Acceleration of Lactic Acid Fermentation by Heat-Labile Substances

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A rapid lactic acid fermentation of either glucose or molasses has been obtained by supplying unheated malt sprouts as a nutrient source for the bacteria. Large-scale laboratory experiments with 64 gallons (240 liters) of unheated molasses mash showed that 12 per cent sugar can be fermented to completion (90 per cent) in 16 to 20 hours. Malt sprouts contain a heat-labile growth factor for *Lactobacillus delbrückii*, which greatly speeds up the fermentation. This factor appears to be stable to heating for 10 minutes at 65° C., but exposure to longer periods at higher temperatures is destructive to it. Pure dextrolactic acid can be produced from molasses by means of aerobic spore-forming organisms and sterilized mash. These organisms also respond to unheated mash, but the lactic acid contains a small amount (10–20 per cent) of the levo form as well as the predominating dextro form. Other optimum conditions for lactic acid fermentation are reported.

THE production of lactic acid by fermentation, as practiced commercially (*5, 7, 15*), is a rather slow process. Five to ten days are required for complete fermentation of a mash containing 10 to 12 per cent sugar in the form of molasses or hydrolyzed starchy materials.

Attempts to shorten the period of fermentation have centered around the choice of better strains of bacteria and the provision of optimum conditions for their activity. *L. delbrückii* is the organism usually employed, and the temperature of fermentation is generally set at 45° to 50° C. The question of nutrients still seems unsettled. Stiles and Pruess (*20*) reported recently that with malt sprouts, steep water, or a combination of one of them with thin grain residue, a molasses mash of 9 to 10 per cent sugar could be completely fermented in 3.5 to 5 days.

Recent researches in bacterial nutrition have established the importance of certain accessory substances for growth as well as fermentation. For the lactic acid bacteria, riboflavin (*14, 17*), pantothenic acid (*18*), nicotinic acid (*18*), and vitamin B_6 (*12*) have been reported as essential growth factors. In view of these results it seemed possible to improve the lactic acid fermentation further by addition of suitable sources of these essential factors or other growth stimulants. Results in this direction are reported in the present paper.

Cultures and Fermentation Procedure

Eight lactobacillus strains were used at the beginning of this work. On the basis of their fermentative power, the following four were chosen for subsequent studies: stock cultures *L. delbrückii 4* (*L. d. 4*) and *L. helveticus* (*B. casei* ϵ Freudenreich), and new isolations *L. delbrückii b* (*L. d. b*) and *L. delbrückii g* (*L. d. g*). These organisms were carried as stab cultures in agar containing 1 per cent malt sprout extract and 0.2 per cent glucose. The cultures were kept in a refrigerator and transferred once a month.

In the preliminary glucose fermentation, 33 cc. of a sterile medium containing 10 per cent glucose, 3 per cent malt sprouts, and 6 per cent calcium carbonate were inoculated with 0.3 cc. of a 24-hour culture in 3 per cent malt sprout medium containing 5 per cent glucose and incubated at 45° C. Sugar analyses at intervals during the fermentation were made by the method of Stiles, Peterson, and Fred (*19*). The curve obtained by plotting the percentage of glucose fermented against time gave a measure of the rate. In the following tables, only the percentage fermentation in the first 24 hours and the time in hours for 95 per cent fermentation as read from the curves are recorded. Whenever necessary, the lactic acid content was determined on the ether extract of the fermented culture by the method of Friedemann and Graeser (*6*).

For the molasses fermentation, Louisiana blackstrap was used and the procedure was essentially the same as for glucose. The medium was analyzed for total sugar after inversion of the sample with 0.4 *N* hydrochloric acid in a boiling water bath for 5 minutes. The results are expressed as amount of invert sugar.

Rate of Glucose Fermentation

With the procedure outlined above, tests on glucose fermentation in sterilized media were made. The effects of temperature and sugar concentration are summarized in Table I. *L. helveticus* fermented as fast at 45° as at 37° C., and *L. d. 4* and *L. d. b.* did best at 45° C. Comparison of the rate of fermentation of 5 per cent glucose with that of 10 per cent shows that, during the early period of fermentation (the first 48 hours), the rate of fermentation, expressed as mg. sugar per cc. per hour, was independent of sugar concentration. For example, in the fermentation of 5 per cent glucose by *L. d. 4*, 17.9 mg. sugar per cc. (35.8 per cent) were fermented in the first 24 hours (i. e., 0.75 mg. per cc. per hour) and in that of 10 per cent glucose, 38.0 mg. were fermented in 48 hours (i. e., 0.79 mg. per cc. per hour). The time for complete fermentation of 5 per cent sugar was less than half that for 10 per cent because the rate decreased in the later stages of fermentation. Table I also shows that the rate of fermen-

TABLE I. EFFECT OF TEMPERATURE AND SUGAR CONCENTRATION ON RATE OF FERMENTATION[a]

Expt. Series No.	Culture	Temp.	Glucose Concn.	Fermentation in 48 Hr.	Time for 95% Fermentation
		° C.	%	%	Hr.
1	L. delbrückii 4	37	10	18.5	250
	L. helveticus	37	10	40.0	165
	L. delbrückii b	37	10	33.5	200
2	L. delbrückii 4	45	10	38.0	200
	L. helveticus	45	10	39.5	250+
	L. delbrückii b	45	10	61.4	95
3	L. delbrückii 4	45–52[b]	10	45.0	>300
	L. helveticus	45–52	10	31.0	>300
	L. delbrückii b	45–52	10	59.2	>300
4	L. delbrückii 4	45	5	35.8[c]	90
	L. helveticus	45	5	45.4[c]	50
	L. delbrückii b	45	5	63.3[c]	38

[a] Malt sprouts 3 per cent; medium sterilized.
[b] 6 hours at 45° C., 12 hours at 48° C., rest at 50–52° C.
[c] Fermentation in 24 hours.

tation varied widely with strains. The new isolation *L. d. b.* was best.

Table II shows the effect of nutrients upon the rate of fermentation. The rate increased with increase in concentration of malt sprouts. Although peptone alone served as a poor source of nutrient for the lactobacilli (data not shown here), its addition to malt sprout medium definitely improved the fermentation. A similar improving effect was observed on adding alcoholic liver extract, potato extract, or yeast extract to the malt sprouts, but whey concentrate was ineffective. The best supplement of the malt sprout medium proved, however, to be the dried soluble portion of the distillation residue obtained in the alcoholic fermentation of grains (designated "grain residue" in Table II). With its help the most rapid fermenter (*L. d. b.*) completed the fermentation of 10 per cent sugar in 40 hours. Under the same condition *L. d. b.* fermented 13 per cent sugar in 3 days and 15 per cent in 5 days. This result agrees with that of Stiles and Pruess (*20*) who believed that grain residue supplied some accessory nutrients.

TABLE II. EFFECT OF NUTRIENTS ON FERMENTATION OF GLUCOSE[a]

Expt. Series No.	Culture	Nutrient[b]	Fermentation in 48 Hr.	Time for 95% Fermentation
		%	%	Hr.
5	L. delbrückii 4	1.5 m. s.	23.0	...
	L. helveticus	1.5 m. s.	Lost	Lost
	L. delbrückii b	1.5 m. s.	33.3	...
	L. delbrückii 4	3.0 m. s.	38.0	200
	L. helveticus	3.0 m. s.	39.5	250+
	L. delbrückii b	3.0 m. s.	61.4	94
	L. delbrückii 4	6.0 m. s.	53.5	105
	L. helveticus	6.0 m. s.	64.4	75
	L. delbrückii b	6.0 m. s.	95.4	48
6	L. delbrückii 4	3.0 m. s. +	50.2	110
	L. helveticus	0.5% peptone	60.5	120
	L. delbrückii b		76.1	75
7	L. delbrückii 4	3.0 m. s. + 0.5 g. r.	47.0	125
	L. helveticus	3.0 m. s. + 0.5 g. r.	56.0	108
	L. delbrückii b	3.0 m. s. + 0.5 g. r.	87.0	59
	L. delbrückii 4	3.0 m. s. + 1.0 g. r.	54.5	98
	L. helveticus	3.0 m. s. + 1.0 g. r.	75.0	72
	L. delbrückii b	3.0 m. s. + 1.0 g. r.	98.2	37

[a] Medium sterilized, sugar 10 per cent, 45° C.
[b] m. s. = malt sprouts; g. r. = grain residue (obtained from A. F. Langlykke of Hiram Walker, Inc.).

Size of inoculum was found to have no effect, which agrees with earlier reports (*8*, *16*). Constant mechanical stirring of a 350-cc. culture was no more effective than shaking three times a day. Probably in fermentations on a larger scale a favorable effect might be shown.

Preliminary Tests on Molasses Fermentation

The first question concerned in the fermentation of molasses was whether the bacteria could ferment sucrose as efficiently as glucose. Tests on pure sucrose showed that *L. helveticus* failed to start at all, and *L. d. b* stopped when 80 per cent of the sugar had disappeared; therefore, both were excluded from further tests on molasses. Cultures *L. d. 4* and *L. d. g*, however, proved suitable.

Fermentation of molasses by *L. d. 4* in sterilized medium failed to go to completion, stopping when 70 per cent of the sugar had disappeared (Figure 1). However, if the molasses–malt sprout mash was steamed for 60 minutes under atmospheric pressure instead of being sterilized, fermentation was faster and more extensive. Fermentation stopped at 90–91 per cent. Probably the remainder was made up of nonfermentable reducing sugars or substances which, although reducing Fehling solution, were not sugar. Apparently molasses contained some additional nutrients because the rate of fermentation was higher than on glucose or sucrose.

The effect of concentration of nutrients upon the rate of fermentation in the steamed molasses medium is shown in Table III. The new isolation *L. d. g* was used in this and the later experiments. It was practically identical with *L. d. 4* except for being a more active strain and showing less variation in rate of fermentation. Table III shows that the rate of fermentation still depended upon the malt sprout concentration and that the grain residue failed to bring about any appreciable improvement. This result can probably be explained on the assumption that blackstrap molasses contained the same type of nutrients as the grain residue. It also seems possible to explain the discrepancy between this result and that of Stiles and Pruess (*20*), who worked with cane sirup molasses (which is poor in nitrogenous matter) and found that the grain residue was an efficient supplement to malt sprout medium.

In the same experiment uninoculated tubes were incubated along with regular fermentation tubes. Spontaneous fermentation occurred in every case, and the rate was almost as high as the inoculated. This raised the question of contamination in steamed molasses as practiced commercially as well as in the work of Stiles and Pruess (*20*) and Saitcew (*16*).

Fermentation of Molasses by Aerobic Spore-Forming Bacteria

Evidently contamination can be eliminated only by carrying out the fermentation under sterile conditions. In the present work two cultures of aerobic spore-forming bacteria which gave a pure lactic fermentation were isolated and shown to be capable of fermenting the sterilized molasses mash to completion. These organisms had one advantage over *L. delbrückii* in that they produced dextrolactic acid while the latter gave the levo enantiomorph (*9*, *13*). The commercial product is usually inactive, probably because of fermentation under nonsterile conditions. The dextrolactic acid is probably of higher value for food purposes than the levo form. For the production of the former, Tatum and Peterson (*21*) prescribed the proper choice of *L. delbrückii* strains, Werkman and Anderson (*24*) reported the use of aerobic sporeforming organisms, and Ward and co-workers

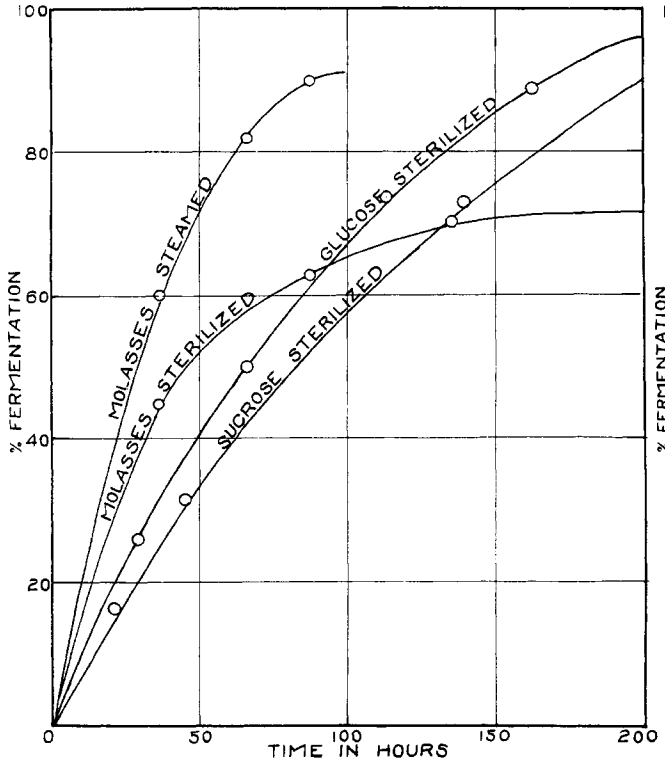

FIGURE 1. RATE OF FERMENTATION OF GLUCOSE, SUCROSE, AND MOLASSES
(Culture *L. d. 4;* malt sprouts 3 per cent; 10 per cent sugar; 45° C.)

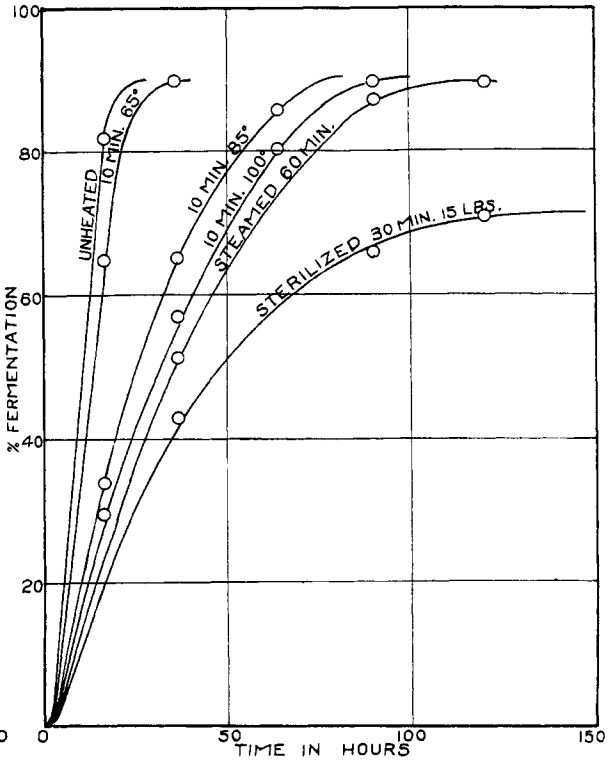

FIGURE 2. EFFECT OF HEAT TREATMENT OF MOLASSES MASH UPON RATE OF FERMENTATION
(Culture *L. d. g;* malt sprouts 3 per cent; 10 per cent sugar; 45° C.)

worked out a fermentation process employing the mold *Rhizopus oryzae* (*22, 23*). The present result seemed to be in agreement with that of Werkman and Anderson. In this connection mention should be made that our *L. helveticus* also forms dextrolactic acid.

One of our aerobic spore-forming organisms was isolated from high-temperature silage and is apparently identical with Demeter's organism (*4*) which he believed to be closely related to *B. calfactor* Miehe (*11*). This organism was designated as culture E. Another culture was isolated directly from the steamed molasses which was undergoing spontaneous fermentation. This organism, designated as culture 6-6, is probably one of Cameron and Esty's flat sour types (*1*), for both are of cane sugar origin (*2*); a test with Cameron and Esty's flat sour organism No. 1608 showed that it was also a fairly active lactic-acid-producing organism. Possibly Werkman and Anderson's organism (*24*) also belongs to this type, although they have not yet given a detailed description of it. The results of the fermentation by these two organisms are given in Table IV; it shows that a sterilized molasses mash containing 10 per cent sugar was completely fermented in 3–4 days, and the yield of lactic acid was 96 per cent of the sugar fermented. Evidently these organisms could be used for the production of dextrolactic acid from molasses.

TABLE III. FACTORS AFFECTING THE FERMENTATION OF MOLASSES[a]

% Nutrient	% Fermentation In 48 hr.	% Fermentation In 96 hr.	Hr. for 90% Fermentation
6 m. s.	90.0	..	46
3 m. s.	61.2	90.5	94
3 m. s. + 0.5 g. r.	63.0	88.0	100+
1.5 m. s.	46.5	69.5	158
1.5 m. s. + 0.5 g. r.	50.0	71.5	150
No nutrient added	22.0	48.0	..
Spontaneous fermentation, no nutrient added	25.8	55.0	..
Spontaneous fermentation, 3 m. s.	45.8	83.0	114

[a] Culture *L. d. g;* molasses steamed and equivalent to 10 per cent sugar; 45° C.

TABLE IV. LACTIC FERMENTATION BY AEROBIC SPORE-FORMING ORGANISMS[a]

	Culture E	Culture 6-6
Initial sugar concn., mg./cc.	103.8	102.5
Hrs. for complete fermentation	84–96	72–90
Residual sugar concn., mg./cc.	8.23	10.4
Fermentation, %	92.2	89.9
Lactic acid, mg./cc.	89.7	86.8
Lactic acid, % of sugar fermented	96.6	95.6
Water of crystallization of zinc lactate, %	12.83	13.12
Sp. rotation of Zn lactate (4%), $[\alpha]_D$	−8.04	−8.02
Form of lactic acid	Dextro	Dextro

[a] Malt sprouts 3 per cent; molasses sterilized and equivalent to 10 per cent sugar; 45° C.

Fermentation of Unheated Molasses by *L. delbruckii*

Since steaming gave a faster fermentation than sterilization, the question arose as to whether pasteurization at lower temperatures would result in a still faster fermentation. It was found that the less heat treatment applied to the molasses mash, the faster became the fermentation. Curves of Figure 2 show that the unheated mash was completely fermented in less than 24 hours.[1] With the assumption that a thermolabile factor was responsible for this rapid fermentation, tests were

[1] The curves for the rapid fermentations are drawn as straight lines with only one or two points determined. As will be shown later, these fermentations did go at a constant rate almost to completion.

TABLE V. EFFECT OF HEAT TREATMENT OF DIFFERENT MEDIA UPON RATE OF FERMENTATION[a]

Treatment	% Fermentation In 12 hr.	% Fermentation In 24 hr.	Hr. for 90% Fermentation
Molasses + m. s., unheated; *L. d. g*	64.8	90.5	20
Molasses, steamed, + m. s., unheated; *L. d. g*	64.5	90.3	20
Molasses, unheated, + m. s., steamed; *L. d. g*	14.8	30.3	128
Molasses + m. s., *steamed separately*; *L. d. g*	17.7	33.2	120
Molasses + m. s., steamed together; *L. d. g*	19.5	38.0	110
Glucose + m. s., unheated; *L. d. b*	53.5	95.0	21[b]
Glucose + m. s., unheated; *L. d. 4*	16.0	80.0	28[c]
Molasses + m. s., unheated; spontaneous fermentation	3.2	31.1	70

[a] Malt sprouts 3 per cent; sugar 10 per cent; 45° C.
[b] 98 per cent fermentation in 28 hours.
[c] 98 per cent fermentation in 34 hours.

made which showed that this factor was contained in the malt sprouts but not in the molasses (Table V). When the malt sprouts were unheated, rapid fermentation took place even though the molasses was heated. The reverse, heated malt sprouts and unheated molasses, gave no better fermentation than when both constituents were heated. Unheated glucose–malt sprout medium fermented as fast as the molasses–malt sprout mash. Table V shows that the rapid fermentation was produced by the inoculum but not by the contaminants, since there was hardly any fermentation during the first 12 hours in the uninoculated tubes, whereas two thirds of the sugar had disappeared by that time in the inoculated ones. Since the fermentation finished in 20 hours, there should be hardly any contamination.

TABLE VI. RELATION BETWEEN INITIAL SUGAR CONCENTRATION AND RATE OF FERMENTATION[a]

Initial Sugar Concn., %	Mg. Sugar Fermented per Cc. in 10 hr.	Mg. Sugar Fermented per Cc. in 20 hr.	Mg. Sugar Fermented per Cc. in 30 hr.	Hr. for Complete Fermentation 90% complete	Hr. for Complete Fermentation Basis, 20 hr. for 9.65% sugar
9.65	48.7	86.8 (complete)	...	20	20
12.4	52.6	107.3	...	24	25.3
14.3	48.6	107.2	126.0	33	29.6
16.0	42.4	87.2	117.0	52	33.2
23.0	11.5	35.6	56.4	>72	41.7

[a] Culture *L. d. g*; malt sprouts 3 per cent; molasses medium unheated; 45° C.

For this rapid fermentation of unheated molasses mash, the optimum conditions were worked out in the usual way. Figure 3 shows the effect of temperature with 45° C. as the optimum. Figure 4 shows the effect of malt sprout concentration; 3 per cent gave almost the highest speed. It also shows that at the expense of a little time, the use of 1.5 to 2.0 per cent malt sprouts was entirely feasible. The rate of fermentation varied within certain limits and seemed to depend on the type of molasses. With Puerto Rican or another sample of Louisiana blackstrap it has been found possible to complete the fermentation in 15 hours with 3 per cent malt sprouts and in 24 hours with 1.5 per cent malt sprouts. Table VI shows the rate of fermentation expressed as mg. sugar fermented per cc. in different intervals in media of various sugar concentrations. Evidently the rate expressed in this way remained practically constant up to 14.3 per cent sugar; further increase resulted in a lower rate.

TABLE VII. UTILIZATION OF SPENT MALT SPROUTS[a]

% Fresh M. S. Added[b]	% Fermentation In 12 hr.	% Fermentation In 24 hr.	Hr. for Complete Fermentation (90%)
2.0	57.5	90.0	22
1.5	50.8	85.8	30
1.0	42.2	75.5	36
3.0 (no spent m. s.)	67.0	90.0	20

[a] Culture *L. d. g*; molasses unheated and equivalent to 10 per cent sugar; 45° C.
[b] *Washed 3 per cent malt sprouts from a previous fermentation were used here.*

Since the cost of malt sprouts is an important factor in a commercial fermentation, the question arose as to whether all the nutrients were extracted and utilized. Attempts were therefore made to utilize the spent sprouts in the next batch. The solids from a fermentation with 3 per cent malt sprouts were filtered off and washed. Fresh media were made up with the residual material, and 1.0, 1.5, and 2.0 per cent additional malt sprouts were supplied. The results are given in Table VII, which shows that the spent sprouts did contain some nutrients. With 2.0 per cent additional sprouts the fermentation could be completed in 22 hours, and even with 1.0 per cent additional malt sprouts 36 hours were long enough for complete fermentation.

Fermentation of Unheated Molasses by Aerobic Spore-Forming Bacteria

Since the aerobic spore-forming organisms produced *d*-lactic acid, it seemed highly desirable to apply the same method—i. e., fermentation of unheated molasses—to these organisms. As Table VIII shows, the organisms did respond to the thermolabile factor but to a far less extent than *L. d. g*. Thirty to fifty hours were required for complete fermentation with 3 per cent malt sprouts. Trials were made to use other nutrient sources—soybean meal, steep water, bean sprouts, alfalfa meal, etc.—in place of malt sprouts, but none of them gave better results than the latter. However, aeration showed beneficial effect and reduced the fermentation period to 24 hours. A still better result was obtained by using an inoculum from a 12-hour culture in unheated malt sprouts–molasses mash. The fermentation was complete in 17 hours with 3

TABLE VIII. FERMENTATION OF UNHEATED MOLASSES BY CULTURE E[a]

Medium	Conditions of Fermentation: Malt sprouts %	Size of inoculum %	Kind of inoculum	Temp. ° C.	Aeration	% Fermentation In 12 hr. %	% Fermentation In 24 hr. %	Time for Complete Fermentation (90%) Hr.	*d*-Lactic Acid %
Sterilized	3	1.5	Sterilized	45	Without	21	42	90	100
Unheated	3	1.5	Sterilized	45	Without	26	60	48	..
Unheated	3	1.5	Sterilized	45	With	37	90	24	..
Unheated	3	1.5	Unheated	45	Without	70	90	18	83
Unheated	3	6	Unheated	45	Without	81	90	17	..
Unheated	1.5	6	Unheated	45	Without	42	70	40	..
Unheated	3	6	Unheated	50–53	Without	35	75	32	85
Unheated	1.5	6	Unheated	50–53	Without	32	60	52	..
Unheated	1.5	6	Unheated	50–53	With	35	70	40	90

[a] Sugar 10 per cent.

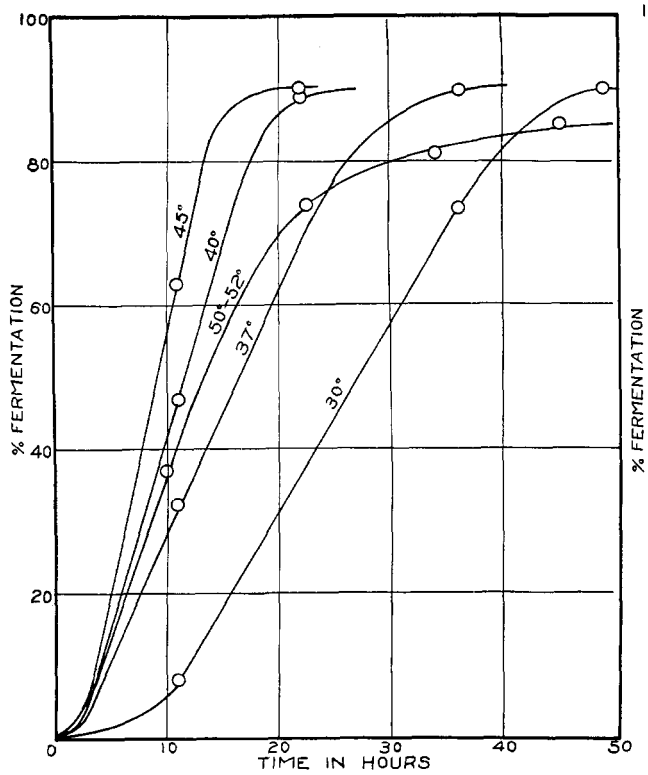

FIGURE 3. EFFECT OF INCUBATION TEMPERATURE UPON RATE OF FERMENTATION
(Culture *L. d. g;* malt sprouts 3 per cent; molasses medium unheated; 10 per cent sugar)

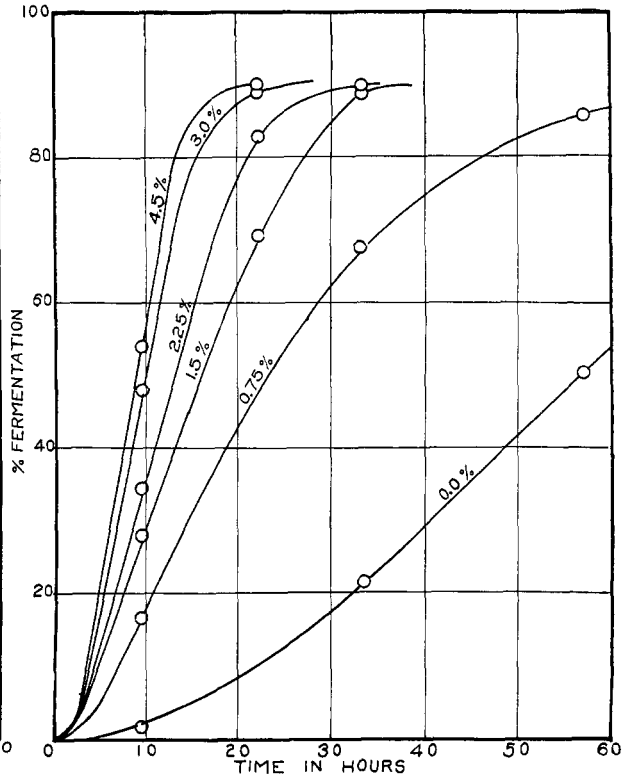

FIGURE 4. EFFECT OF MALT SPROUTS CONCENTRATION UPON RATE OF FERMENTATION
(Culture *L. d. g;* molasses medium unheated; 10 per cent sugar; 45° C.)

per cent malt sprouts and in 40 hours with 1.5 per cent malt sprouts. The lactic acid, however, was not optically pure, probably because of contaminating organisms in the fermentation. It contained only 83 per cent dextro acid.

In order to eliminate contamination, fermentations at a higher temperature, 50–53° C., were tried. The result showed that the purity of the lactic acid was improved but the rate of fermentation was decreased. Aeration seemed to improve both rate and purity; fermentation was complete in 36–40 hours, and the product was 90 per cent dextro acid. The results of these tests are summarized in Table VIII where the data on culture E are given. Those of culture 6-6 were practically the same.

Large-Scale Fermentation of Unheated Molasses by *L. delbrückii*

With the optimum conditions for a rapid fermentation known, a trial was made on a large scale. A 90-gallon (350-liter) open copper tank, provided with a heating coil, was used for this purpose. Sixty-four gallons (240 liters) of diluted molasses (12.6 per cent invert sugar) were mixed with 14.7 pounds (6.67 kg.) malt sprouts (2.8 per cent) and warmed to 45° C. Four liters of a 24-hour culture of *L. d. g* in 3 per cent malt sprouts–10 per cent molasses medium (steamed) were added, followed by the addition of 33 pounds (15 kg.) of calcium carbonate. During fermentation the temperature was maintained at 44–46° C. and the medium was occasionally stirred. Analyses of sugar at different intervals showed that fermentation started in 3 hours and went at a constant rate to completion in 21 hours. The data for residual sugar, lactic acid content, etc., are summarized in Table IX (run 1). The yield of lactic acid was 95.7 per cent of the sugar fermented or 87.3 per cent of the sugar present in the original molasses. The final pH of the fermented mash was 4.85. After the culture was neutralized to pH 7–8, steamed for 5 minutes, and filtered, the calcium lactate could be crystallized directly.

In order to establish its practicability, the large-scale fermentation was repeated. Puerto Rican blackstrap molasses was used in the second run. It had a higher sugar content than the Louisiana blackstrap, but the fermentability of the sugar was a little lower (87–88 per cent). A mechanical agitator was employed in this fermentation to facilitate neutralization and to bring about uniformity of temperature. As a result of these effects, the duration of fermentation was decreased to 16 hours. The complete data are given in Table IX, run 2.

The form of the lactic acid as determined at the end of the fermentation was pure levo which indicated a pure *L. delbrückii* fermentation. Absence of contamination during the fermentation was further confirmed by plating the mash on

TABLE IX. DATA OF LARGE-SCALE FERMENTATIONS[a]

	Run 1	Run 2[b]
Molasses used, lb.	120.2	100.0
Sugar content of molasses, %	55.9	59.5
Malt sprouts, lb.	14.7	14.5
$CaCO_3$ added, lb.	33.0	32.5
Total volume, gal.	63.8	63.0
Duration of fermentation, hr.	21	16
Initial sugar concn., g./100 cc.	12.6	11.32
Final sugar concn., g./100 cc.	1.10	1.46
Fermentation, %	91.3	87.0
Lactic acid, g./100 cc.	11.0	9.35
Yield, % sugar fermented	95.7	95.0
Yield, % sugar in molasses	87.3	82.6
Sp. rotation[c] of Ca lactate (4%), $[\alpha]_D$	+6.16	+6.06
Form of lactic acid	levo	levo

[a] *L. d. g*, used; 44–46° C.
[b] Puerto Rican blackstrap molasses.
[c] Value of $[\alpha]_D$ for calcium lactate is 6.13 (*3*).

malt sprouts–glucose–agar; only typical *L. delbrückii* colonies developed. On standing, the lactic acid tended to become inactive. This was probably caused by the racemase of other organisms which would have a chance to grow after the fermentation was over. Many bacteria are known to produce this enzyme (*10*), and in mixed cultures the lactic acid consists of equal quantities of the dextro and levo enantiomorphs.

Discussion

Aside from the strain of bacteria and the effect of temperature, the amount of nutrients (e. g., malt sprouts) is apparently the main factor governing the rate of lactic fermentation. In the fermentation of glucose, a number of nutrient sources, especially grain residue, can be used to supplement malt sprouts, but in fermentation of molasses, malt sprouts alone serve as the proper nutrient source. The rapid fermentation in unheated medium shows that malt sprouts contain heat-labile substances which can greatly stimulate the lactic fermentation.

For the production of edible dextrolactic acid, *L. helveticus* seems highly efficient for glucose fermentation, whereas the aerobic spore-forming cultures (E and 6-6) prove to be proper organisms for molasses fermentation. Sterile conditions are necessary for these fermentations.

For the production of low-grade acid for industrial uses, the form of acid is not important. The proposed rapid fermentation of unheated molasses by *L. delbrückii* shows decided advantages over the usual commercial process of slow fermentation of pasteurized mash in open tanks. These advantages are simple operation, ease of control (e. g., of contamination, slowing down of the fermentation, etc.), saving of time, and saving of labor. With the aerobic bacilli under proper conditions, lactic acid containing 90 per cent of the dextro form can also be produced from unheated materials. It is possible to reduce the malt sprouts concentration to 1.0 or 1.5 per cent and to run the fermentation at 14–15 per cent sugar concentrations without much difficulty. This means that malt sprouts at a rate below 10 per cent of the sugar is sufficient for fermentation, which is highly desirable in commercial practice.

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Viscosity of Gases and Vapors at High Pressures

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THE rapid development of high-pressure processes has increased the need for methods of equipment design which are applicable to any fluid. Such methods require, first, a knowledge of the effect of pressure on the physical properties of fluids. Considerable information is available relative to the effect of pressure on the density of gases and vapors, and methods of predicting this effect based on the concept of corresponding states (*4*, *19*) have been in use for some time. Similar methods are needed for viscosity, thermal conductivity, and diffusivity.

A limited amount of data is available on the viscosity of gases and vapors at high pressures. These indicate that variations with pressure at constant temperature sometimes amount to several hundred per cent. These variations are important where they influence rates of fluid flow and rates of heat transfer and diffusion.

Methods and equations for expressing the effect of pressure on the viscosity of gases and vapors have been suggested by Meyer (*7*), Batschinski (*1*), Boyd (*2*), and many others. These equations all contain arbitrary constants which must be evaluated by measurements at high pressures. They are therefore suited for interpolating and extending existing data obtained under high-pressure conditions rather than for predicting the viscosity where no pressure-viscosity data exist. The method to be described predicts the viscosity of gases and vapors at high pressures and requires only the viscosity at atmospheric pressure and the critical temperature and pressure of the pure compound. The method follows logically from the concept of viscosity used in the kinetic theory of gases and by analogy to the approximate equality of the compressibility factors for a wide variety of compounds at equal reduced temperatures and pressures. Instead of the