meter temperature was obtained by increase in weight of the calcium chloride tube. This volume of air was then converted to the exit-air temperature and the relative humidity (Grosvenor) thus obtained.

Results

It will readily be seen that water flow was of greater importance than the other variables. The humidity of the exit air increased very rapidly at first, passed through a transition period, and thereafter rose very slowly. Since the power required to circulate the water increased more rapidly than the rate of circulation increased the optimum conditions for operation occur in the transition region, indicated in the graphs by the rapid change in curvature. Under these conditions the relative humidity was about 90 per cent.

Literature Cited

(1) Walker, Lewis, and McAdams, "Principles of Chemical Engineering," p. 391, McGraw-Hill, 1927.

Continuous Fermentation in the Production of Lactic Acid[1]

E. O. Whittier and L. A. Rogers

RESEARCH LABORATORIES, BUREAU OF DAIRY INDUSTRY, DEPARTMENT OF AGRICULTURE, WASHINGTON, D. C.

A method for continuous lactic acid fermentation of the lactose of sweet whey has been devised and operated on both the laboratory and the plant scale. This method with suitable modifications should be applicable to other industrial fermentations.

THE output of plants employing industrial fermentation processes is limited by the size of the fermentation vats and the time required for the cycle of filling, sterilizing, actual fermentation, emptying, and cleaning. Since a complete cycle may in some cases be as long as ten days, the vat capacity in many such plants is necessarily made large.

Efforts have been made to reduce the length of the operating cycle by the use of more active bacteria and special treatments to accelerate the chemical action of the bacteria. These means have been found to be valuable, but to a limited degree.

During the time required for cleaning, sterilizing, and emptying the equipment and for the growth of the inoculating organism to its maximum effective numbers, very little actual fermentation takes place. If these time-consuming factors could be eliminated and the actual fermentation process carried on continuously, the ratio of equipment cost to production could be considerably reduced.

When a suitable medium is inoculated with a culture of the lactic type, growth and fermentation pass through a definite series of phases. There is first the lag period, in which certain physiological changes take place in the cell but little or no multiplication occurs. This is followed by a period of active multiplication and fermentation until the normal population is reached and various factors come into play to limit further activity of the cells. If transfers are made into new medium at the right stage of the growth curve, the lag period may be greatly reduced or entirely eliminated. The method described in this paper of providing the culture with a continuous flow of fresh materials is equivalent to an infinitely rapid transfer and maintains the culture at its most active phase.

The authors have applied the idea of continuous fermentation to the production of lactic acid from the lactose of whey, with the hope that their results may be applied, not only in lactic acid production, but also, with suitable modifications, to other commercial fermentations.

Theoretical

The fermentation of sugars to lactic acid is frequently encountered. The souring of milk and the production of sauerkraut are common examples. The reaction involved is, if we disregard the probable formation of intermediate products, of the general type,

$$C_6H_{12}O_6 \longrightarrow 2CH_3.CHOH.COOH$$

When the better known organisms are the causative agents, the proportion of side products is very small, consisting of volatile acids of low molecular weight, frequently acetic acid.

Of the large number of organisms known to produce lactic acid to some extent, the best known are the common *Streptococcus lactis* of souring milk and several of the lactobacilli such as *L. bulgaricus* and *L. casei*. The lactobacilli have the advantage of being able to continue the fermentation until a pH value is reached approximately one unit lower than is possible when *S. lactis* is used. This advantage becomes of greater importance when the acid is partly neutralized from time to time, as is done in the commercial fermentation. With the accumulation of lactates in the solution, a limiting concentration of undissociated lactic acid becomes more definitely the factor inhibiting the fermentation and thus the actual limiting pH value gradually increases (*1*, *2*). The higher the pH value in the fermenting solution, the greater the likelihood that contaminating organisms will succeed in utilizing part of the sugar and in converting it to undesired products, such as butyric acid. Hence the advantage of using an organism able to withstand high acidity and high concentration of undissociated lactic acid, when practically complete utilization of sugar is desired. A mycoderm in a culture of a lactobacillus has the associative effect of accelerating the action of the lactobacillus and is frequently used for this purpose.

The abandonment of preliminary sterilization of equipment and media in industrial sugar fermentations is possible only when other means can be used successfully to prevent loss of sugar and formation of undesired products. If the fermentation can be carried out within pH and temperature ranges unfavorable to foreign fermentations, it is a very desirable situation. This is the case in the lactic fermentation. At 44° C. and between pH 5.0 and pH 5.8 the lactic fermentation may be kept clean of active contamination.

Preservation of unsterilized sugar media may be accomplished by use of low temperatures, high acidity, or high alkalinity. Obviously, the means used to bring the preserved media to the proper condition for fermentation should not

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introduce any complication into the process. If acid is the preserving agent, it would seem desirable to develop it by the fermentation itself. If alkali is the preservative, it should be the alkali used for neutralization and the medium thus preserved should be fed to the fermentation vat in lieu of both medium and part or all of the neutralizing agent.

Experimental

The first results obtained were a by-product of successful attempts to maintain bacterial cultures at normal population in a continuous flow of medium (*3*). Sterilized 6 per cent lactose broth and suspended calcium carbonate were fed separately and continuously to a flask in which a mixed culture of a lactobacillus and a mycoderm was growing. The overflow was collected, measured, and analyzed for lactic acid and unfermented sugar. Rate of feed and other conditions were varied, both intentionally and accidentally, so that continuously uniform results were not obtained; but it was established that in 24 hours, under favorable conditions, the sugar in a volume of broth equal to the working volume of the flask could be practically entirely fermented. The average yield of lactic acid was approximately 80 per cent of theoretical. This run was continued for 20 days.

A similar fermentation was carried out later in a glass-lined tank of 30 gallons' working capacity. The tank was sterilized previous to the run. Swiss cheese whey was fed through a tubular preheater which continuously sterilized the whey under pressure at 120° C. The rate of flow that was maintained insured an exposure of the whey to this temperature for at least 30 minutes. Dry calcium carbonate was fed continuously by means of an endless-chain device. This run went smoothly for 2 days and then the sterilizer became clogged with coagulated milk proteins. The run was continued successfully for another 24 hours with raw whey. It was demonstrated that there is no inherent difficulty in carrying out sterilization continuously. The trouble was due to the heat-coagulability of certain components of the raw material.

Another run was made in the same equipment over a period of 14 days. Raw whey pretreated with hydrated lime as a temporary preservative was fed to the fermentation tank. Dry hydrated lime was fed at such a rate as would maintain the reaction in a range favorable to lactic fermentation but unfavorable to butyric acid production. It was again demonstrated that a volume of whey equal to the working volume of the fermentation vat could be practically entirely fermented in 24 hours under conditions of continuous flow. Crude calcium lactate was recovered from a 6-liter sample of the overflow and analyzed for its percentage of lactic acid. Calculations showed that the yield was 90 per cent of theoretical based on the lactose originally present.

Two runs each lasting a week were carried out in larger equipment at the Grove City Creamery, Grove City, Pa. A fermentation vat of 4500 pounds capacity was used and the product of the fermentation was isolated as crude calcium lactate. The earlier figures as to rate of fermentation and yield were substantiated.

Recommendations regarding equipment and procedure are outlined in the following paragraphs.

Recommended Equipment and Procedure

EQUIPMENT—*Whey storage.* Feeding constantly to the fermentation tank at a rate such that the contents of the fermentation tank will be displaced every 24 hours.

Lime reservoir and feeding device. Feeding hydrated lime at such a rate as to maintain a reaction between pH 5.0 and 5.8 in the fermentation tank.

Fermentation tank. Insulated, covered, equipped for slow-speed agitation and means of maintaining temperature of contents at 43° ± 1° C.

Storage tank. One-fourth the capacity of the fermentation tank, receiving the overflow from the fermentation tank, delivering to the coagulation tank except when a charge is being coagulated.

Coagulation tank. Of the same (or half) working capacity as the fermentation tank, with facilities for heating contents to boiling, delivering via filter press or centrifuge to evaporator.

Evaporator. Single-effect, discharging to crystallizing pans.

MATERIALS PER 24 HOURS—*Sweet whey.* Quantity equal to the working capacity of the fermentation vat.

Hydrated lime. Four per cent of the weight of the whey.

PRODUCT PER 24 HOURS—*Wet cake.* Ten per cent of the weight of the whey, containing 50 per cent calcium lactate, equivalent to 40 per cent lactic acid or 80 per cent of the theoretical yield.

PROCEDURE—The fermentation tank, clean but not necessarily sterile, is filled with sweet whey to the operating level. The whey should be brought to 43° C. and the contents of the fermentation tank should be kept as close as possible to this temperature during operation. The whey is inoculated with an active culture of a lactobacillus and the agitation started. As soon as the reaction is at pH 5.0, which should require about 12 hours, the lime feed is started and regulated to hold the reaction between pH 5.0 and 5.8. After about 24 hours an analysis should be made for lactose, and subsequent analyses should be made every 12 hours until the fermenting whey contains less than 1.0 per cent lactose. This point should be reached in from 48 to 72 hours after inoculation. The whey feed is then started. A volume of whey equal to the volume of the fermentation tank should be fed each 24 hours at first and until a more efficient rate can be determined. The whey in storage should be pretreated with hydrated lime to prevent undesired fermentations.

The necessary controls are the following: The whey in storage should be tested with phenolphthalein paper after each addition of a new supply. If the test paper does not show pink, hydrated lime should be added until it will. The reaction of the contents of the fermentation tank should be tested frequently to insure that the pH value is kept between 5.0 and 5.8. Bromocresol green paper may be used (*4*). It gives a green color in this range. Potentiometric measurements may be desirable at first. The lactose percentage of the overflowing whey as it comes from the fermentation tank should be determined once each day and the whey feed gaged thereby to give maximum fermentation efficiency. Probably 0.5 per cent lactose is about the proper goal, since fermentation continues in the coagulation tank.

As often as sufficient fermented whey collects, it is heated to boiling in the coagulation tank and the boiling is continued until the protein is completely coagulated. Lime is then added till the solution turns litmus paper blue to convert the excess lactic acid to calcium lactate. The coagulum is then removed by filtration and the solution is evaporated to a density of about 25° Bé. (10 to 1). The hot liquid is run into crystallizing pans. When it is cold, the cake of calcium lactate is broken up and dried.

If it is desired to purify the calcium lactate somewhat, the condensing ratio may be made 5 to 1. Cooling will then give a semi-fluid crystal mass, which may be filtered in a centrifuge and lightly washed. The filtrate and washings may be returned to the evaporator or, if the lactose content is appreciable, fed to the fermentation vat.

In case it is desired to make lactic acid directly, sulfuric acid may be added to the hot coagulated whey to convert

the calcium lactate to the free acid and then the coagulum and calcium sulfate filtered off together. The filtered lactic acid solution may then be evaporated to the desired concentration.

Application to Other Fermentations

The method of using alkali as a preservative for medium in storage is obviously particularly applicable to fermentations producing acid. The procedure of inoculating the medium in storage with the culture used in the fermentation, whereby the acidity of the partially fermented medium acts as its own preservative, is another possible means of accomplishing the same purpose. For many fermentations, however, it would be necessary to sterilize raw medium either continuously or intermittently. The prevention of contamination of the fermenting medium with organisms producing undesirable substances may frequently be prevented by proper control of H-ion concentration. It must be remembered in this connection that the growth of acid-producing organisms is usually inhibited by the undissociated form of the acid produced, the concentration of which is a function of both the H-ion concentration and the total concentration of the acid (*1*).

Literature Cited

(1) Kolthoff, *Tijdschr. vergelijk. Geneeskunde*, **11**, 288 (1925).
(2) Rogers and Whittier, *J. Bact.*, **16**, 211 (1928), and unpublished work.
(3) Rogers and Whittier, *Ibid.*, **20**, 127 (1930).
(4) Whittier, *J. Dairy Sci.*, **14**, 26 (1931).

The Rate of Calcination of Limestone[1,2]

C. C. Furnas

North Central Experiment Station, U. S. Bureau of Mines, Minneapolis, Minn.

Calcination of limestone takes place in a very narrow zone which is the phase boundary between calcium carbonate and calcium oxide. This zone advances from the outside to the inside of the piece at a constant rate for each temperature, independently of particle size or degree of calcination. Curves and data are given for rates of calcination and temperature histories of particles. Most of the resistance to heat transfer into the piece appears to be in the narrow zone of calcination, and not in the body of the calcined material. The calcination data may be used to determine the surface area of the particles.

IN THE United States alone approximately 5 million tons of limestone per year are burned for use as lime; 20 million tons are calcined in metallurgical furnaces to be used as flux, and several times this much, of the order of 75 to 100 million tons, are calcined in the manufacture of cement. Yet there is but little information available on the rate at which calcination takes place. As far as the author knows, there are only three published investigations on the matter. In the first (*2*), the data were very meager and indefinite. The size of material used was not specified, and other attending conditions of the system were not mentioned.

In the second (*3*) it was apparently assumed that heat transfer and calcination were synonymous or, more correctly, that they occurred together, which is not necessarily true. A particle of limestone may acquire a calcining temperature and remain that way for a long time before calcination actually takes place. This second paper, then, is a clever correlation of the data of temperature acquisition in limestone, but its applicability to lime burning is doubtful.

In the third piece of work (*7*) nine different limestones were calcined at various temperatures for varying lengths of time. The data show the relative ease with which different limestones may calcine, but the actual rates reported are hardly applicable to burning limestone in practice, for the material used was mostly of small size (−4 mesh) and the calcining was done in porcelain crucibles heated from the outside. The results obtained are really those for a confined bed of fine material and do not give much information as to the specific rate of calcination within the limestone itself. It may be said, however, that none of the data reported in Linzell's paper are qualitatively different from those of this present paper.

Because of its importance in the heat-transfer phenomena of the blast furnace, the Bureau of Mines has undertaken a short study of the rate of calcination of limestone, and the data are reported in this paper.

Summary of Results

The data obtained may be summarized in such a simple manner that it seems best to present a statement of the results first and then to offer the experimental proof just as in geometry a theorem is first stated and then proved.

Calcination proceeds only from the outside of the piece inwards over a very narrow zone, practically a line. The data reported in this paper are given as rates of advance of this line of calcination from the outside to the inside of the piece. As a first approximation, this line of calcination advances at a constant linear rate (measured in centimeters per hour), dependent only on the temperature of the surroundings and independent of size or shape of particle, degree of calcination, or amount of previous heating.

The summarized data are given in Figure 1. The equation of the plotted curve is very simple, being

$$\log_{10} R = 0.003145t - 3.3085 \qquad (1)$$

where R = rate of advance of the line of calcination in centimeters per hour
t = temperature, ° C.

This equation is purely empirical and no theoretical importance should be attached to it.

Obviously, since the rate of penetration of the line of calcination is constant throughout the entire period, the length of time required to calcine is directly proportional to the size of the piece. In Figure 2 are given computed curves for the time required for complete calcination of pieces of different sizes at different temperatures. The size is defined as the greatest thickness of the piece, where thickness is defined as the smallest of the three dimensions as contrasted with breadth and length.

This makes the problem of time of calcination a very simple one. Undoubtedly, particle size and degree of calcination do have an effect on the rate, but under the conditions of size and temperature studies these effects, if they were there, were

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