic acid production during the constant rate period in the steady-state fermentations could be changed at will by altering the temperature of the mash. These variations provided data for calculation of the Q_{10} coefficient and the activation energy for the fermentation.

For this purpose, steady-state fermentations similar to run 19 were carried out and the temperature was purposely changed during what would otherwise have been the constant rate period of acid production. Run 7 was such a fermentation. In this run 200 gm of sugar and 40 gm of corn steep liquor concentrate were initially combined with distilled water and salt solution to prepare 4000 ml of media. During the fermentation, 120 gm of sterile corn steep liquor concentrate was aseptically added as a dilute solution. The pH was controlled at 5.5 ± 0.03 pH units. Table II and Fig. 3 show the variations in the instantaneous rate of acid production which developed in the constant rate period when the fermentation temperature was changed during this run.

TABLE I

RATE OF ACID PRODUCTION
FOR STEADY AND UNSTEADY STATE LACTIC ACID FERMENTATIONS

Hours after Inoculation	Ml of 3.4N alkali added/hr	
	Run 19	Run 20
0	0.0	0.0
1	1.0	1.0
2	3.0	2.0
3	5.0	5.0
4	9.0	10.0
5	14.0	17.0
6	20.0	28.0
7	28.0	42.0
8	35.0	54.0
9	45.0	59.0
10	48.0	56.0
11	47.0	50.0
12	45.0	47.0
13	42.0	44.0
14	40.0	41.0
15	40.0	39.0
16	40.0	37.0
17	40.0	35.0
18	40.0	33.5
19	40.0	32.0
20	40.0	30.0
21	40.0	0.0
22	38.0	
23	0.0	

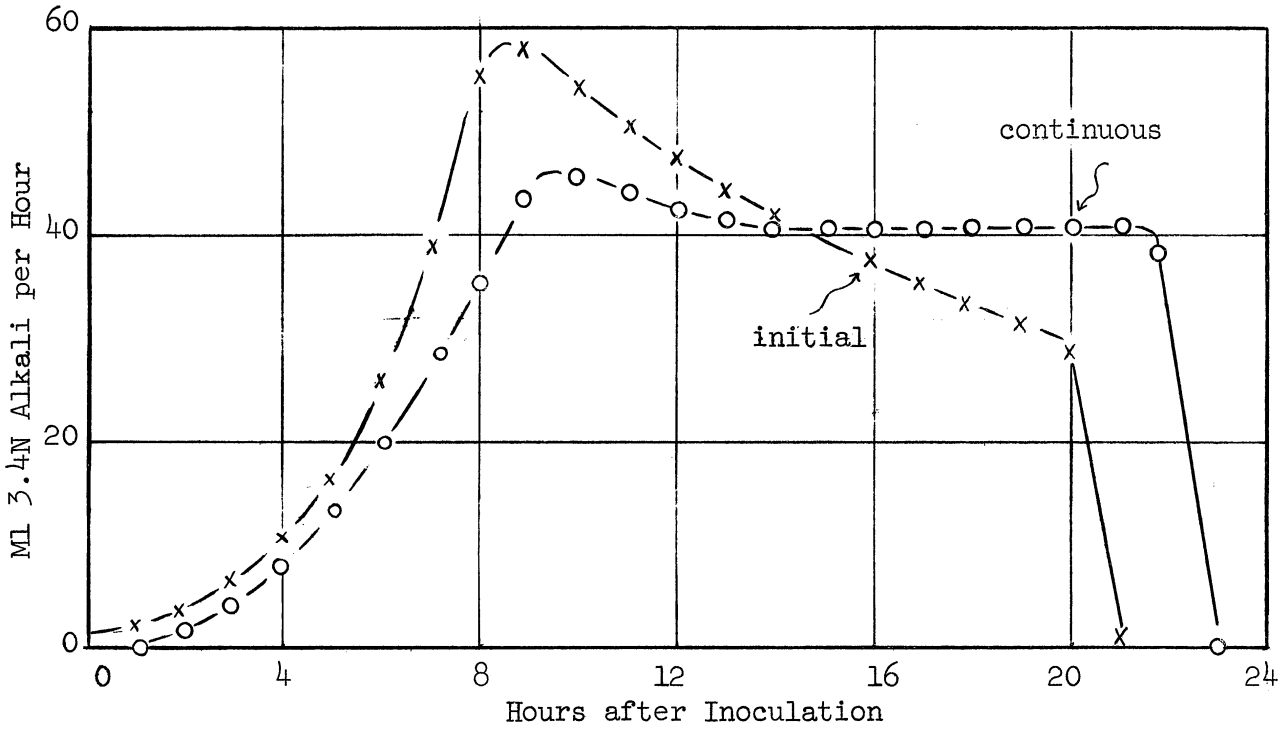

Fig. 2. Effect of Manner of Nutrilite Addition on the Instantaneous Rate of Lactic Acid Production by Lactobacillus Delbrueckii.

TABLE II

EFFECT OF TEMPERATURE ON THE RATE OF ACID PRODUCTION DURING THE STEADY STATE IN A LACTIC ACID

Hours after Inoculation	Temp °C	Ml 3.4 N Alkali Added per Hour
16	40.4	18.5
17	40.4	18.5
18	40.4	18.5
19	--	16.0
20	36.8	15.0
21	36.8	15.0
22	--	14.0
23	--	12.0
24	32.8	10.0
25	33.0	10.0
26	32.0	10.0
27	30.0	10.0
28	--	9.0
29	--	8.0
30	30.0	7.0
31	30.0	7.0
32	30.0	7.0
33	30.0	0.0
34	--	6.0
35	27.2	5.0
36	25.1	5.0
37	25.1	5.0
38	25.1	5.0
39	--	0.0

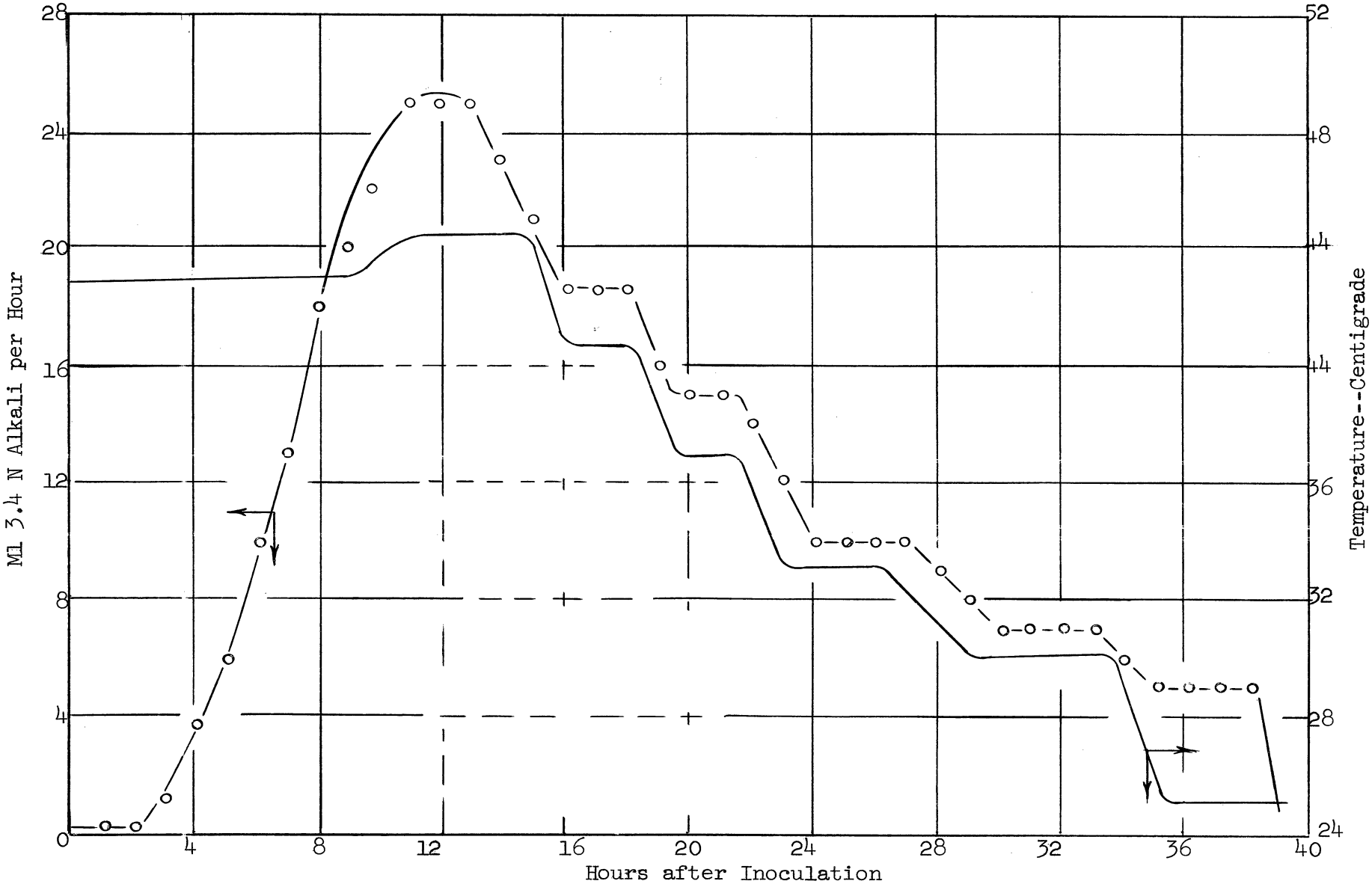

Fig. 3. Effect of Temperature on the Rate of Acid Production by Lactobacillus Delbrueckii when Corn Steep Liquor Was Introduced into the Mash both Initially and during the Fermentation.

III. CALCULATIONS

The overall equation for the formation of lactic acid by homofermentative lactic acid bacteria can be represented by

$$\underset{(A)}{C_6H_{12}O_6} \leftrightarrows \underset{(B)}{2CH_3\ CHOHCOOH} \ .$$

When molar concentration of (A) is sufficiently large to saturate the enzyme systems involved, it can be considered as unity. Under this condition, C_A will not affect the rate of the reaction (6) so:

$$-\frac{dC_A}{dt} = K'C_A = K' \ ,$$

but $-(dC_A/dt)$ in this fermentation is proportional to the rate of lactic acid formation so:

$$-\frac{dC_A}{dt} = \frac{1}{2}\frac{dC_B}{dt} = K'$$

$$\frac{dC_B}{dt} = 2K' = K \ .$$

Therefore, the value of K was determined experimentally at different temperatures from:

$$K = \frac{0.97SN}{1000\ V} \ .$$

This assumed that 97 percent of the acid formed was lactic acid (2). Substitution of the value for the slope of the line from Fig. 4 into the following equation

$$\text{slope} = \frac{-A}{2.303\ R}$$

gave a value of 17,100 cal/gm mol for A. The calculated values for K are shown in Table 3 and Fig. 4.

Using this value for A and substituting in

$$2.303 \log \frac{k_2}{k_1} = \frac{A\ (10)}{(T_1 + 10)(T_1)} \ .$$

TABLE III

VARIATION OF THE CONSTANT RATE OF LACTIC ACID PRODUCTION WITH TEMPERATURE IN RUN NO. 7

Temp. °K	Mash Volume, Liters	K, in mols of Lactic acid/hr/liter
313.6	4.355	0.0140
310.0	4.440	0.0112
306.3	4.500	0.0074
303.2	4.530	0.0051
298.3	4.605	0.0036

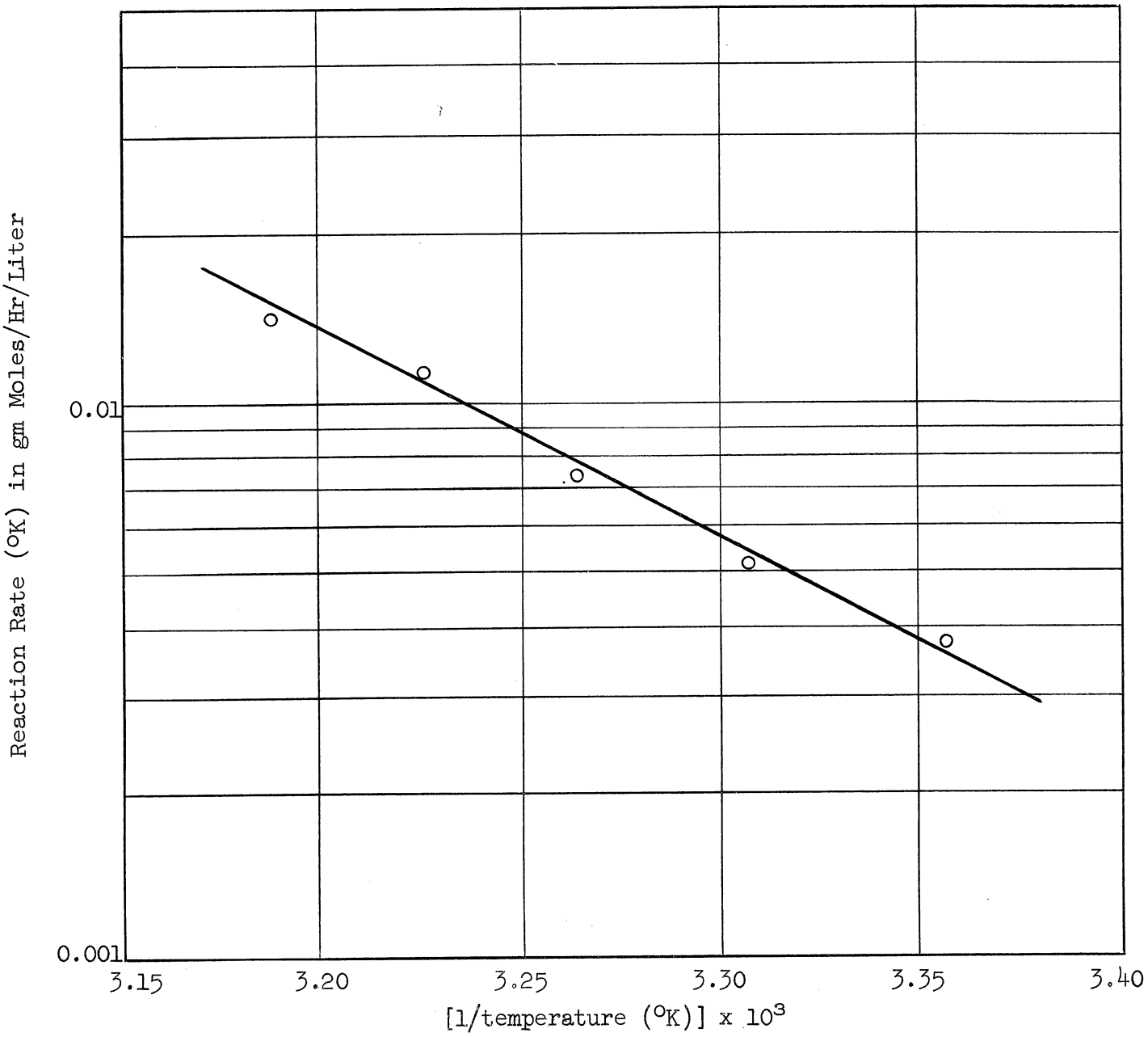

Fig. 4. Arrehnium Plot for the Lactic Acid Fermentation.

Q_{10} was found to be 2.51 between 30°C and 40°C and 2.64 between 30°C and 20°C.

IV. DISCUSSION

The constant rate of lactic acid production that was developed when nutrilites were continuously added to the fermenting mash provided a sensitive tool for studying the effects of various environmental changes on this fermentation. The data showed that temperature changes roughly doubled the instantaneous rate of lactic acid production for each ten degree centigrade temperature rise. Since the Q_{10} values thus obtained were in the normal range for enzymatically catalyzed reactions, it was interesting to prepare the Arrehnius activation plot. Here the data produced a straight line function over the feasible fermentation temperature range. This suggested that the same reactions were operative over the temperature interval that was studied. The calculations further suggested an activation energy of 17,100 cal/gm mol for this fermentation which is similar to those normally ascribed to biological reactions (3). The actual significance of the activation energy for a fermentation process consisting of many consecutive reactions is not entirely clear. If only one reaction were involved, the activation energy value would unquestionably be significant for this reaction. But even in the series of reactions that are involved here, it is likely that the rate of the total process is regulated by the slowest reaction in the chain (4). On this basis we can speak of the energy of activation of the lactic acid fermentation.

The basic observation, that the rate of fermentation at controlled pH and temperature was a constant, predictable value when nutrilites were added continuously provides a basis for the calculation of lactic acid yields in a continuous process. In such a system, the nutrilites could be added along with carbohydrate and other mash constituents and the fermented

mash could be removed simultaneously. This would develop a typical continuous reactor system.

The use of low nutrilite concentrations in the original mash has previously been shown to reduce the importance of side reactions and to increase the yield of lactic acid produced from sugar (2, 5). However, low nutrilite concentrations also cause low rates of fermentation (2, 5). It has been shown in this paper that the combination of low initial nutrilite concentration with continuous addition of such material causes a high overal rate of lactic acid production. Further, it has been observed that nutrilite sources continually break down when present as dilute components of the mash, hence much of these usually expensive materials can be wasted (2). Continuous addition of the nutrilite reduces this loss to a minimum. Hence, even in batch fermentations it is probable that proper combinations of initial and continuous growth factor additions would reduce the nutrilite cost and increase both the yield and rate of lactic acid production in the mashes.

V. CONCLUSIONS

Steady-state lactic-acid fermentations can be used to determine the effect of temperature on the rate of lactic acid production. A Q_{10} value of 2.5 between 30°C and 40°C and an activation energy of 17,100 cal/gm mol were calculated for this fermentation.

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