MICROORGANISMS AND HEAT PRODUCTION IN SILAGE FERMENTATION

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INTRODUCTION

Heat formation is characteristic of silage fermentation. The amount of heat liberated varies as affected by different factors. The average temperature limits of fermenting forage in the center of the silo range between 30° and 40° C. This represents the temperature of normal fermenting silage. There is a marked difference in the degree of heat noted between the fermenting forage at the top and center of the silo. The amount of oxygen present governs to a large extent the amount of heat formed. More oxygen is present in the surface forage than in the center of the silo, which accounts for the higher temperature at the top of the silo.

In European countries silage is referred to as sweet or sour silage, the amount of heat produced governing the type of fermentation. Sweet silage results when the temperature rises to 50° C., while sour silage is formed if the temperature does not exceed 40° C.

PREVIOUS INVESTIGATIONS

Heat production in fermenting forage has never been satisfactorily explained. While it can not be interpreted from previous investigations that this heating results from the major fermentation processes in silage, it seems highly probable. Investigators differ widely regarding the causative agents concerned in silage ripening. Their conclusions may be briefly summarized as follows: Fermentation processes in silage are due to—

(1) Intramolecular respiration of the tissue cells.

(2) Intramolecular respiration of the tissue cells, and microorganisms. The former action is essential, while the latter is of secondary importance.

(3) Microorganisms.

Fry $(7)^1$ and Babcock and Russell (1, 2, 3) support the first hypothesis, contending that heat production results from the activity of the plant cells. Russell (12), Kayser (10, p. 367-390), and Samarani (13) state that intramolecular respiration is the most important, but that certain bacilli exert a secondary action. Wollny (15, p. 444-460) likewise believes that heat production is caused by the activity of the plant cells, but that the lactic and acetic acids formed are from the action of bacteria.

¹ Reference is made by number to "Literature cited, pp. 82-83.

Journal of Agricultural Research, Washington, D. C. ip Vol. X, No. 2 July 9, 1917 Key No. Kans.---7 Esten and Mason (6) conclude that microorganisms are the predominating factor in silage ripening, but that heat formation is the result of the activity of plant cells. On the other hand, Burrill (4) states that the high temperature attained in his investigations was caused by two or more species of rodlike bacteria to which butyric-acid production could be attributed. Lafar (11, p. 199–203) and Conn (5, p. 112–114) discuss heat production in silage as the result of bacterial action. Griffiths (8) describes several groups of bacteria, important in silage fermentation, but makes no statement regarding silage heating. In a recent article Hunter and Bushnell (9) show that microorganisms are the essential cause of silage ripening. Sherman (14) suggests the probable importance of acid-producing bacilli in the curing of corn silage.

Evidence is sufficient to warrant the assumption that microorganisms are the influential factor in forage fermentation. It is logical to assume, therefore, that the heating of ripening forage is a result of their activities. With this hypothesis in mind the following investigations were planned.

METHOD OF PROCEDURE

Alfalfa, corn, cane, and kafir forage, siloed under laboratory conditions, were used for silage production. The forage was finely cut and packed tightly in 1-quart thermos fruit jars and hermetically sealed. Thermos jars were used in order to prevent as much heat radiation as possible. Care was taken each time to entirely fill the jars before sealing, as such air spaces would afford opportunity for the growth of molds. The heat thus liberated by their activities would offer a source of error in the interpretation of results. In order to bring heat radiation to a minimum, the thermos bottles were kept at a fairly uniform temperature. In a majority of the experiments this temperature ranged between 35° to 37° C. In a few cases, however, they were kept near 20°. The general course of action was the same in each case.

The temperature readings were determined by the use of thermometers and thermo-resistance coils. A type of thermometer was used which allowed the extended mercury end of the thermometer to be inserted to the center of the jar, while the graduations remained above the neck of the bottle. It was graduated to 0.1° C.

The resistance coil consisted of 40 feet of black-enameled magnet wire No. 36, wound around a small-sized spool. A thin coat of paraffin covered the coil in order to insure perfect insulation. The wire leads connecting the coil and resistance box were incased in small glass tubing from the coil to the outside of the thermos jar. This was to avoid breaking the threadlike wires, especially during the filling of the jars. Each was standardized with a thermometer and the resistance readings converted into degrees. The coils also registered to 0.1° C. All thermometers and resistance coils were standardized against each other, and the necessarv temperature corrections noted. The general plan of procedure for determining the relative importance of microorganisms and intramolecular respiration of the plant cells in the heating of siloed forage provided checks of different types on microbial activity. Heat production was observed in—

(a) Normal fermenting forage;

(b) Forage treated with a weak antiseptic;

(c) Forage treated with heat;

(d) Heated forage inoculated with bacteria;

(e) Cured or dried forage.

Normal fermentation, used as a check, was provided by siloing the untreated forage. When alfalfa was used, 2 to 5 per cent of cane sugar was added to supply an available source of carbohydrate for the ferments.

Forage treated with a weak antiseptic offered favorable conditions for intramolecular respiration of its tissue cells, while the action of the microbial flora was checked. Two to three per cent of chloroform was used for this purpose.

The action of both microorganisms and tissue enzyms was prevented by heating the forage for one to two hours at 100° C. In this way all plant enzyms and the majority of the essential microorganisms were killed. Forage thus made inert was treated with chloroform to check the action of any organisms not killed by the heat and those which entered during the siloing of the heated forage. Such treated forage was used chiefly as a control both for heat production and chemical changes.

Heated forage was also inoculated with a pure culture of the Bulgarianlike organism isolated from silage.

Cured and dried forage, to which the proper amount of moisture had been added to insure conditions for fermentation, was likewise siloed. Owing to the destruction of large quantities of plant enzyms by drying, such forage offered little or no opportunity for tissue activity.

Total acidity determinations were made by the method described by Hunter and Bushnell (9). The results are expressed in terms of lactic acid per gram of dry forage.

EXPERIMENTAL DATA

Only representative records of the different kinds of forage are reported from the large amount accumulated, as all exhibited the same essential characteristics. The temperature readings in each case are plotted as curves. Figures I to IO, inclusive, indicate the heat-producing ability of the different kinds of forage. The untreated and inoculated forage all exhibited a marked increase in acid production, while the chloroformed and heated samples exhibited no increase.

Good clean-flavored silage resulted in every instance from the fermentation of the untreated, green, cured, and inoculated forage. The treated



FIG. 1.—Curves representing the heat-producing ability of cured alfalfa, untreated and treated with chloroform.



FIG. 2.—Curves representing the heat-producing ability of dry kafir fodder, untreated and treated with chloroform.

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forage, that saturated with chloroform and that heated, exhibited no characteristics of silage. The total acidity determinations noted on figures 1, 7, 8, 9, and 10, respectively, indicate the fermentation ability of the various types of forage treated differently.



FIG. 3.—Curves representing the heat-producing ability of dry corn fodder, untreated and treated with chloroform and heat, respectively.

Heat production was only observed in the untreated and inoculated forage. The treated samples offered no indications of heating. The differences noted in the comparative amounts of heat production are probably due to the varying amounts of oxygen incorporated in the jars at the time of siloing the forage.





The temperature curve representing the chloroformed forage followed the curve of the heated forage and that of the outside temperature very closely. However, there will be noticed a rapid rise of the treatedforage curves the first few days, which at the first glance might signify heat production. This increase stops at a temperature corresponding

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FIG. 5.—Curves representing the heat-producing ability of green alfalfa, untreated and treated with chloroform and heat, respectively.



FIG. 6.—Curves representing the heat-producing ability of cured alfalfa, untreated and treated with chloroform and heat, respectively.



FIG. 7.—Curves representing the heat-producing ability of green alfalfa, untreated and treated with chloroform and heat, respectively,



FIG. 8.—Curves representing the heat-producing ability of green corn fodder, untreated and treated with chloroform and heat, respectively.



FIG. 9.—Curves representing the heat-producing ability of green kafir inoculated with Bacterium bulgaricus and treated with heat.





with the outside temperature and remains practically the same throughout the experiment. The rise of temperature therefore does not represent heat production in this case, but heat absorption. This is demonstrated by the results in figure 10. Here the temperatures of the untreated and inoculated forage at time of siloing were slightly lower than the temperature of the room $(36^{\circ} \text{ to } 37^{\circ} \text{ C.})$ in which the jars were kept, while the temperatures of the treated, chloroformed, and heated were higher. The records in figure 10 indicate a decrease in the temperature readings of both treated samples the first few days, until they corresponded with the temperature outside the jars. The untreated and inoculated samples, however, exhibited heat production, their curves showing a steady rise, exceeding the room temperature and followed by the customary decline.

The fact that dry forage will undergo normal silage fermentation when water is added is significant. Such material can offer no manifestation of cell respiration. However, microorganisms are present and silage is produced. The conclusions are plainly evident.

Temperature curves obtained from the fermentation of dried forage are noted in figures 1, 2, 3, and 6 and are comparable with the fermentation records of green forage.

It is concluded from these investigations that heat production in forage fermentation results from microbial activity and not from intramolecular respiration of the tissue cells.

LITERATURE CITED

(I) BABCOCK, S. M., and RUSSELL, H. L.

1900. CAUSES OPERATIVE IN THE PRODUCTION OF SILAGE. In Wis. Agr. Exp. Sta. 17th Ann. Rpt. [1899]/1900, p. 123-141, fig. 17.

1901. CAUSES OPERATIVE IN THE FORMATION OF SILAGE. (SECOND PAPER.) In Wis. Agr. Exp. Sta. 18th Ann. Rpt. [1900]/01, p. 177-184, fig. 44.

(3) ------1902. DIE BEI DER HERSTELLUNG VON GÄRFUTTER (SILAGE) WIRKENDEN URSACHEN. In Centbl. Bakt. [etc.], Abt. 2, Bd. 9, No. 3/4, p. 81-88.

(4) BURRILL, T. J.

1889. THE BIOLOGY OF ENSILAGE. In Ill. Agr. Exp. Sta. Bul. 7, p. 177-194. (5) CONN, H. W.

1897. THE STORY OF GERM LIFE. 199 p., 34 fig. New York.

(6) ESTEN, W. M., and MASON, C. J.

1912. SILAGE FERMENTATION. Conn. Storrs Agr. Exp. Sta. Bul. 70, 40 p., 3 charts. Bibliography, p. 37-40.

(7) FRY, George.

(2) -

1885. THE THEORY & PRACTICE OF SWEET ENSILAGE. 66 p. London. (8) GRIFFITHS, A. B.

1894. ON THE MICROBES INVOLVED IN THE ENSILAGE OF GREEN FODDER. In Chem. News, v. 70, no. 1828, p. 273-275.

(9) HUNTER, O. W., and BUSHNELL, L. D.

1916. SOME IMPORTANT FERMENTATIONS IN SILAGE. Kans. Agr. Exp. Sta. Tech. Bul. 2, 32 p. Bibliography, p. 32. (10) KAYSER, Edmond.

1910. MICROBIOLOGIE AGRICOLE. 481 p. 95 fig. Paris.

(11) LAFAR, Franz.

1910. TECHNICAL MYCOLOGY. Translated by C. T. C. Salter. v. I. London. (12) RUSSELL, E. J.

1908. THE CHEMICAL CHANGES TAKING PLACE DURING THE ENSILAGE OF MAIZE. In Jour. Agr. Sci., v. 2, pt. 4, p. 392-410.

(13) SAMARANI, Franco.

- 1913. PREPARATION OF ENSILAGE. (Abstract.) In Mo. Bul. Agr. Intel. [Internat. Inst. Agr., Rome], year 5, no. 12, p. 1625-1626. 1914. (Original article in Bol. Min. Agr., Indus., e Com. [Italy], anno 12, ser. C, fasc. 8/12, p. 87-103. 1913. Not seen.)
- (14) SHERMAN, J. M.

1916. A CONTRIBUTION TO THE BACTERIOLOGY OF SILAGE. In Jour. Bact., v. 1, no. 4, p. 445-452. Bibliography, p. 452.

- (15) WOLLNY, Ewald.
 - 1897. DIE ZERSETZUNG DER ORGANISCHEN STOFFE UND DIE HUMUSBILDUNGEN. 479 p., 52 fig. Heidelberg.

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