CCL. THE PRODUCTION OF MUCUS DURING THE DECOMPOSITION OF PLANT MATERIALS.

III. THE EFFECT OF PARTIALLY AEROBIC AND ANAEROBIC CONDITIONS.

By JAGESHWAR GOPAL SHRIKHANDE.

*From the Indian Institute of Science, Bangalore, and Tea Research Institute
of Ceylon, Talawakella.*

(Received 9 June 1936.)

THE conditions necessary for the production of stickiness in manures described in the first two parts [1933, 1, 2] of this series were studied chiefly under aerobic conditions. While conducting experiments on the applicability of the Edelmist process to the composting of town refuse under tropical and sub-tropical conditions, it was thought worth while to follow the production of stickiness under partially aerobic conditions. Ragi straw, *Eleusine coracana*, was therefore decomposed in the presence of different sources of available nitrogen, the carbon-nitrogen ratios being adjusted to 30 : 1 and 10 : 1. The amount of mucus produced was measured by the physical test described in Part I [1933, 1]. No attempt was made to analyse the different structural constituents of the straw as no correlation was found to exist between the rates of their disappearance and the formation of mucus [1933, 2].

EXPERIMENTAL.

The physical test was conducted on fermented straws obtained under conditions described below.

Ragi straw was rotted in the presence of a mixed natural flora at 35° with the following changes in aeration, intervals of time and adjustment of reaction.

(1) Initial aerobic fermentation of 14 days followed by loose packing in bottles and analysing at intervals of 14, 28 and 56 days.

(2) As in (1) except that the bottles were tightly packed.

(3) Completely anaerobic conditions throughout the experiment, attained by packing air-tight in bottles and analysing at intervals of 14, 28 and 56 days.

(4) Completely aerobic conditions throughout the experiment and analysing on the 28th and 56th days after commencement.

(5) Adjustment of the final pH to a constant value independent of the source of nitrogen.

TECHNIQUE.

Ragi straw was chaffed and weighed quantities were fermented aerobically in large bottles with different amounts of nitrogen in various forms. The moisture content was maintained at about 60 %. After 14 days the contents were weighed and distributed into small bottles for subsequent decomposition. Weighed quantities of the thoroughly mixed material were taken and packed into bottles as required. The bottles were fitted with paraffined corks carrying exit tubes and pinch-cocks. The enclosed air for experiments in series II and III was sucked

away and the bottles were then incubated at 35°. The pinch-cocks were opened at intervals of 3 days to let out the accumulated gases. This process had to be repeated more frequently when night soil was used as a source of available nitrogen, the fermentation being very vigorous in the first 4 or 5 days. At the end of the desired period the bottles were opened and weighed and the contents subjected to stickiness test.

Table I. *Loss of dry matter per 100 g. of original straw and variation in stickiness under different conditions of fermentation.*

Series I unrestricted air supply (U) followed by partially restricted air supply (PR).

Time in days	Loss of dry matter	Physical test g.	Time in days	Loss of dry matter	Physical test g.
(NH ₄) ₂ SO ₄ , 30 : 1.			NaNO ₃ , 30 : 1.		
U 0	0	—	U 0	0	—
14	14.3	437	14	16.9	4444
PR 28	13.9	107	PR 28	17.2	3009
42	15.2	0	42	27.0	3140
70	26.2	0	70	33.1	2856
(NH ₄) ₂ SO ₄ , 10 : 1.			NaNO ₃ , 10 : 1.		
U 0	0	—	U 0	0	—
14	3.6	0	14	6.2	2069
PR 28	7.5	0	PR 28	13.9	1096
42	11.5	0	42	19.6	2778
70	19.9	0	70	27.8	1997
(NH ₄) ₂ CO ₃ , 30 : 1.			Night soil, 30 : 1.		
U 0	0	—	U 0	0	—
14	11.7	2190	14	24.7	5450
PR 28	16.9	1876	PR 28	25.7	3000
42	18.8	869	42	29.3	1076
70	27.7	798	70	43.7	1133
(NH ₄) ₂ CO ₃ , 10 : 1.			Night soil + (NH ₄) ₂ SO ₄ , 10 : 1.		
U 0	0	—	U 0	0	—
14	3.6	0	14	19.8	4370
PR 28	12.9	2049	PR 28	16.7	987
42	19.9	775	42	21.1	923
70	27.3	810	70	24.5	0

RESULTS.

Series I. Variation in stickiness of manures obtained by a preliminary aerobic followed by partially aerobic fermentation. Table I contains figures for stickiness and losses of dry matter obtained with such fermentation. The losses of dry matter after 14 days of aerobic decomposition are comparable with those previously recorded but the rate of decomposition appears to slow down as the period of restricted supply of air increases. The maximum losses occur with sodium nitrate and night soil as the sources of available nitrogen. In both of these cases the pH was higher than with ammonium sulphate or carbonate. The decomposition, therefore, depends upon the final reaction of the manure. Stickiness also runs parallel with the losses of dry matter and the final reaction of the manure. That there exists a relationship between the reaction and stickiness can be more clearly seen from the last two sections of Table I. Night soil with ammonium sulphate has produced less stickiness than night soil alone.

Table II. *Loss of dry matter per 100 g. of original straw and variation in stickiness under different conditions of fermentation.*

Series II unrestricted air supply (U) followed by completely restricted air supply (CR).

Time in days	Loss of dry matter	Physical test g.	Time in days	Loss of dry matter	Physical test g.
(NH ₄) ₂ SO ₄ , 30 : 1.			NaNO ₃ , 30 : 1.		
U 0	0	—	U 0	0	—
14	13.9	564	14	18.9	6459
CR 28	27.8*	678	CR 28	24.0	2897
42	20.0	107	42	27.3	676
70	24.7	110	70	29.6	208
(NH ₄) ₂ SO ₄ , 10 : 1.			NaNO ₃ , 10 : 1.		
U 0	0	—	U 0	0	—
14	14.8	459	14	10.5	2875
CR 28	23.1	203	CR 28	23.4	2060
42	26.7	0	42	25.9	555
70	29.7	0	70	29.1	210
(NH ₄) ₂ CO ₃ , 30 : 1.			Night soil, 30 : 1.		
U 0	0	—	U 0	0	—
14	23.1	2871	14	21.9	3597
CR 28	24.1	708	CR 28	23.2	1050
42	27.0	171	42	23.7	1025
70	30.8	0	70	28.4	0
(NH ₄) ₂ CO ₃ , 10 : 1.			Night soil + (NH ₄) ₂ SO ₄ , 10 : 1.		
U 0	0	—	U 0	0	—
14	10.5	2344	14	18.7	3049
CR 28	19.8	1019	CR 28	28.2	889
42	22.5	0	42	30.8	114
70	27.5	0	70	32.1	506

* Gone aerobic.

Incorporation of ammonium sulphate lowers the pH and the greater acidity reduces the stickiness. Sodium nitrate and night soil give the maximum stickiness. The stickiness obtained on aerobic fermentation gradually disappears as the supply of air gets less. This is perhaps due to the amounts of organic acids liberated during partially anaerobic conditions. The bottles when opened smelt of lactic acid. These points are more clearly brought out in the following series.

Series II. Variation in stickiness of manure obtained by a preliminary aerobic followed by anaerobic fermentation. The contents of the bottles were highly foul-smelling and smelt of organic acids, mostly acetic and lactic. The decomposition is practically of the same order though the rate is lower than in Series I. The values for stickiness recorded in Table II are also much lower than those in Table I and the rate of fall in stickiness is greater as the period of anaerobic decomposition increases. Such differences both in the losses of dry matter and stickiness can safely be attributed to the total absence of air. More acids are generated during anaerobic fermentation, which destroy the organisms responsible for a satisfactory rot and the production of stickiness. Sodium nitrate and night soil maintain some stickiness even at the end of 70 days because of their comparatively high final pH. It is well established that under anaerobic conditions nitrates are rapidly denitrified in presence of organic matter, leaving a strong base which serves as a neutralizing agent. Night soil too during fermentation in the early stages gives out ammonia which to a considerable extent neutralizes these acids.

Series III. Variation in stickiness of manure obtained under entirely anaerobic conditions. The losses of dry matter and the rate of decomposition as seen in Table III are extremely poor when compared with the first two series. The

Table III. *Loss of dry matter per 100 g. of original straw and variation in stickiness under different conditions of fermentation.*

Series III. Completely restricted supply of air throughout the experiment (CR).

Time in days	Loss of dry matter	Physical test g.	Time in days	Loss of dry matter	Physical test g.
(NH ₄) ₂ SO ₄ , 30 : 1.			NaNO ₃ , 30 : 1.		
CR 0	0	—	CR 0	0	—
14	6.9	0	14	3.3	0
28	7.5	0	28	5.0	0
56	21.9*	110	56	6.9	0
(NH ₄) ₂ SO ₄ , 10 : 1.			NaNO ₃ , 10 : 1.		
CR 0	0	—	CR 0	0	—
14	14.7	0	14	15.3	593
28	17.0	0	28	10.5	471
56	16.9	0	56	16.9	0
(NH ₄) ₂ CO ₃ , 30 : 1.			Night soil, 30 : 1.		
CR 0	0	—	CR 0	0	—
14	10.8	0	14	12.8	116
28	9.5	0	28	14.6	107
56	16.4	0	56	16.2	0
(NH ₄) ₂ CO ₃ , 10 : 1.			Night soil + (NH ₄) ₂ SO ₄ , 10 : 1.		
CR 0	0	—	CR 0	0	—
14	21.5*	670	14	14.5	130
28	14.1	0	28	14.5	110
56	13.5	0	56	19.5	0

* Gone aerobic.

manure was also non-sticky. Results in Table III, when compared amongst themselves, show that the maximum loss of dry matter was obtained with sodium nitrate and night soil owing to the reaction being more nearly neutral. The contents of the bottles were not so foul-smelling as in Series II, but smelt strongly of acetic acid. The colour was also peculiarly pale yellow, thus differing from that in the foregoing series which was distinctly brown. The complete absence of stickiness must, therefore, be ascribed to anaerobic conditions preventing the growth of those aerobic types of fungi and bacteria which are mostly responsible for the production of stickiness, as has been suggested in Part II [1933, 2].

Series IV. Variation in stickiness of manure obtained under entirely aerobic conditions. This series was conducted by way of comparison. It has been discussed in more detail in Part I [1933, 1]. As previously recorded sodium nitrate gives the stickiest manure, followed by night soil and ammonium carbonate. On comparing all the results of the first three series with those in Table IV it will be clear that manure obtained under entirely aerobic conditions is the stickiest, whilst manure is absolutely non-sticky when obtained under anaerobic conditions. Presence of a plentiful supply of air is, therefore, an essential condition for the production of stickiness in a manure heap.

Series V. The effect of the modification of the final pH on the production of stickiness obtained under various conditions detailed above. The pH in all the

Table IV. *Loss of dry matter per 100 g. of original straw and variation in stickiness under unrestricted supply of air (U)—Series IV.*

Time in days	Loss of dry matter	Physical test g.	Time in days	Loss of dry matter	Physical test g.
	(NH ₄) ₂ SO ₄ , 30 : 1.			NaNO ₃ , 30 : 1.	
U 14	23.0	200	U 14	28.9	4120
56	39.4	270	56	37.5	7353
	(NH ₄) ₂ SO ₄ , 10 : 1.			NaNO ₃ , 10 : 1.	
U 14	14.8	185	U 14	10.5	2689
56	39.0	210	56	37.1	7244
	(NH ₄) ₂ CO ₃ , 30 : 1.			Night soil, 30 : 1.	
U 14	23.1	3450	U 14	41.9	5978
56	47.4	4500	56	54.3	6010
	(NH ₄) ₂ CO ₃ , 10 : 1.			Night soil + (NH ₄) ₂ SO ₄ , 10 : 1.	
U 14	20.5	3252	U 14	18.7	4856
56	54.9	4553	56	56.0	5873

bottles fell considerably during the course of fermentation. Under restricted supply of air, ammonium sulphate gave the lowest and sodium nitrate the highest *pH*. The present series was undertaken to demonstrate the effect on stickiness of artificially raising the *pH* to 9.0 by adding sodium carbonate. This *pH* of 9.0 is usually attained by a sodium nitrate rot which has lost about 40 % of dry matter in about 5 weeks and is accompanied by a maximum stickiness of nearly 7000 g. Table V includes the results of such experiments. No appreciable

Table V. *Effect of the modification of pH on the production of stickiness.*

(pH adjusted to 9.0 with Na ₂ CO ₃ .)		Physical tests g.			
	Source of nitrogen	Loss of dry matter	Final pH	Before pH adjustment	After pH adjustment
From series I Ratio 30 : 1	(NH ₄) ₂ SO ₄	13.9	4.8	107	203
	(NH ₄) ₂ CO ₃	18.8	5.2	869	997
	NaNO ₃	27.0	6.8	3140	3808
	Night soil	29.3	6.2	1076	1300
From series II Ratio 30 : 1	(NH ₄) ₂ SO ₄	20.0	4.5	107	276
	(NH ₄) ₂ CO ₃	27.0	5.1	171	549
	NaNO ₃	27.3	6.4	676	1050
	Night soil	23.2	6.2	1050	1529
From series III	(NH ₄) ₂ SO ₄ , 30 : 1	—	4.5	—	—
	(NH ₄) ₂ CO ₃ , 30 : 1	—	5.1	—	—
	NaNO ₃ , 10 : 1	10.5	6.0	417	500
	Night soil, 30 : 1	14.6	6.0	107	110

increase in the stickiness after adjustment of the *pH* to 9.0 in any of the samples is noticeable. This must be due to the lower losses of dry matter and also to the entirely different nature and course of decomposition under partially aerobic and anaerobic conditions.

DISCUSSION.

The experiments described above suggest that the decomposition of plant tissues is much slower under anaerobic than under aerobic conditions. The losses in dry matter with narrow C : N ratio are in general more than those recorded with wide C : N ratio. This observation is in accord with those of previous

workers. Organic acids are generated during anaerobic fermentation the accumulation of which is responsible for the retardation of fermentation and destruction of stickiness. Fowler & Joshi [1920] observed depressed fermentation of cellulosic materials when the acid concentration reached a level of 1 %. Experiments on anaerobic decomposition of rice straw by Acharya [1935] and the author's own results [1933, 1] on aerobic decomposition of oat straw show that the change is influenced by the reaction during fermentation. A pH of 7.5–8.0 is found to be suitable for a satisfactory rot. Non-production of stickiness under partially restricted and totally restricted supply of air must be due partly to the strongly acid reaction and partly to the different nature and course of the decomposition. Anaerobic conditions modify the flora completely. Essentially aerobic fungi and mucus-producing bacteria like *Spyrochaeta cytophaga*, which have been described as essential in Part II [1933, 2] for the production of stickiness, do not develop under anaerobic conditions. Consequently there is no synthesis of the material responsible for stickiness because of the possible absence of interaction between fungal tissue and the gum from a bacterium. For a manure heap to be sticky, therefore, it must be maintained alkaline and should be kept thoroughly aerobic. This will ensure a satisfactory rot with a good growth of fungi and mucus-producing bacteria leading to the synthesis of the material responsible for stickiness.

SUMMARY.

1. Decomposition of Ragi straw, *Eleusine coracana*, under different conditions of fermentation in presence of different sources of available nitrogen has been studied from the point of view of stickiness. The results obtained confirm those previously recorded [1933, 1, 2].
2. Strong acidity and a different course and nature of decomposition under partially aerobic and anaerobic conditions prevent the development of stickiness during fermentation.
3. Wide C : N ratio produces a greater loss than narrow C : N ratio. High doses of nitrogen appear to have no effect on the production of stickiness.
4. Final adjustment of the pH to 9.0 with sodium carbonate has practically no effect on the stickiness obtained during fermentation.

REFERENCES.

- Acharya (1935). *Biochem. J.* **29**, 528.
Fowler & Joshi (1920). *J. Indian Inst. Sci.* **3**, 39.
Shrikhande (1933, 1). *Biochem. J.* **27**, 1551.
— (1933, 2). *Biochem. J.* **27**, 1563.