Comparison of Gamma Radiation and Heat for Sterilization of Fermentation Mashes

ROBERT A. GILLIES[1] and LLOYD L. KEMPE

Bacteriology Department, University of Michigan, Ann Arbor, Mich.

Radiation sterilization of fermentation media using radiation from cobalt-60 was investigated as an alternative to the usual heat sterilization procedure. Increases of 40 to 73% in the rate of fermentation and no change in the yield were found when malt sprouts were sterilized by irradiation rather than heat. A decrease in both the rate of fermentation and in the yield was observed when corn steep liquor was substituted for the malt sprouts. Sterilization of the complete mash with irradiation was found to be undesirable, as a marked decrease in the rate of fermentation occurred. Gamma radiation may be valuable in situations where heat causes undue damage to the media constituents or in fermentations that cannot currently be conducted under aseptic conditions. The rate of product formation in some biological processes may be increased by the use of irradiation for sterilization.

THE USE OF IONIZING RADIATIONS has been suggested as an alternative for heat sterilization in the preparation of fermentation mashes (*13*), but no experimental data have been reported for this application. Therefore, the characteristics of fermentations in which the nutrilites had been sterilized in this manner were investigated. Nutrilites include all organic substances that are important in minute amounts for the nutrition of microorganisms (*17*).

For pure culture fermentations, mashes are sterilized with steam and no reliable alternatives are available. This is unfortunate because heat often damages some of the constituents of the medium. Pan and coworkers (*11*) have shown that the yield and rate of lactic acid production by fermentation vary inversely with the severity of the heat treatment used in preparing the mash. Furthermore, some industrial fermentations are not conducted under aseptic conditions. This is necessary, as heat would either destroy the nutrilites employed or would inactivate essential enzymes present in raw materials such as malt. Because there is no appreciable temperature rise accompanying sterilization with ionizing radiations the important heat-labile substances or enzymes would probably not be damaged.

Lactic acid organisms require a great variety of nutrilites (*12*) and are sensitive to small changes in the nutrilite content of the media. Consequently, fermentations employing these organisms should offer a sensitive test system, as radiation damage of the nutrilites would be reflected by changes in the yield and rate of fermentation.

[1] Present address, California Packing Corp., 215 Fremont St., San Francisco 19, Calif.

Apparatus and Methods

A diagram of the equipment is shown in Figure 1. The fermentor and automatic pH control apparatus were similar to those described by Kempe (*9*), but modifications permitted sterilization of the fermentor in order to start with an initially sterile system.

The rate-recording equipment was the same in principle as that used by Finn (*3*) with a kymograph drum rotated by an electric clock motor being used instead of the modified Stevens hydrograph. This equipment continuously measured and recorded the rate of addition of neutralizing solution. Graphical differentiation of the curve recorded on the kymograph chart showed the instantaneous rate of acid formation at any time during the fermentation, as at constant pH the amount of alkali used is equivalent to the amount of acid produced. From analytical data and this fermentation-rate curve, the rate of lactic acid production per unit time was calculated.

Rates of lactic acid formation are expressed as grams of lactic acid formed per 100 ml. of mash per hour. Only the maximum rates of lactic acid formation were used in comparing fermentations, as the time after inoculation, until the maximum rate was reached, was constant for any controlled pH level regardless of the nutrilite concentration. Suitable corrections were made for neutralized acid that was not lactic acid. The amount of undissociated lactic acid present in the media at the pH values used was calculated to be less than 2%, so it was neglected in the rate calculations. The specific details of the rate calculations as well as the equipment have been described (*7*).

The basal fermentation mash consisted of 200 grams of dextrose, 70 ml. of salt solutions, and 3930 ml. of dis-

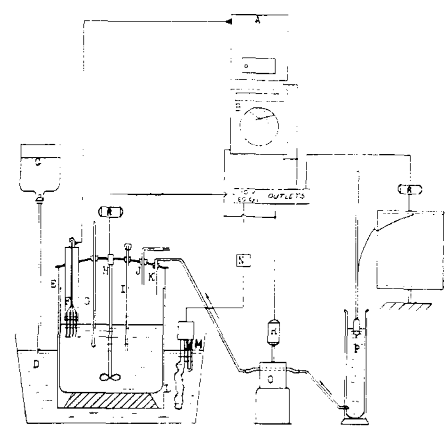

Figure 1. Equipment for fermentation, pH control, and rate recording

A. Beckman pH meter
B. Bristol Pyromaster potentiometer
C. Water level control system
D. Water bath
E. Fermentor
F. Electrode assembly
G. Thermometer
H. Agitator
I. Inoculating and sampling tube
J. Optional addition line
K. Alkali addition line
L. Electric heater coil
M. Thermoregulator
N. Thermostatic delay mechanism
O. Alkali pump
P. Alkali reservoir
Q. Rate recording drum
R. Electric motors

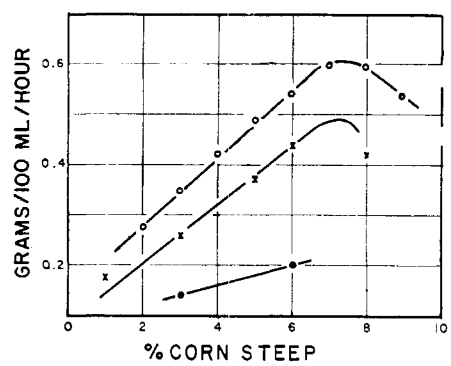

Figure 2. Maximum rates of lactic acid production in *Lactobacillus delbrueckii* fermentations of corn steep liquor–glucose mashes at pH 5.5 and 43° C.

—O— Heated corn steep
—X— Irradiated corn steep
—●— Entire mash irradiated

tilled water. The salt solutions described by Kempe, Halvorson, and Piret (*9*) were employed, except that ammonium dibasic phosphate and sodium dibasic phosphate in amounts of 125 grams per liter of stock solution were substituted for the sodium hexametaphosphate. Appropriate amounts of corn steep liquor or malt sprouts were added to the basal mash prior to sterilizing with heat. In cases where the nutrilite sources were sterilized by irradiation, the nutrilites were added aseptically to the basal media which had been previously heat sterilized.

Fermentations were carried out in the following general manner. The basal medium and nutrilites were mixed, the pH was adjusted to 6.4, and the mash was sterilized by autoclaving at 15 pounds per square inch gage steam pressure for 45 minutes. After cooling the medium

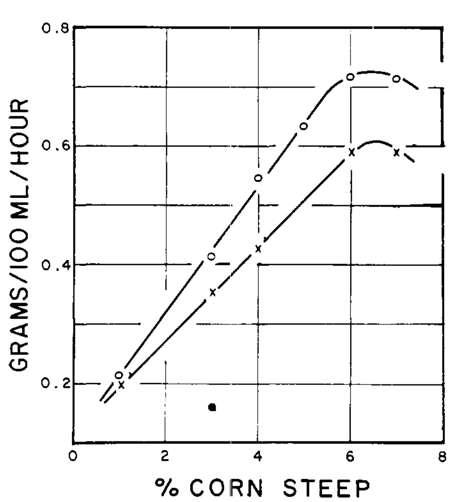

Figure 3. Maximum rates of lactic acid production in *Lactobacillus delbrueckii* fermentations of corn steep liquor–glucose mashes at pH 6.2 and 43° C.

—O— Heated corn steep
—X— Irradiated corn steep
—●— Entire mash irradiated

to the control temperature of 43° C., the equipment illustrated in Figure 1 was emplaced. The pH was adjusted to a value just above the control pH chosen for the fermentation and 60 ml. of an actively growing culture of *Lactobacillus delbrueckii* NRRL-B445 were added aseptically to initiate the fermentation.

As acid was produced the automatic pH regulating system caused 3.4*N* alkali (equimolar concentrations of sodium hydroxide and potassium carbonate) to be pumped into the fermentor. Fermentations were considered complete when the addition of alkali ceased. At this time, a 100-ml. sample was withdrawn from the fermentor; this was acidified with sulfuric acid, frozen, and kept in this condition until sugar and lactic acid analyses were made (*8*).

Gamma radiation was supplied by the cobalt-60 source at the University of Michigan (*1, 10*). This source developed 2.2 × 10^5 rep (roentgens-equivalent-physical) per hour in the center well, where the irradiations were carried out. A rep is that amount of radiation which will result in the absorption of 93 ergs per gram of tissue. Radiation doses were determined by the ferrous sulfate method of Weiss (*16*).

Samples of malt sprouts and corn steep liquor were irradiated in glass containers. A radiation dose of 2.5 megarep was employed unless otherwise stated. Sterility tests were carried out on the irradiated samples and this dose was found sufficient to accomplish sterilization.

Sugar was determined by the method of Shaffer and Somogyi (*14*) using the copper reagent described by Somogyi (*15*) in 1952. The amount of fermentable and nonfermentable reducing sugar initially present in the mash was known accurately from the amount of glucose added and from analyses of the corn steep liquor and malt sprouts used to prepare the mashes.

The samples were analyzed for lactic acid by the method of Friedemann and Graeser (*5*), using colloidal manganese dioxide as the oxidizing agent (*6*). The customary precautions were taken to remove the interfering substances by copper hydroxide precipitation (*4*). Standard lactic acid solutions were prepared from lithium lactate or calcium lactate.

Yields of lactic acid are based upon the initial available fermentable sugar and fermentation efficiency is expressed in terms of lactic acid yield and sugar conversion.

Experimental and Discussion

Two series of fermentations were carried out using corn steep liquor. These were conducted at pH 5.5 and pH 6.2. Independent variables at each pH level were the amount of corn steep

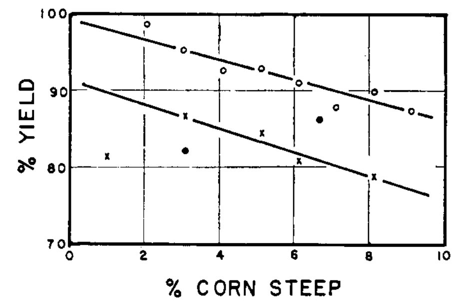

Figure 4. Yields of lactic acid in *Lactobacillus delbrueckii* fermentations of corn steep liquor–glucose mashes at pH 5.5 and 43° C.

—O— Heated corn steep
—X— Irradiated corn steep
—●— Entire mash irradiated

liquor and the method of sterilization. Figures 2 and 3 show the maximum rates of lactic acid production for the two series as a function of the amount of corn steep liquor employed. With concentrations of less than 7% corn steep liquor, the fermentation rate increased with increasing amounts of this nutrilite. However, at both pH levels mashes employing irradiated corn steep liquor showed consistently lower fermentation rates than those which had been heat sterilized. Furthermore, when the entire mash was irradiated, especially low rates were observed when compared with those found when the corn steep liquor was irradiated independently. As expected, the fermentation rates at pH 6.2 were higher than those for corresponding fermentation at pH 5.5 (*3*).

Figures 4 and 5 show the yields of lactic acid for the two series as a function of the concentration of corn steep liquor and indicate that the yield of lactic acid decreased with increasing amounts of corn steep liquor in the medium. At both pH levels, however, mashes containing irradiated corn steep liquor showed consistently lower yields than those which had been heat sterilized. Also, slightly lower yields were ob-

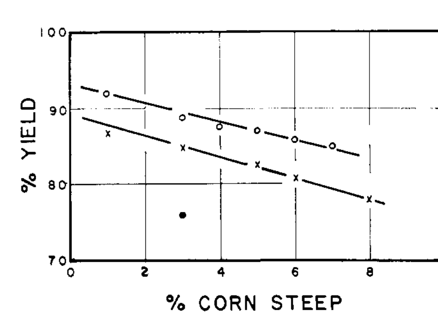

Figure 5. Yields of lactic acid in *Lactobacillus delbrueckii* fermentations of corn steep liquor–glucose mashes at pH 6.2 and 43° C.

—O— Heated corn steep
—X— Irradiated corn steep
—●— Entire mash irradiated

tained when the mash containing the corn steep liquor was irradiated.

In the heat-sterilized mashes, the amount of fermentable sugar utilized decreased in both series of fermentations as the corn steep liquor concentration was increased. Also the fermentable sugar utilized remained constant when irradiated nutrilites were used. This indicated that the amount of side products formed increased with the corn steep liquor concentration.

The results have shown that gamma radiation was more damaging to corn steep liquor than heat. However, corn steep liquor is subjected to high temperatures in its production which would have already destroyed the heat-labile substances. As radiation and heat may cause different kinds of damage, a decrease in the rate of fermentation would be expected by virtue of the increased number of nutrilites affected. The evidence with corn steep liquor supports this view but does not entirely rule out the possibility that the mechanism of nutrilite inactivation could be the same for heat and irradiation, with the difference being only quantitative in nature. This suggested the use of a natural source of nutrilites such as malt sprouts, as these would not have been damaged by previous heat treatment and thus should contain most of their initial complement of nutrilites.

Two series of fermentations were carried out using malt sprouts to parallel the corn steep liquor work. These were at pH 5.5 and pH 6.2 and the independent variables were the amount of malt sprouts and the method of sterilization. Table I shows the results of these two series of fermentations. There was an increase in fermentation rate when the malt sprout concentration in the mash was increased from 2 to 3%. It is significant that at both pH levels fermentations employing irradiated or unsterilized malt sprouts showed consistently higher fermentation rates of 40 to 73% than in mashes which had been heat sterilized. The irradiated malt sprouts allowed higher fermentation rates than the unsterilized malt sprouts. This would indicate that the malt sprouts were not materially affected by the radiation.

In every case 78 to 85% of the fermentable sugar was converted to lactic acid. This indicates that the yields of lactic acid obtained and the sugar utilization were not affected by the method of sterilization.

Increasing the irradiation dose from 2.5 megarep to 4.5 megarep decreased the rate of fermentation only slightly and the rate was still substantially higher than could be obtained with heat-sterilized malt sprouts. Therefore, a relatively high irradiation dose did not materially damage the malt sprouts with regard to their efficiency as a nutrilite source in fermentation mashes.

For industrial application it is fortunate that less damage to the nutrilites was found when they were sterilized separately with irradiation, because it would be easier and less expensive to sterilize small volumes of nutrilites with ionizing radiation than to treat large volumes of mash similarly. In this connection Elliott, Brownell, and Gross (*2*) found that, when dilute media were irradiated, the media failed to support the growth of *Tetrahymena pyriformis*. However, when the components of the media were irradiated independently, the adverse effects of irradiation were much less marked.

The results indicate that gamma radiation may be valuable in situations where heat causes undue damage to the media constituents or in fermentations that cannot be conducted under aseptic conditions. Furthermore, the rate of product formation in some biological processes may be increased by the use of irradiation for sterilization as shown here in fermentations employing malt sprouts.

The future application of ionizing radiations for peacetime purposes is uncertain. However, both industry and government are intensely interested in their potentialities.

Table I. Rate of Acid Production and Lactic Acid Yield for *Lactobacillus delbrueckii* Fermentations of Malt Sprout-Glucose Mashes at 43° C.

Run	pH of Run	Malt Sprout Concn., %	Method of Sterilization	Lactic Acid Yield, %	Maximum Rate of Acid Formation, Gm./100 Ml./Hr. (as lactic)	Increase in Rate of Acid Production[a], %
73	6.2	2	Heat	84.8	0.313	..
74	6.2	2	Irradiation	81.4	0.542	73
87	6.2	2	Unsterilized	80.3	0.513	64
75	6.2	3	Heat	78.4	0.436	..
76	6.2	3	Irradiation	84.9	0.702	61
88	6.2	3	Unsterilized	79.2	0.645	48
80	6.2	3	Irradiation[b]	80.8	0.669	53.5
82	5.5	2	Heat	81.2	0.300	..
78	5.5	2	Irradiation	78.0	0.424	41.5
86	5.5	2	Unsterilized	82.7	0.436	45.5
81	5.5	3	Heat	79.2	0.398	..
77	5.5	3	Irradiation	89.1	0.597	50
85	5.5	3	Unsterilized	81.1	0.570	43
83	5.5	3	Irradiation[b]	79.3	0.537	35

[a] Increase in rate of acid production in fermentations employing irradiated malt sprouts as compared to rates of acid production in fermentations employing heated malt sprouts.
[b] Irradiation dosage used was 4,500,000 rep; all other samples received 2,500,000 rep.

Acknowledgment

The authors gratefully thank the Michigan Memorial Phoenix Project for making the irradiation facilities available and J. T. Graikoski for his assistance in irradiating the samples.

Literature Cited

(1) Brownell, L. E., Meinke, W. W., Nehemias, J. V., Coleman, E. W., *Chem. Eng. Progr.* **49,** 569–76 (1953).
(2) Elliott, A. M., Brownell, L. E., Gross, J. A., *J. Protozool.* **1,** 193–9 (1954).
(3) Finn, R. K., Halvorson, H. O., Piret, E. L., *Ind. Eng. Chem.* **42,** 1857–61 (1950).
(4) Freeman, G. G., Morrison, R. T., *Analyst* **71,** 511–20 (1946).
(5) Friedemann, T. E., Graeser, J. B., *J. Biol. Chem.* **100,** 291–308 (1933).
(6) Friedemann, T. E., Kendall, A. I., *Ibid.*, **82,** 23–43 (1929).
(7) Gillies, R. A., Ph. D. thesis, University of Michigan, Ann Arbor, Mich., 1955.
(8) Kempe, L. L., Ph. D. thesis, University of Minnesota, Minneapolis, Minn., 1948.
(9) Kempe, L. L., Halvorson, H. O., Piret, E. L., *Ind. Eng. Chem.* **42,** 1852–7 (1950).
(10) Nehemias, J. V., Brownell, L. E., Meinke, W. W., Coleman, E. W., *Am. J. Phys.* **22,** 88–92 (1954).
(11) Pan, S. C., Peterson, W. H., Johnson, M. J., *Ind. Eng. Chem.* **32,** 709–14 (1940).
(12) Peters, V. J., Snell, E. E., *J. Bacteriol.* **67,** 69–75 (1954).
(13) Pfeifer, V. F., Vojnovich, C., *Ind. Eng. Chem.* **44,** 1940–6 (1952).
(14) Shaffer, P. A., Somogyi, M., *J. Biol. Chem.* **100,** 695–713 (1933).
(15) Somogyi, M., *Ibid.*, **195,** 19–23 (1952).
(16) Weiss, J., *Nucleonics* **10,** 28–31 (1952).
(17) Williams, R. J., *Biol. Revs., Cambridge Phil. Soc.* **16,** 49 (1941).

Received for review July 13, 1956. Accepted February 23, 1957. Division of Agricultural and Food Chemistry, 127th Meeting, ACS, Cincinnati, Ohio, March-April 1955. Submitted by R. A. Gillies in partial fulfillment of the requirements for the Ph.D. degree in the University of Michigan.