MICROBIAL THERMOGENESIS IN THE DECOMPOSITION OF PLANT MATERIALS

PART II. FACTORS INVOLVED[1]

R. E. CARLYLE AND A. G. NORMAN

Soils Subsection, Iowa Agricultural Experiment Station, Ames, Iowa

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HISTORICAL

Much of the earlier work on the heating of moist plant materials was concerned with the nature of the possible agencies responsible, and this has been reviewed fully by Browne (1929). The term "thermogenesis" was coined by Cohn (1888, 1893) who appears to have been the first to realize and demonstrate the part played by microorganisms. He isolated *Aspergillus fumigatus* from heating malt and claimed that this and other fungi were active in raising the temperature far above 35° which was the limit that could be reached by the respiratory activities of the germinating grain. Much of the significant work in microbial thermogenesis has been carried out on hay. Duggeli (1906) sampled haystacks and made total counts of bacteria in the samples. He concluded that microorganisms are responsible for temperature increases to 70° or thereabouts, and stated that, as the temperature of the hay rises, different groups of organisms in turn become dominant.

Both mixed and pure culture studies were carried out by Miehe (1907) with hay packed in a series of wire backets, one enclosing another, to provide insulation and some protection from contamination. He claimed that if the plant tissues are dead, microorganisms are responsible for the whole process of heating, and described certain organisms, among them *Bacillus cale-*

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factor and *Oidium lactis* which possessed thermogenic properties. Much later, Miehe (1930) restated and amplified his conclusions as a result of many additional experiments, the later ones mostly in Dewar vacuum flasks. Hildebrandt (1927) studied the heating of hay, proving the microbial nature of the process by the use of disinfectants, and tested in pure culture the activity of certain unidentified bacteria and an *Aspergillus*. Temperatures above 40° he believed to be due to the association of organisms of the type of *B. calefactor* with *Actinomyces thermophilus*.

Extensive investigations of heat evolution by moistened corn meal, cured hay, and uncured alfalfa were reported by James, Rettger, and Thom (1928) using as a fermentation vessel a heavily insulated Dewar flask provided with means for aeration. Temperatures above 60° and as high as 73.5° were obtained on aeration with oxygen. Bacterial examinations made at different stages showed large increases in the number of mesophilic organisms up to a temperature of 50°, above which marked decreases occurred. Twelve strains isolated from corn meal, including some fungi and common spore formers, were tested in pure culture, and most of them proved to be vigorously thermogenic. This property, however, seemed to be lost by cultivation on laboratory media for several weeks. By analysis it was shown that considerable losses of dry weight occurred during the heating of corn, and that approximately 75 per cent of the loss could be accounted for by removal of "carbohydrate."

More recent workers have confined themselves largely to a study of the activity of fungi, and although perhaps not implicitly claimed, the impression has been given that under natural conditions in a mixed flora the fungi are probably more important than bacteria in this process. For example, various fungi were found by Miehe (1930) to raise appreciably the temperature of hay and other substrates and he expressed the opinion that in mixed floras, up to about 65°, the molds are primarily concerned, but that the final ascent to a maximum of 75° must be due to the activities of thermophilic bacteria.

Norman (1930), using straw as substrate, showed a number of fungi isolated therefrom to be thermogenic in pure culture.

In simple combinations, however, the maximum temperature attained was close to that given by the organism with the lower thermogenic power. The evolution of carbon dioxide paralleled closely the temperature changes in Dewar flasks. In both moist grain and hay, fungi were found by Gilman and Barron (1930) and Harrison (1934), respectively, to be active in bringing about decomposition with evolution of heat.

The rate of heat evolution and decomposition of materials initially of low nitrogen content, such as cereal straw, is enhanced by the addition of available nitrogen. Gaskill and Gilman (1939) investigated the influence of different sources of nitrogen on the maximum temperatures produced by five species of fungi in pure culture. Additions of asparagine were found to be most effective, but this was possibly due to the fact that asparagine is also a soluble energy source.

The discontinuity of this review of literature indicates the gaps in our understanding of the phenomenon of thermogenesis. Fundamentally, heat evolution is an incidental and not an essential feature of the microbiological decomposition of the substrate. The biochemical aspects of the problem, such as the influence of composition of the plant material, have as yet hardly been touched upon, nor have the intricate biological problems of mixed floras, which are complicated by the fact that the heat evolved affects the subsequent activities of the population.

EXPERIMENTAL

A. Experiments in Dewar flasks

The more recent investigations in this field have almost invariably made use of Dewar vacuum flasks as containers for the plant material. Additional insulation or exterior temperature control has sometimes been provided. Some have made provision for positive aeration with air or oxygen, and others have relied on diffusion through a cotton plug. These variations in technique have undoubtedly substantially influenced the results. In order to understand the relative importance of certain of these factors, and particularly to have a basis of comparison between the results obtained in such a manner with those in the adiabatic

apparatus described in a previous paper (Norman, Richards and Carlyle, 1941), a large number of preliminary experiments were carried out in quart Dewar flasks. Each contained 40 grams air dry oat straw cut into short lengths moistened with 110 ml. water containing 1.3 gram ammonium nitrate, the last so that insufficiency of nitrogen cannot be a factor limiting the rate of decomposition.

Different methods and rates of aeration were compared with diffusion through a cotton plug. The last gave results as satisfactory as any other method, with least likelihood of heat loss. When it was required to determine CO_2 evolution, the most

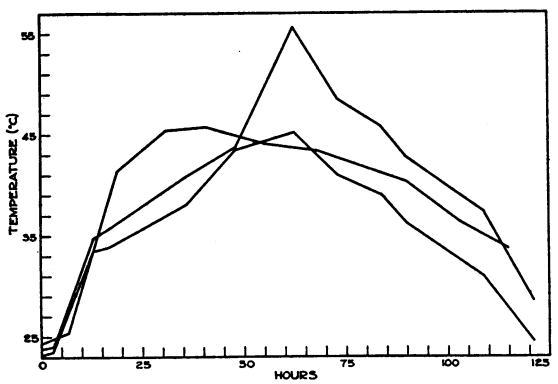

FIG. 1. TEMPERATURE CHANGES IN STRAW DECOMPOSITIONS IN DEWAR FLASKS

satisfactory procedure was to aerate continuously from the bottom of the flask at a rate of 12 to 16 liters per day, the flow being controlled by a microvalve on a compressed air cylinder.

Results typical of the Dewar flask experiments carried out without aeration in a constant temperature bath are shown in figure 1. The maximum temperature attained varied somewhat in different experiments under apparently identical conditions, but usually fell between 45° and 48°. Temperatures above 50° or not exceeding 40° occurred occasionally. Considerable variation occurred also in the length of time elapsing before the peak was reached. It seems certain that such mixed flora decompositions are not absolutely reproducible. The balance between

active organisms in the population must be delicate and easily disturbed. Probably the most variable factor is lack of uniformity in packing the straw, which influences the rate of heat transfer and loss. However, the results in general resemble those of other workers. One outstanding difference is apparent in comparing these figures with those given earlier by Norman (1930) for oat straw. Whereas the temperature range is approximately the same, the peak temperatures in his work were not attained until the sixth or seventh day as against 36 to 60 hours in these experiments. His flasks, however, were initially at a temperature always well below 20° (usually about 17°) whereas

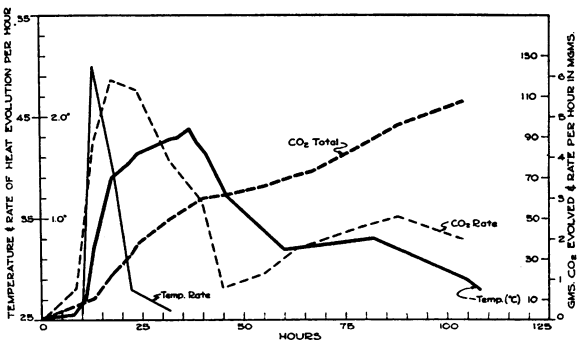

FIG. 2. TEMPERATURE READINGS, CO_2 EVOLUTION, RATE OF TEMPERATURE CHANGE AND RATE OF CO_2 EVOLUTION FROM A STRAW DECOMPOSITION IN DEWAR FLASKS

in these experiments the bath was maintained at 25°. Initial temperatures lower than 25° apparently lengthen considerably the time taken to establish rapid decomposition, and heat evolution.

The loss in weight of the straw by the time the temperature had receded was always appreciable. In the three experiments shown in figure 1 the losses fell between 12.3 and 13.5 per cent of the dry weight, but this was often exceeded.

The rate of evolution of CO_2 follows the rate of increase of temperatures, as shown in figure 2. In this experiment aeration was provided at a rate of about 14 liters per day. A second temperature peak occurred at about 80 hours and this is a feature that

was occasionally observed. The downward part of the temperature curve, after the first maximum, approached in slope the cooling curve for the flask, and it has to be concluded that heat evolution almost ceases or is considerably less than the rate of loss from the system.

The suggestion has been advanced that the apparent cessation of heat production, once a temperature in the neighborhood of 45 to 50° has been reached in a Dewar flask, is due to the exhaustion of a substance or substances in the substrate, the

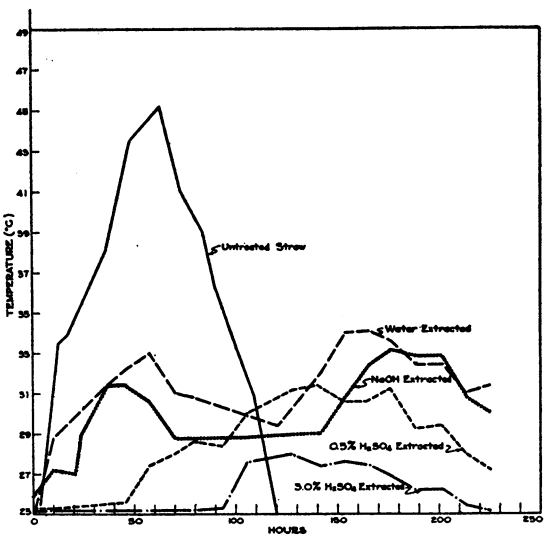

FIG. 3. EFFECT OF EXTRACTION ON TEMPERATURE CHANGES IN STRAW DECOMPOSITION IN DEWAR FLASKS

decomposition of which is accompanied by heat evolution. This theory, while possible in some cases, would seem to be at variance with the practical observation that similar plant materials in large piles often attain much higher temperatures. The effects of modification of the substrate by extraction with various reagents were therefore determined. The same oat straw cut into short lengths was extracted with (1) water, by being raised slowly to the boil (2) 0.5 per cent NaOH, heated gently for 30 minutes (3) 0.5 per cent H_2SO_4, heated at the boil for 1 hour (4) 3 per cent H_2SO_4, heated at the boil for 30 minutes. Each

treatment was followed by extensive washing to neutrality. The effect of the treatments was to change drastically the temperatures reached when placed as before in Dewar flasks (fig. 3). The maximum temperatures attained were far lower than in untreated straw. Of the four treatments, hot water had the least severe effect, and the temperature curves for the water- and NaOH-extracted straw resemble each other in that two clear temperature peaks are evident, the first between 40 and 60 hours and the second after 150 hours. The hot water treatment probably did not completely remove all water-soluble constituents because the straw was not finely divided. Even so it seems clear that *rapid* thermogenesis is associated with the decomposition of readily available water-soluble constituents in the straw. One temperature peak only occurred on the acid extracted straws, and the 3 per cent acid apparently removed the greater part of the available plant constituents since the temperature rise was only 3° in over 100 hours. The general conclusion that such material is non-thermogenic and incapable of heating to high temperatures might easily be reached, but as will be seen later, this would be entirely erroneous under slightly different conditions.

B. Experiments in an adiabatic apparatus

1. Rate of heat evolution with a mixed flora. The heat losses from a Dewar flask are by no means inappreciable and have a considerable influence on temperature changes and the route of the decomposition. If all heat loss is avoided, as in the adiabatic apparatus described in the preceding paper, an entirely different picture is obtained, as may be seen in figure 4 and table 1. These give typical results for 40 grams air-dry oat straw, readings of temperature and CO_2 evolution having been taken hourly. A final temperature of 69.8° was obtained in the surprisingly short time of 44 hours. In some other experiments the time has been even shorter. The temperature curve at first sight appears to consist of two flattened sigmoid curves, one succeeding the other. When the rate of temperature increase is plotted in hourly increments, the curve gives more information. Two distinct maxima are evident, one at 40° and another at 60.2° with a low

minimum intervening. This suggests that a mesophilic population may be responsible for the first maximum, but, as the temperature rises further past the optimum for this group, the activities of the organisms are diminished progressively, and the rate of temperature change reaches a low point from about 52° to 55°. However, a thermophilic population is becoming established, with the result that a second maximum is reached at 60°, after which there is again a decline in the activity of the organisms. The rate of carbon dioxide evolution was closely related to these

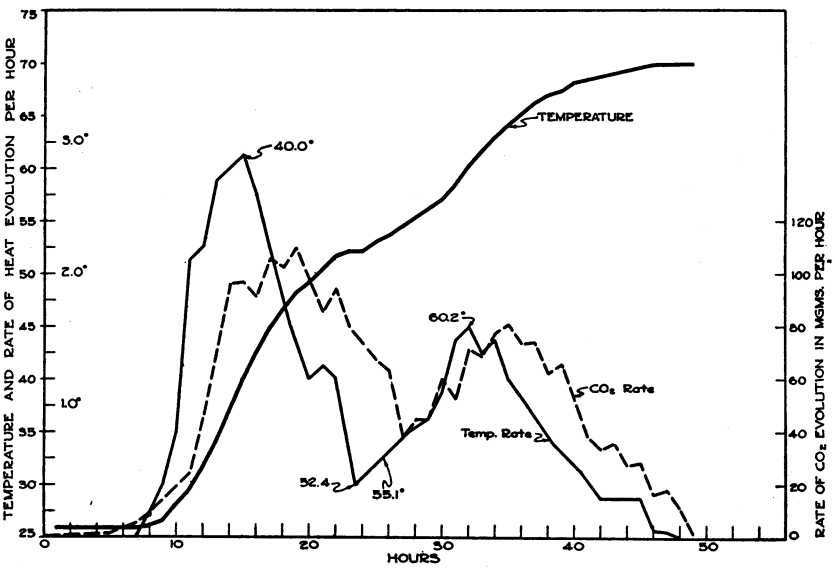

FIG. 4. TEMPERATURE READINGS, RATE OF HEAT EVOLUTION AND RATE OF CO_2 EVOLUTION FROM STRAW DECOMPOSITION IN ADIABATIC APPARATUS

temperature changes, and similar peaks occurred. The curve plotting rate of CO_2 evolution per hour lags behind the temperature increments curve, because the CO_2 is swept out of the fermentation vessel only slowly by the air stream. The CO_2 evolved in any one hour is not quantitatively removed in that hour. Minor irregularities are noticeable in the CO_2 rate curve, and these are primarily due to the incomplete flushing of the fermentation vessel in any one interval between readings.

The amount of decomposition occurring during one temperature

ascent to 70° or thereabouts was always small, and in the experiment cited, was only 2.6 per cent. The range observed in a number of similar experiments, not all continued for identical times, was from 5.2 per cent to 1.1 per cent, figures that are much

TABLE 1

Temperature readings, and carbon dioxide evolution from straw in adiabatic apparatus

TIME	TEMPERATURE	CARBON DIOXIDE	TIME	TEMPERATURE	CARBON DIOXIDE
hours	*°C.*	*mgm. per hour*	*hours*	*°C.*	*mgm. per hour*
0	25.8		25	53.2	67.7
1	25.8		26	53.7	53.2
2	25.8		27	54.4	38.0
3	25.8		28	55.2	45.6
4	25.8		29	56.1	44.9
5	25.8	1.3	30	57.1	60.0
6	25.8	2.1	31	58.6	52.5
7	25.8	2.1	32	60.2	71.9
8	26.0	3.5	33	61.6	68.4
9	26.4	6.9	34	63.0	77.4
10	27.2	12.4	35	64.2	80.8
11	29.4	24.2	36	65.2	73.2
12	31.6	44.9	37	66.2	73.9
13	34.3	69.8	38	66.8	62.2
14	37.1	96.0	39	67.5	65.6
15	40.0	96.7	40	68.0	52.5
16	42.6	91.2	41	68.6	38.7
17	44.8	105.7	42	68.8	33.2
18	46.4	102.3	43	69.1	36.0
19	48.0	109.9	44	69.4	27.6
20	49.2	97.4	45	69.8	28.3
21	50.5	85.7	46	69.8	16.6
22	51.7	94.7	47	69.9	18.0
23	52.1	79.5	48	69.9	11.7
24	52.6	73.0	49	69.9	1.4

Per cent decomposition 2.6.

lower than those given in the Dewar flasks. Decomposition is clearly not extensive, and only a small portion of the total plant constituents is removed in attaining these high temperatures.

An approximate figure for the calories produced has been obtained. In early experiments a double-walled vacuum flask was

used as the fermentation vessel, and attempts were made to determine accurately the water equivalent of the vessel and its contents by generating electrically a known amount of heat in the vessel partially filled with a known volume of water. Variations in the volume of water present considerably affected the apparent water equivalent of the system, and since there was no way of determining accurately the volume of water which would give a value equal to that provided by the contact of 40 grams of moistened straw with the wall, a single-walled glass jar was substituted. This, being completely submerged, may be considered to be heated entirely by the bath. The thermopile, thermocouples, etc., inside the vessel were weighed and their water equivalents obtained by calculation. The specific heat of oat straw was determined and found to be 0.37. In this way the approximate water equivalent of the system was calculated to be 131 calories, by far the greatest contribution being made by the water used to moisten the straw. In the experiment reported in table 1 the temperature rise was 44.1°, so the total number of calories produced was in the neighborhood of 5890. Other experiments in which the ultimate temperature was higher gave figures a little over 6000 calories. The heat evolved at the first maximum in rate of heat liberation in the experiment given in table 1 was 380 calories per hour and 210 calories per hour at the second peak. At the lowest point between the two peaks, 52.1°, the rate fell to the low figure of 56 calories per hour.

2. Substrate exhaustion. Inasmuch as the amount of material removed during one temperature ascent was so low, attempts were made to ascertain the length of time necessary to exhaust the straw of constituents the decomposition of which are accompanied by a rapid rise in temperature. The method exployed was to allow the temperature of the straw to rise to a high figure, and then to lower it by rapidly cooling the water bath, the air stream being cut off during the cooling period. When equilibrium was reached, air was again supplied, and temperature readings continued. This was repeated until no further significant increase in temperature was obtained. The results are shown in figures 5 and 6 and tables 2 and 3. A complicating biological factor

enters into these experiments in that, as the temperature rises, the active flora is altered. The flora may consist of both spore-forming and non-spore-forming bacteria as well as fungi. As the temperature rises above 60°, some of the non-spore-forming mesophilic bacteria and fungi will be destroyed while the spore-formers will be able to survive. In the upper temperature range,

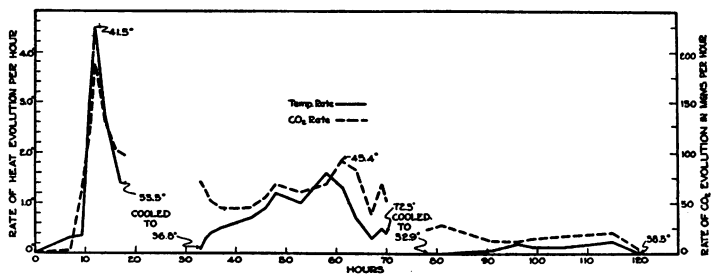

FIG. 5. RATES OF EVOLUTION OF HEAT AND CO_2 FROM STRAW (TWO ASCENTS)
Temperature lowered from 72.8° to 21.7° and from 64.5° to 31.7°
Per cent decomposition = 5.6

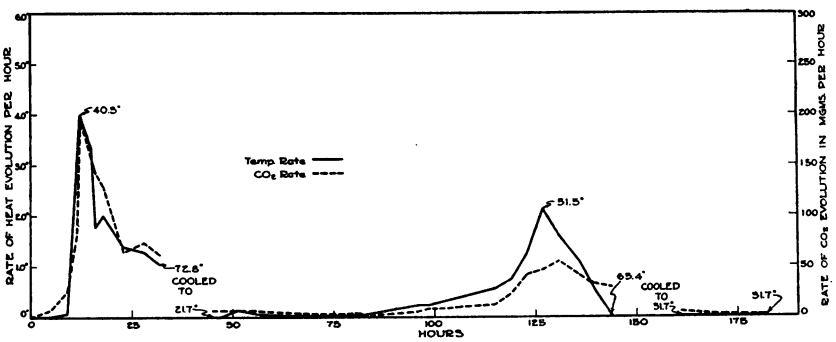

FIG. 6. RATES OF EVOLUTION OF HEAT AND CO_2 FROM STRAW (TWO ASCENTS)
Temperature lowered from 53.5° to 36.8° and from 72.5° to 32.9°
Per cent decomposition = 10.4

therefore, the effect on the population may be virtually that of pasteurization. Hence, it is possible that changes so extreme could take place in the microflora that substrate effects might not be clearly apparent. In one experiment (fig. 5) the temperature was allowed to reach 53.5° and then was lowered. In the other (fig. 6) the temperature reached 72.5° before the fermenta-

tion was cooled. In the former the effect on the activity of the population was not drastic, for in 37 hours more the temperature attained was 73.2°. After cooling a second time, the third temperature ascent was so slow that in 40 hours more a rise of less than 6° took place. In the second experiment the initial temperature ascent was allowed to continue to 72.8° before cooling to 21.7°. Under such severe treatment the population required almost 50 hours to recover its activity, but it is noteworthy that, eventually, heat evolution recommenced at a substantial rate, and a temperature of 65.4° was reached. On cooling again, this time to 31.7°, no further rise occurred and CO_2 evolution ceased. In the former experiment, lasting in all 120 hours, the amount of decomposition was only 5.6 per cent, whereas, in the latter, 10.4 per cent was lost in 187 hours. Both these figures, however, are less than many obtained in Dewar flask experiments. The population can obviously withstand severe treatment as far as temperature changes are concerned. Lessening of the rate of heat evolution may have been due to substrate exhaustion, but because of the difference in the percentage decomposition in the two cases, the evidence cannot be considered clear-cut on this point.

TABLE 2

Temperature readings, carbon dioxide evolution and bacterial counts

(Bacterial counts in parallel fermentations)

TIME	TEMPERATURE	CARBON DIOXIDE	BACTERIAL NUMBERS PER GRAM OF OVEN DRY RESIDUE
hours	*°C.*	*mgm. per hour**	
0	28.0		26 × 10^5
9	30.6	66.0	588 × 10^5
12	37.8	146.0	16570 × 10^5
15	45.6	141.9	2065 × 10^5
21	51.6	64.8	155 × 10^5
26	56.6	80.6	17 × 10^5
31	63.1	99.1	2.3 × 10^5
45	68.4	43.4	1.3 × 10^5

* Rate for the hour previous to sampling for counts.

In view of the earlier observation that rapid thermogenesis

seems to be associated with the presence of water-soluble constituents, an experiment was carried out in which the water extract of 40 grams straw, after filtration and concentration under

TABLE 3

Temperature readings, temperature increments, carbon dioxide evolution and bacterial counts in mixed flora experiments

(Bacterial counts in parallel fermentations)

TIME	TEMPERATURE	TEMPERATURE RISE	CARBON DIOXIDE	BACTERIAL NUMBERS PER GRAM DRY RESIDUE	
				Incubated at 30°	Incubated at 60°
hours	*°C.*	*°C. per hour*	*mgm. per hour*		
0	25.3			149 × 10^5	
8	29.7	0.54	9.8	447 × 10^5	2200
9	32.5	2.79	55.1		
10	36.0	3.59	102.5	2745 × 10^5	2000
10.75	40.1			3510 × 10^5	2300
11	41.0	5.00	155.4		
12	45.1	4.02	181.8	1368 × 10^5	
13	48.6	3.53	196.2		
14	50.4	1.83	123.9	284.5 × 10^5	10,400
16	52.9	1.23	93.4		
17.5	54.1	0.80	76.3		
18.5	55.4			524 × 10^5	
19	56.3	1.32	74.7		
20	58.9	2.56	82.4		
20.5	60.3			12.6 × 10^5	659,000
21	61.9	3.07	102.5		
22	64.6	2.65	90.5		
22.25	65.0			1.8 × 10^5	1,575,000
23	66.4	1.80	118.2		
24	67.3	0.93	97.5		
27	69.2	0.62	65.6		
28.5	70.1			2.8 × 10^5	214,200
30	70.6	0.47	39.6		
34	72.0	0.36	25.0		
38	73.6	0.39	11.3		
43	75.6	0.39	7.1	1.3 × 10^5	5,750

reduced pressure to an appropriate volume, was used to moisten an equal weight of straw in the fermentation vessel. The plan followed was to allow the temperature to rise to approximately 65° then to lower it to 30°, and to repeat this a number of times.

In figure 7 each curve represents the temperature changes in each successive temperature ascent. An increase of 14.9° in 3 hours at an almost uniform rate took place between 35.5° and 50.4° in the first ascent, and 66° was reached in 24 hours. This peak rate of heat evolution is considerably higher than the usual maximum (about 3.0° per hour) and was longer maintained, no doubt due to the presence of the added soluble material. These figures demonstrate the remarkable possibilities of biological thermogenesis when plenty of available energy is present. By roughly doubling the amount of water-soluble material present, more than twice as many temperature ascents were obtained as in the two

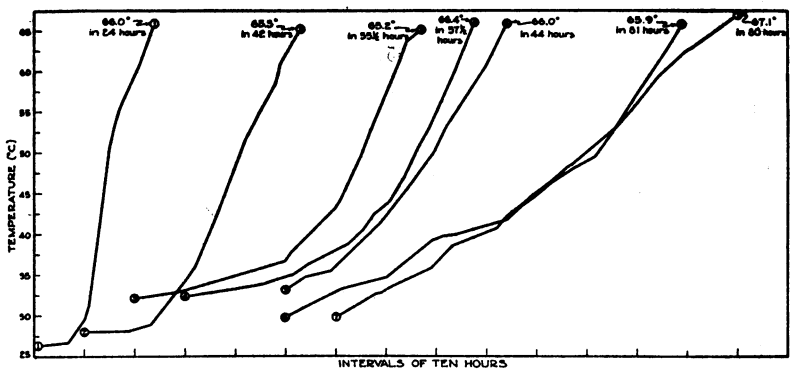

FIG. 7. TEMPERATURE READINGS IN STRAW WITH ADDITION OF HOT WATER EXTRACT (SEVEN ASCENTS)
Temperature lowered six times. Per cent decomposition = 12.9

previous experiments. Every time the temperature was lowered, the straw took longer to reach 65° (except in the case of ascent 5 which was quicker than ascents 3 and 4). The seventh and last ascent required 80 hours, the maximum rate being 0.8° per hour from 40.8° to 42.4°. In all, the experiment ran for a total period of 394 hours, and the amount of decomposition was 12.9 per cent, excluding the added water-soluble material.

This experiment does not indicate any sharp exhaustion of substrate, but shows clearly that, as the more readily available constituents are removed, the rate of heat evolution progressively slackens. The population was not so severely affected by the

temperature changes as in the previous experiments, in each of which the temperature was once allowed to exceed 70°.

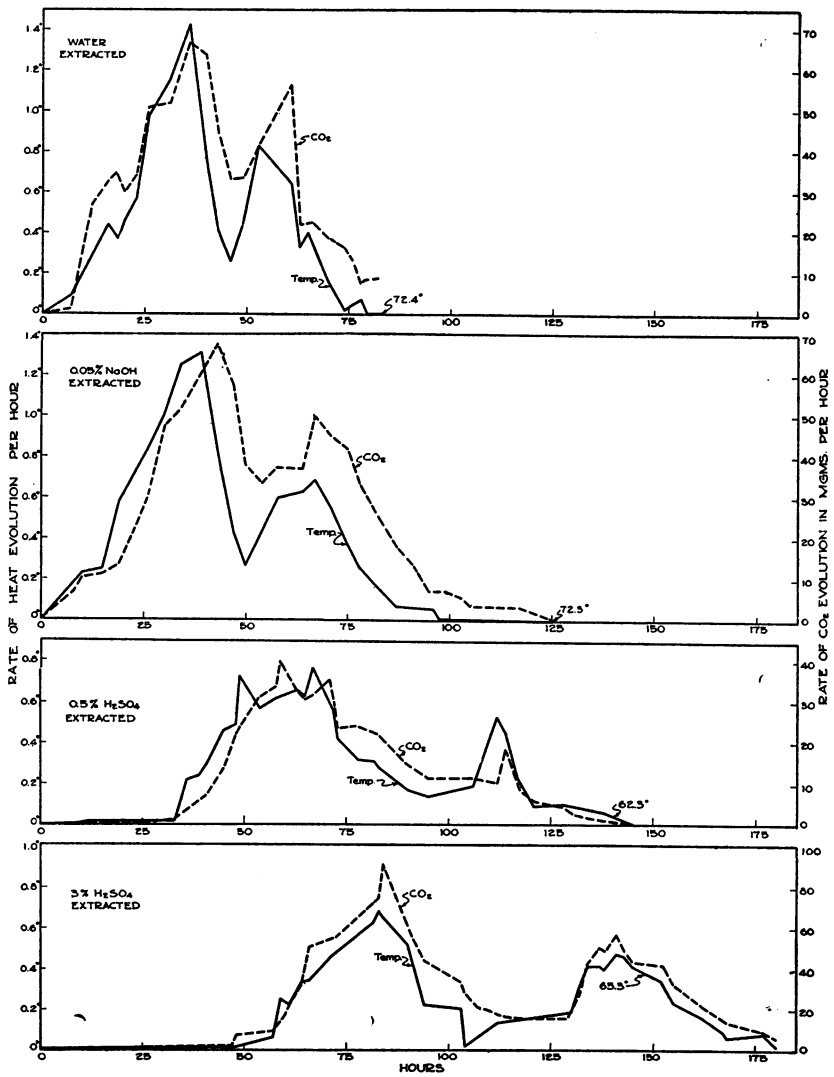

FIG. 8. EFFECT OF EXTRACTION ON RATE OF EVOLUTION AND CO_2 FROM STRAW IN ADIABATIC APPARATUS

In order further to examine the effect of modifying the substrate the decomposition of the extracted straws previously de-

scribed was studied. Each was, of course, re-inoculated after extraction by adding to the water used for moistening a small volume of cold wash water from untreated straw. Pre-extraction with hot water, dilute alkali, 0.5 per cent and 3 per cent acid, treatments of increasing severity in the removal of available constituents, diminished the rate of thermogenesis in the order named but had no serious effect on the maximum temperature attained (fig. 8). Even the 3 per cent acid-extracted straw, which gave a rise of only 3° in a Dewar flask, finally attained a temperature of 65.3°, but required 180 hours to do so, against about 35 hours for untreated straw. There are also evident large differences in the time elapsing before active populations became established and fermentation commenced. In each case the temperature change and CO_2 curves are closely related as before, and two maxima occurred in the temperature rate curve just as in fermentations carried out on untreated straw, confirming the view that these have their origin in sequential population changes.

3. Nitrogen requirements. Previous workers carrying out natural flora experiments in Dewar flasks have frequently found heavy fungal growth, and, in pure culture experiments with fungi, have made it a practice to add additional available nitrogen to meet the structural needs of the organisms if the plant material is low in nitrogen. In all the experiments described so far, such an addition was made, but in view of the fact that the extent of decomposition was relatively small, and since few fungi could be isolated from samples taken during a fermentation in the adiabatic apparatus it seemed probable that the active population was almost exclusively bacterial and that the nitrogen additions were therefore unnecessary. Accordingly, experiments were carried out without the addition of ammonium nitrate, and no significant differences could be detected in rate of heat evolution. Typical results are given in Figure 9. A temperature of 73.4° was reached in 44 hours with a maximum rate of heat evolution of 3.1° per hour from 40.3° to 43.4°. The absence of additional nitrogen, therefore, had no detrimental effect on the course of the fermentation.

4. Activity of thermophilic organisms. In all the experiments

so far described two peaks in rate of temperature increase have been apparent, one in the neighborhood of 40° and one near 60°. The former has always exceeded the latter; for example, in figure 1 the two maxima were 2.9° and 1.6° per hour, or in figure 8, 3.1

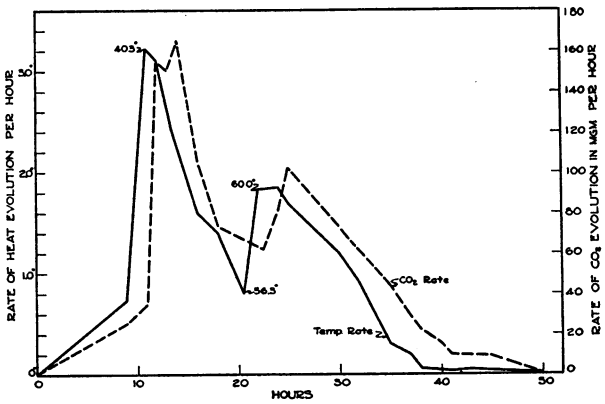

FIG. 9. RATES OF EVOLUTION OF HEAT AND CO_2 FROM STRAW WITHOUT ADDED AVAILABLE NITROGEN
Final temperature 73.4°. Per cent decomposition = 3.3

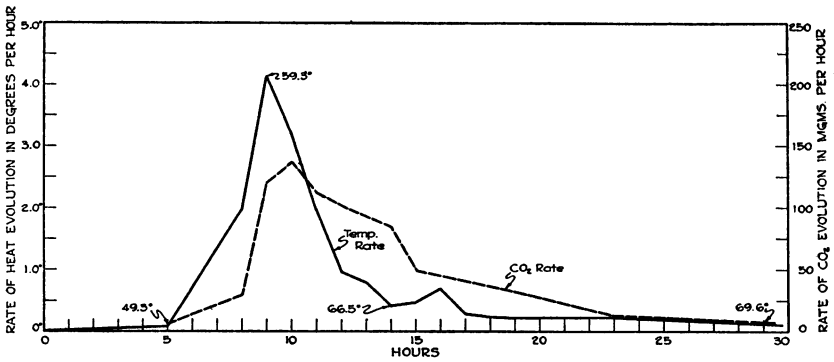

FIG. 10. RATES OF EVOLUTION OF HEAT AND CO_2 FROM STRAW INITIALLY AT 48.9°

and 1.9°. This might be explained by a less vigorous thermophilic population, or because the mesophilic population had removed some of the most readily and rapidly fermentable material. To investigate this point, experiments were commenced at 48.9° (fig. 10). After a period of 6 or 7 hours heat evolution

occurred rapidly and a peak rate of temperature increase of 4.1° per hour was found from 55.2° to 59.3°. The maximum of 69.6° was reached in only 29 hours. The thermophilic flora capable of developing on straw is therefore potentially as active in thermogenesis as the mesophilic flora and the difference usually apparent must be due to the prior removal of some of the most readily available constituents by the latter.

5. Bacterial counts. The rapid rate of evolution of heat and carbon dioxide observed in many experiments indicates great microbial activity, and, accordingly, some attempts were made to

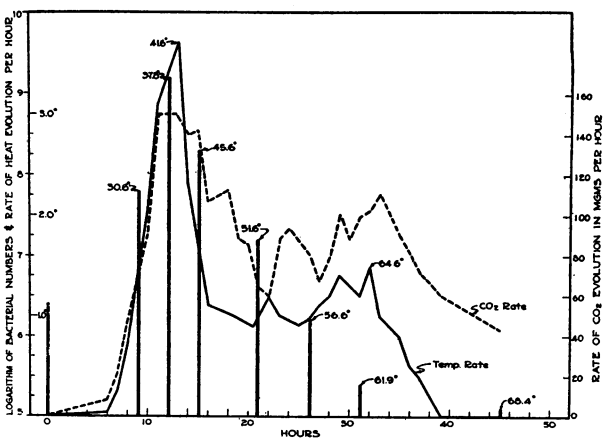

FIG. 11. RATES OF EVOLUTION OF HEAT AND CO_2, AND CHANGES IN BACTERIAL NUMBERS IN STRAW
(Bacterial counts made at 30° in parallel decompositions)

follow approximately the changes in population that occur as the temperature rises from 25° to 70°. Although fungi were present on the straw initially, little evidence of fungal development could be obtained by plating at intervals, and bacteria appeared to constitute the active flora in all mixed flora experiments in the adiabatic apparatus. The opening of the fermentation vessel at intervals to withdraw samples for counts was obviously impractical, and accordingly the count samples were taken from satellite vessels of the same size surrounding the fermentation vessel and immersed in the bath. Each contained moistened straw equal in amount to that in the central vessel, the changes

in which controlled the temperature of the bath. At appropriate intervals one of these vessels was removed, and a generous sample withdrawn. After thoroughly mixing, a 10-gram portion was taken for preparation of dilutions. The moisture content was simultaneously determined, and the numbers finally calculated on the basis of organisms per gram of dry residue. In one experiment (fig. 11 and table 2) the plates were incubated at 30° and the organisms counted, therefore, were of the mesophilic group. The changes in bacterial numbers up to 55° have the same general trend as the temperature rate curve. At a temperature of 37.8° just prior to the highest rate of heat evolution the number of bacteria reached the enormous figure of $16{,}570 \times 10^5$ organisms per gram only to fall again rapidly as the temperature rose out of the mesophilic range. This figure is many times greater than the maximum reported by James *et al.* (1927) on corn meal. At the close of the experiment only 130,000 organisms per gram could be found. Final figures of this order and lower have been repeatedly obtained. The number appears to be affected to some extent by the length of time the apparatus is allowed to remain at the end point, and it has to be remembered that it is a count of mesophilic organisms. The plates in an experiment of this type are qualitatively of great interest, for the increase of temperature is accompanied by an obvious reduction in the number and variety of species present. This aspect of the problem is of course being further investigated.

The rapid fall in numbers of mesophilic organisms at the higher temperatures and the low final counts suggest that the active population that flares up initially is composed mainly of non-spore-formers. In preliminary pure culture experiments with a mesophilic spore-former, no reduction in count has been observed as the temperature rises beyond the point of maximum rate of heat evolution, which is in sharp contrast to the great reduction shown in table 2.

In other experiments, counts have been made on plates incubated also at 60° in an attempt to obtain information as to the population changes in the thermophilic temperature range. These counts have not been entirely satisfactory because of the

presence of spreaders and pin point colonies. Growth of the former is very rapid, with the result that the plates have to be closely watched and the colonies counted 12 to 15 hours after the time of plating. The results of such an experiment in which samples were taken at 5° intervals from 30° to 75° are given in

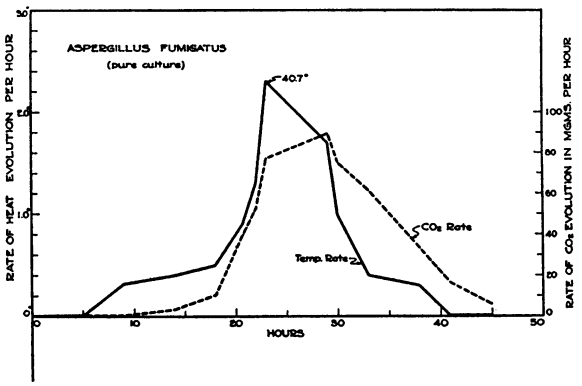

FIG. 12. RATES OF EVOLUTION OF HEAT AND CO_2 FROM STERILE STRAW INOCULATED WITH ASPERGILLUS FUMIGATUS
Final temperature 54.8°

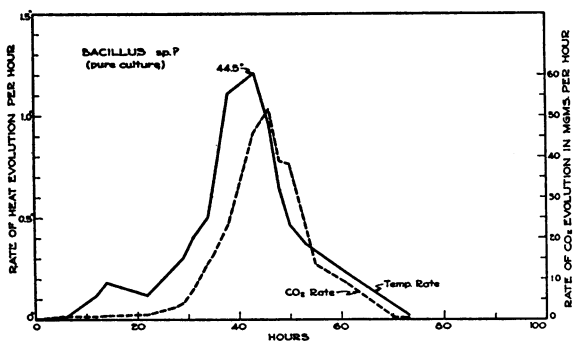

FIG. 13. RATES OF EVOLUTION OF HEAT AND CO_2 FROM STERILE STRAW INOCULATED WITH AN UNIDENTIFIED BACILLUS
Final temperature 54.3°

table 3. The highest count of mesophilic organisms occurred just prior to the first maximum in rate of heat evolution, but the highest thermophilic count occurred at 65°, somewhat past the second maximum, thereafter declining rapidly.

6. Pure culture studies. A large number of species of bacteria

and fungi have been isolated from the original straw and picked from the count plates. Two organisms have been used in pure culture fermentations in the adiabatic apparatus, one a fungus, *Aspergillus fumigatus*, and the other a bacillus as yet unidentified. *A. fumigatus* has been repeatedly stated to have powerful thermogenic properties. Elaborate precautions were necessary to ensure the sterility of the fermentation vessel, its contents and controls. The air stream was passed through concentrated sulphuric acid after removal of CO_2, and the water in the bead tower was replaced with 1:1000 $HgCl_2$. With the mold (fig. 12), an ultimate temperature of 54.8° was reached in 38 hours, with a peak rate of 2.3° per hour at 40.7°. Although the straw was initially inoculated with a heavy suspension of spores there was little obvious sign of mycelial development at the end of the experiment. On plating, however, much growth was obtained, without contamination.

A similar maximum temperature was obtained in the fermentation with the spore-former (fig. 13). The inoculation in this case was a cell suspension centrifuged out from liquid culture. More than 20 hours elapsed before any increase in temperature occurred. The peak rate of 1.2° was reached between 38.4° and 44.5° and a maximum temperature of 54.3°.

Both these organisms are thermogenically active over a considerable range and to a temperature rather higher than perhaps would be expected of an ordinary mesophil. However, the trough in the curve for rate of temperature increase in mixed culture has so consistently been in the neighborhood of 53 to 55° that it seems likely that this is the upper limit of activity of the mesophils. So far, attempts to secure a pure culture fermentation with a thermophil have been unsuccessful.

DISCUSSION

Broad generalisations on the subject of thermogenesis would be premature until the biological factors and population effects have been more fully examined. The use of the adiabatic apparatus has permitted the study of this phenomenon over the full biological temperature range and has made it certain that the rate of heat evolution during decomposition is by no means uni-

form. Two well-defined maxima are apparent, with an intervening trough, indicating that the normal mixed population can be divided distinctly into mesophilic and thermophilic groups, the active ranges of which barely overlap. It would seem that, in the past, workers have to some extent predetermined their results in the choice of apparatus. Those using the less rigorous conditions given by Dewar flasks have usually obtained only the first, or mesophilic, phase of the full thermogenic decomposition, because the flasks lost heat at a faster rate than would be evolved by the decomposition as it moved into that critical region between the mesophilic and thermophilic range. The practical implications of this observation would seem to be that, other factors being equal, the amount of heating will depend on the rate of heat loss, or inversely, the degree of insulation of the heating mass. Thus in loose, comparatively small, piles of hay, heating takes place to a limited extent, while in compost heaps and hotbeds, which are more compact and better insulated, higher temperatures may be reached and maintained. Finally, in large haystacks or barns filled with quantities of moist hay, the centre is extremely well insulated, and as a result the maximum biological temperature is reached and the stage set for those chemical oxidations that may eventually lead to spontaneous ignition.

The adiabatic apparatus cannot be accurately described as a calorimeter for reasons that were discussed on page 708, and because of the impossibility of determining the water equivalent of the system by introducing electrically a known amount of heat in any manner which would be expected to raise the temperature of sterile moistened straw uniformly through the mass. However, in an adiabatic system the figure for total calories generated is only a simple multiple of the initial and final temperature differential, if there is no significant change in mass. Because the specific heat of straw is low, a loss of dry matter of 6.5 per cent would be required to obtain a reduction of 1 per cent in the calculated water equivalent of the contents of the fermentation vessel under the conditions described. In all experiments in which the temperature was allowed to ascend once only, the amount of straw decomposed rarely exceeded half of this figure.

Hence, a knowledge of the total amount of heat evolved is of little added advantage because in ascending through the same temperature range, acid-extracted straw, for example, would produce almost exactly the same number of calories as an equal mass of untreated straw, the great difference being in the time taken to evolve the heat. The determination of the rate of heat evolution is therefore of more value as an index of microbial activity than the total calories evolved and may be expressed equally well in terms of degrees rise per hour as calories per hour.

Pure cultural studies have shown that organisms differ in thermogenic ability, and it has been at least implied, if not clearly stated, that there are also some organisms, capable of effecting decomposition, which are non-thermogenic and therefore do so without evolution of heat. Moreover, James *et al.* (1928) commented on the apparent loss of thermogenic ability undergone by some species when carried through several transfers on glucose agar, as though thermogenic ability was independent of fermentative ability. Inasmuch as microorganisms are not efficient in the utilisation of the energy available in the dissimilation processes they bring about, heat is always evolved in biological decomposition. However, the evolution is not recognized unless there is sufficient accumulation to raise the temperature of the substrate or medium appreciably. Organisms ordinarily recognized as thermogenic, therefore, are organisms which carry on decomposition fast enough so that the rate of heat evolution is more rapid than the heat losses from the system. It would seem likely that a powerfully thermogenic organism is one that can ferment some portion of the substrate extremely rapidly and a non-thermogenic organism is one which ferments it so slowly that the rate of heat evolution is less than the rate of heat loss at that temperature. Moreover, in the former case the rising temperature itself, within the optimum limits of the organism, accelerates the fermentation process and therefore the rate of heat evolution. The heat losses from Dewar flasks, particularly when immersed in a water bath, are by no means negligible and it is usually by experiments in Dewar flasks that organisms have been classified as thermogenic or non-thermogenic. According to this concept, then, the loss of thermogenic power described by

James *et al.* (1928) as a result of culturing on laboratory media would be a reduction in fermentative ability but not necessarily its complete disappearance.

The theory has been advanced (Norman 1930) that certain constituents of the substrate are capable of decomposition with the evolution of a considerable amount of heat, whereas from others little heat is obtained. This suggestion arose out of experiments in Dewar flasks similar to those given in figure 1, in which the temperature rises to a maximum of perhaps 50° and then slowly declines to the initial temperature or a little above it. It can be readily ascertained that decomposition is still proceeding and that CO_2 is still being evolved. The decomposition is, however, at the expense of the less readily available cell-wall constituents and proceeds slowly so that the rate of heat evolution is balanced by heat losses. From the Dewar flask experiments shown in figure 3, it might have been said that the acid-extracted straw was nonthermogenic, or almost so, inasmuch as its temperature rose but 3° in over 100 hours. In the adiabatic apparatus, however, the same material rose to almost the same temperature as untreated straw, but took 180 hours to do so.

In brief, under conditions not strictly adiabatic only those constituents which are fermented with the evolution of heat at a rate faster than the rate of loss have been considered important in thermogenesis, and the dividing line therefore has been the purely incidental one of the degree of insulation of the system.

There is little doubt that the initial rapid thermogenesis of straw is due to the fermentation of water-soluble constituents, since removal of this fraction so markedly reduced the rate of decomposition. The nature of the substances concerned has not been ascertained. There was practically no free sugar, or easily hydrolyzable water-soluble carbohydrates in the straw employed, and little water-soluble nitrogen. Perhaps the most surprising feature of these investigations was the small amount of material removed in one temperature ascent in the adiabatic apparatus. It is interesting to note, however, that 6000 calories would be liberated in the complete oxidation of about 1.6 gram hexose, if all the available energy appeared as heat, and that the losses from

40 grams plant material in one temperature ascent were usually of this order.

SUMMARY

In preliminary experiments in Dewar flasks, moistened oat straw attained temperatures usually between 45° and 48° in times varying from 36 to 60 hours. Pre-extraction of the straw with water, dilute acid, or dilute alkali much reduced the maximum temperature reached and increased the time elapsing before the maximum was reached.

In the adiabatic apparatus, temperature maxima of 70° to 73° were obtained in 40 hours or less. The rate of heat evolution was not uniform and two well-defined maxima were apparent, one about 40° and one about 60°, coinciding with the points of maximum activity of mesophilic and thermophilic populations. The extent of decomposition was less than 4 per cent. The rate of heat evolution and the rate of evolution of carbon dioxide were closely related.

Experiments involving several temperature ascents, the purpose of which was the exhaustion of readily fermentable constituents, were not conclusive, inasmuch as the active flora is modified by the high temperatures. The addition of an aqueous extract of straw caused a temperature of 66° to be reached in 24 hours with a maximum rate of 14.9° in 3 hours between 35.5° and 50.4°. In subsequent ascents the rate of heat evolution progressively decreased as the readily available constituents were removed. Pre-extraction with reagents of increasing severity markedly diminished the rate of heat evolution but had no serious effect on the maximum temperature obtained. Two maxima were invariably found in the temperature rate curves.

The absence of additional available nitrogen had no effect on the thermogenic processes in one temperature ascent.

With a mixed population, the first maximum in rate of heat evolution (about 3° per hour at 40°) was always greater than the second maximum (less than 2° per hour at about 60°). This was due to the prior removal of some of the most readily available constituents by the mesophilic flora, since in experiments commenced at 50° the thermophilic peak was much higher.

Numbers of mesophilic and thermophilic bacteria were found to increase enormously at the time of most rapid heat evolution in their respective ranges. Low final counts of mesophilic organisms were obtained suggesting that the active mesophilic population is composed mainly of non-spore formers.

Pure culture decompositions were carried out with *Aspergillus fumigatus* and an unidentified bacillus. Both proved to be active only in the mesophilic range, but to be capable of raising the temperature of the straw from 25° to nearly 55°.

Thanks are due to Dr. J. C. Gilman for his helpful interest in the progress of these investigations.

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