MICROBIAL THERMOGENESIS IN THE DECOMPOSITION OF PLANT MATERIALS

PART I. AN ADIABATIC FERMENTATION APPARATUS¹

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INTRODUCTION

The phenomenon of heat evolution by moist plant materials has long been recognized, and is a common occurrence in incompletely dried hay or grain, and in compost piles, or manure heaps. Investigators have held at various times that this phenomenon of heating was due to one, or some combination of, the three following agencies: microbial action, plant enzymic activity in tissue respiration, and an autoxidative process perhaps aided by inorganic catalysts. The present view is that in most circumstances microbial activity is primarily and often exclusively responsible. The dissimilation of available plant constituents furnishes energy for the maintenance of heterotrophic microorganisms. Because of the low specific heat and insulating properties of plant materials, the excess of energy appearing as heat is not at once dissipated but may cause a considerable rise in temperature. This may not have any undesirable effect if not excessive, but in other circumstances the temperature may rise to such a level that the quality of the material is damaged for its ordinary use, or even that "spontaneous" combustion may occur.

Many of the biological aspects of this phenomenon of heating are incompletely understood, despite a number of extensive investigations. It is probably true to say that so far progress has been mainly in the direction of the recognition of factors involved

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in such phenomena, but that the relative importance and interaction of the various factors are not yet fully known. As a result the method of investigation selected may predetermine the conclusions, and, as will become evident later, there are studies in which this has undoubtedly occurred. The evolution of heat is the phenomenon under investigation but at the same time the temperature of the heating mass is perhaps the most potent factor in determining the further course of the fermentation. Ordinarily the end product of a reaction has either no effect or an inhibiting effect when it accumulates, but in this case the accumulation of heat resulting in a rise of temperature may at first increase and then later decrease the activity of the organisms present as their temperature optima are passed. When a mixed flora is present fascinating biological problems arise, since the rapid change of temperature must profoundly affect the nature of the microbial population active in any particular range. If progress is to be made in approaching these problems the temperature factor must be completely controlled and, if possible, heat evolution placed on a quantitative basis. Investigations of some of the factors involved in thermogenic decompositions will be described in succeeding papers.

HISTORICAL

Previous investigators have in most cases carried out their experiments in Dewar vacuum flasks and have simply determined by means of thermometers or thermocouples the rise and subsequent fall in temperature of the decomposing plant material. Miede (1907), whose studies on hay have been most extensive, at one time made use of a number of different sizes of wire baskets, the innermost and smallest containing the experimental material, which was protected from contamination and insulated by the same material packed into baskets surrounding it. James (1927) describes an apparatus in which heat losses from a Dewar flask were reduced by adding heavy cork insulation, and, in addition, means were provided for controlled aeration of the material in the flask by a steady stream of oxygen. More recently, Gilman and Barron (1930) and Norman (1930) made use of Dewar flasks, both with and without extra insulation. All seemed to

have ignored or underestimated the heat losses from flasks of this type and only in one or two cases have cooling curves for the system been given. Even with this knowledge, however, it would be difficult to obtain any satisfactory quantitative estimate of heat evolved and this was not attempted.

EXPERIMENTAL

1. The requirements of an apparatus for the study of thermogenesis by plant materials

In designing an apparatus in which to follow the heat evolution by decomposing plant materials, there are a number of requirements that must be met.

1. As in the case of any calorimeter, heat loss from the system should be at a minimum, or if heat transfer takes place, the quantity should be readily determinable so that corrections may subsequently be made.

2. The volume of the fermentation vessel should be great enough to accommodate at least 30–50 grams of plant material in order that the loss on decomposition may be determined with sufficient accuracy.

3. Aeration has to be provided, a problem that presents some difficulties in that heat must neither be lost nor gained as the air enters and leaves the fermentation vessel. This also involves control of the relative humidity of the air stream, which should preferably be saturated.

4. Temperature increases must be accurately determined and for this purpose thermocouples are desirable.

5. The system should be capable of operating over a wide temperature range, because heat increases of 50° may be obtained.

6. Finally, the apparatus must be so designed that pure culture investigations can be carried out. This requirement calls for easy sterilization of certain parts of the apparatus and the prevention of subsequent contamination.

2. Construction and description of apparatus

The apparatus described below meets the requirements outlined in the previous section and in effect consists of an adiabatic fermentation calorimeter. In principle it is similar to that recently

described by Winzler and Baumberger (1938) though widely different in range. The fermentation vessel is a glass quart fruit jar, approximately seven inches in height and three and one-half inches in diameter. Over the fruit jar is fitted a flanged cover, made of brass with four threaded openings into which are screwed four sleeves, two small and two slightly larger. The smaller openings accommodate the glass air inlet and air outlet, the former reaching to the bottom of the vessel. One of the two larger openings accommodates a thermocouple and the other a two-junction thermopile, both being held in place by small rubber stoppers which act as air- and water-tight gaskets, and each extending into the center of the plant material in the vessel. The cover is fastened over the top of the glass jar by means of a clamp, a rubber gasket being used to prevent leaks of air or water.

The fermentation vessel is immersed in a cylindrical water bath, the temperature of which is both controlled by, and remains the same as, the fermenting material in the jar. In this way an adiabatic system is obtained with no heat loss or gain during the fermentation.

The bath is constructed from an ordinary galvanized garbage can, 24 inches high, 20 inches in diameter and capable of holding approximately 32 gallons of water. A larger galvanized container is built around the garbage can so that the latter is insulated on its bottom and sides by a four-inch thickness of packed glass wool. This insulating space is closed off at the top by a closely fitted wooden rim, sealed in place with putty and paint, leaving only the opening of the garbage can exposed. Over this opening can be placed the cover of the garbage can, also heavily insulated in a manner similar to the can itself. The result is a well-insulated, covered, cylindrical water bath, which proved to lose little heat even at a temperature of 75°.

Two openings are provided, one above the other, through the wall of the bath to allow for the introduction of the cooling, aeration and electrical systems which are described later. Heavy pyrex boiler tubing, of one-inch inside-diameter, is sealed into the openings and the water seal around the various service lines passing through these tubes is made with rubber stoppers. The

lower opening is situated about one inch from the bottom of the bath and the upper one just above the water line. Around the inside wall, several feet of $\frac{1}{4}$ " copper tubing are spiralled and connected through the lower opening to a cold water tap, in this way providing a cooling system similar to that of a constant temperature bath. Water is circulated only when the bath is operated near room temperature or if it is desired to cool the system rapidly.

Two chromium-plated, knife-type electric heaters (250 watt) are fastened opposite each other on the inside wall of the bath so that the blades are immersed. Electric leads to the heaters pass through the upper side wall opening. A motor-driven Cenco centrifugal stirrer is also fastened to the inside wall of the bath to circulate the water vigorously.

An aerating system is necessary to provide oxygen to the fermentation. This unit is constructed so that the air stream neither adds nor removes any heat. The air is released from a compressed air cylinder by means of a microvalve, bubbled through concentrated KOH and baryta to remove the carbon dioxide and passed by means of copper tubing through the lower side wall opening into the bath. Upon entering the bath the air passes through several feet of copper tubing coiled on the bottom of the water bath. In this way the air attains the same temperature as the water, which in turn is controlled by the temperature of the fermentation. Then by means of rubber pressure tubing the copper coil connects to a suction flask with a wet bead tower sealed into it, below the surface of the water, so that as the air bubbles up through the tower it becomes saturated. Rubber tubing connected to the top of the tower leads the air to the fermentation vessel from which it passes through the upper opening to a carbon dioxide absorption tower containing standard NaOH. This system provides for regulated aeration of the fermenting material with no loss or gain of heat and also for the determination of carbon dioxide produced by the fermentation.

The temperature of the bath is automatically maintained the same as that of the fermenting material by means of a controlling system composed of two two-junction thermopiles. One thermo-

pile is fastened into the fermentation jar as already described, and the other is suspended in the water bath. The like wires of the two thermopiles are connected directly to the poles of a Leeds and Northrup Type P wall galvanometer (fig. 1). A bright beam of light is focused on the galvanometer mirror in such a manner that when the bath and fermenting material are at the

FIG. 1. DIAGRAM OF ADIABATIC APPARATUS FOR STUDY OF THERMOGENESIS BY PLANT MATERIAL

G_3 , a wall galvanometer used in temperature control circuit; T_1 and T_2 , copper-constantan thermocouples; T_{p1} and T_{p2} , copper constantan thermopiles (two junction).

same temperature the galvanometer mirror reflects the filament image through a slit onto the sensitive section of a photoelectric cell. The photoelectric cell is in turn connected to a relay which controls the heaters in the bath. Thus, when the temperature in the flask rises above that of the bath, the galvanometer mirror is deflected so that the filament image is thrown off the photoelectric cell. This closes the relay and switches on the electric

heaters until the bath and flask are once more at the same temperature. This system of control proved to be sensitive enough to cause the relay to switch the heaters on and off several times a minute when heat evolution was rapid, so that no temperature differences between the flask and the bath could ordinarily be detected. The sensitivity of the controlling system is within that of the measuring system. A difference in temperature of 0.1° between bath and fermentation vessel causes a 8.5 mm. deflection of the filament image.

FIG. 2. WIRING DIAGRAM FOR THE TEMPERATURE MEASURING SYSTEM

G_1 , a portable galvanometer used in the standard cell circuit; G_2 , a wall galvanometer used in temperature measurement; S_1 , a mercury well switch; S_2 , a double-pole double-throw knife switch; J , a cold junction; T_1 and T_2 , copper-constantan thermocouples; T_{p1} and T_{p2} , copper-constantan thermopiles (tow junction); r , 1000 ohm resistance.

The temperature measurements are made with copper-constantan thermocouples using a Leeds and Northrup potentiometer and a Leeds and Northrup Type P galvanometer,² as shown in figure 2. A resistance box is included in the circuit as a potential divider so that practically the full length of the potentiometer slide wire is required to balance the output of the

² We are indebted to Dr. J. W. Woodrow of the Physics Department, Iowa State College for the loan of the galvanometers used in this apparatus.

thermocouples over the desired temperature range. The measuring system is calibrated against an accurate thermometer and a detailed curve drawn for the desired temperature range so that potentiometer readings can be converted directly into degrees centigrade. The thermocouples are constructed from 30 gauge copper and constantan wire and are placed in glass tubes with a thin glass bulb blown on one end. The junction is pushed down into the glass bulb and secured there by means of sealing wax. Two such thermocouples are used, one in the water bath and the other in the fermentation vessel, so that when a reading is taken the bath temperature can also be ascertained.

3. Procedure

The procedure in using this apparatus is as follows: The fermentation is commenced ordinarily at a temperature in the neighborhood of 25°. An appropriate quantity of plant material cut into short lengths is brought to a moisture content of 75 per cent by the addition of water in which a nitrogen source may be present. (In many experiments to be described 40 grams of oat straw was moistened with 110 ml. water containing 1.3 gram ammonium nitrate.) In mixed culture studies no inoculation is made unless the plant material has previously received a chemical pretreatment.

The lid is sealed tightly and aeration commenced at a rate of approximately 16 liters per day. The circuit controlling the heaters is switched on when the straw in the fermentation vessel comes into equilibrium with the water bath. Some heat is generated by the motor driving the water agitator, and this in the first few hours is balanced by circulating a slow stream of cold water through the coil around the bath. As the fermentation proceeds, temperature readings are made at frequent intervals. The carbon dioxide evolved and carried out in the air stream is absorbed in standard NaOH in a Truog tower and determined at the same intervals. In the region of maximum heat evolution hourly readings are not too frequent. When the temperature has ceased to rise or in some cases when the heat increments per hour are small, the fermentation vessel is removed from the bath

and the loss of dry matter ascertained by weighing the wet residue and determining its moisture content in triplicate. In some cases plate counts are made of bacteria in the wet residue.

SUMMARY

The requirements of an apparatus for the study of microbial thermogenesis by decomposing plant materials are outlined. The construction and use of an adiabatic apparatus meeting most of these requirements are described. By means of a photo-electric system the temperature of the bath surrounding the fermentation vessel is controlled by the temperature of the contents, in this way avoiding all heat loss.

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