CXL. STUDIES ON THE ANAEROBIC DECOMPOSITION OF PLANT MATERIALS.

III. COMPARISON OF THE COURSE OF DECOMPOSITION OF RICE STRAW UNDER ANAEROBIC, AEROBIC AND PARTIALLY AEROBIC CONDITIONS.

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THE experiments reported in this paper form a continuation of those already described [Acharya, 1935, 1, 2] and comprise a comparative study of the rate and course of decomposition of rice straw under anaerobic, aerobic and partially aerobic (*e.g.* water-logged) conditions.

The procedure and analytical details adopted were the same as those previously reported except for the modifications noted below. Ammonium carbonate equivalent to 1 % N on the straw, 0·5 g. K_2HPO_4, 0·05 g. $MgSO_4$, $7H_2O$ and 10 ml. of an extract from a rice soil were added to 20 g. portions of chaffed rice straw. The aerobic decomposition was carried out in open dishes, keeping the mass at a straw : water ratio of about 1 : 4 by additions of water from time to time. Water-logged conditions were obtained by having about an inch of water above the straw which was kept in beakers. Every few days the mass was stirred and fresh water added to replace evaporation losses. Decomposition under anaerobic conditions was carried out in stoppered bottles as already described [1935, 1, 2]. It was observed that abundant fungal growth accompanied decomposition under aerobic conditions and that even under water-logged conditions a thin fungal layer formed on the surface of the water when the mass was not stirred.

In addition to the above comparisons the effect of a regulated admission of air was tested by incubating samples in stoppered bottles and bubbling air through them at weekly intervals, after the accumulated gases had been tapped off for analysis. In one set of bottles a current of air was drawn for 5 minutes through the bottles just over the surface of the liquid; this is termed "mild aeration." In another set, air was bubbled through the liquid for 5 minutes so as to replace the dissolved gases by air and this is termed "strong aeration."

The results obtained are given in Table I. They show that the rate of decomposition is highest under aerobic conditions and lowest under anaerobic conditions, the partially aerobic (*e.g.* water-logged) systems occupying an intermediate position. At the end of 6 months the losses in ash-free insoluble dry matter are 65·0, 47·5 and 55·5 % for aerobic, anaerobic and water-logged fermentations respectively. The relative ease with which the cellulose and hemicellulose fractions of straw are attacked varies, however, in the different cases. Under anaerobic conditions, the hemicelluloses are the first to be attacked; at the end of one month 26·8 % of the amount originally present has been decomposed as against 9·8 % of the cellulose. At the end of the same period under aerobic conditions the loss of hemicelluloses is 62·4 % and of cellulose 56·2 %; the water-logged straw loses 38·0 % of hemicelluloses and 32·0 % of cellulose. The water-logged system thus resembles the aerobic system in the relative proportions of cellulose to hemicellulose destroyed, though the actual losses are much lower in the former case. The greater loss of cellulose in the early stages in these two systems, as compared with the anaerobic system, is probably due to the activity of cellulose-decomposing fungi.

The greatest loss of lignin occurs in the water-logged straw, whether determined on the material without any treatment or after a preliminary hydrolysis with 5 % H_2SO_4 [Norman and Jenkins, 1933, p. 826]; the losses are 39·6 % and 33·1 % of the initial value by these two methods. The corresponding losses under anaerobic conditions are 28·5 % and 28·5 %, and under aerobic conditions 33·5 % and 29·8 % respectively.

A striking difference between the three methods of fermentation lies in the protein content of the insoluble residue. In the aerobic system this rises from 2·88 g. per 100 g. straw at the start to 5·45 g. at the end of 6 months, whereas under anaerobic and partially aerobic conditions losses of this constituent are found. Of these two latter residues, the anaerobic contains less protein (2·01 g.) than the water-logged (2·69 g.); this difference is probably associated with the more intense reducing conditions present in the anaerobic system which help to bring more of the protein into solution.

The loss of added ammonia is most rapid under aerobic conditions, about 46 % of it being lost by volatilisation in a fortnight. The loss is less rapid from the water-logged straw, about 18 % being lost during the same period. The added ammonia is much better conserved under anaerobic conditions, the loss over a period of 6 months amounting to only 15 %; this no doubt is due to the closed nature of the system and the limited number of occasions on which the accumulated gases are let out for analysis.

The differences in the nature of the products obtained under aerobic and anaerobic conditions have been dealt with in a previous communication [1935, 1]. The formation of organic acids and the evolution of combustible gases characteristic of anaerobic decomposition are absent in the former case, where intermediate products if any are rapidly oxidised to CO_2. Water-logged conditions resemble the anaerobic system in the rapid accumulation of organic acids in the early stages, but these are decomposed much more rapidly than in the latter case.

The influence of partial aeration on anaerobic decomposition is shown in Tables I and II under the heads "mild aeration" and "strong aeration." The significance of these terms has been explained already.

With "mild aeration" the anaerobic conditions in the liquid portion are not appreciably disturbed as shown by the accumulation of organic acids and the evolution of methane and hydrogen. There is a marked increase in the amount of CO_2 evolved, presumably due to the admission of air, and a decrease in the amount of CH_4 formed. The amount of H_2 evolved shows a great increase as compared with anaerobic conditions. This difference, it is suggested, is associated with the oxidation-reduction potential of the system under these conditions.

The decomposition with "strong aeration" resembles that under aerobic conditions as shown by the small amount of organic acids, CH_4 and H_2 formed and the large amounts of CO_2 evolved (Table II). The high proportion of free nitrogen contained in the gases evolved in both aeration treatments is derived from the air passed through the bottles.

The nitrogen relationships under the different treatments are of special interest. It has already been noted that the amount of protein in the insoluble residue is lowest in the anaerobic system and highest in the aerobic system.

Table I. *Decomposition of rice straw at 30° under aerobic, anaerobic and partially aerobic conditions.*

		Aerobic				Anaerobic				Partially aerobic					
										Water-logged				Mild aeration	Strong aeration
	100 g. original straw g.	2 weeks g.	1 month g.	3 months g.	6 months g.	2 weeks g.	1 month g.	3 months g.	6 months g.	2 weeks g.	1 month g.	3 months g.	6 months g.	6 months g.	6 months g.
						Losses of constituents.									
Dry residue less ash	74·1	24·1	35·7	45·4	48·2	10·2	15·2	22·9	35·2	18·2	24·5	32·2	41·1	36·8	34·4
Cellulose (including xylan)	44·55	17·25	25·05	33·01	35·25	2·12	4·38	10·47	21·26	10·20	14·25	19·20	25·95	24·60	23·60
Xylan in cellulose	11·73	4·28	6·38	8·88	9·93	0·91	1·54	3·25	6·36	2·08	3·18	4·98	7·23	6·03	5·93
Total xylans	21·04	9·59	13·14	17·44	18·24	4·12	5·64	8·78	12·79	5·09	7·99	11·59	15·44	13·04	11·99
Lignin (direct)	10·67	1·52	2·77	3·37	3·57	0·72	0·87	1·47	3·04	1·75	2·27	3·47	4·22	2·63	2·93
Lignin (after hydrol.)	9·12	1·37	1·97	2·37	2·72	0·61	1·20	1·99	2·60	1·58	2·02	2·77	3·02	2·22	2·67
Protein	2·88	+1·35	+1·83	+2·25	+2·57	0·82	0·87	0·87	0·87	0·15	0·16	0·18	0·19	0·28	0·19
						Present in aqueous extract.									
Organic acids	—	Nil	Nil	Nil	Nil	6·42	7·50	7·26	1·30	10·82	3·58	1·50	0·12	7·38	0·73
Protein-N	—	0·050	0·075	0·100	0·125	0·135	0·153	0·138	0·207	0·315	0·350	0·375	0·425	0·310	0·360
Nitrate-N	—	0·025	0·050	0·050	0·050	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
NH_4-N	—	0·275	0·125	0·050	Nil	0·824	0·812	0·781	0·704	0·525	0·210	0·095	0·040	0·651	0·515
p_H of medium	—	8·8	9·0	9·2	9·2	7·0	7·2	7·2	7·8	7·0	7·4	8·2	8·8	7·2	8·8
						Nitrogen relationships.									
Protein-N in residue	0·461	0·677	0·754	0·821	0·872	0·330	0·320	0·324	0·323	0·438	0·435	0·432	0·431	0·416	0·431
Protein-N in aq. extract	—	0·050	0·075	0·100	0·125	0·135	0·153	0·168	0·207	0·315	0·350	0·375	0·425	0·310	0·360
Total protein-N present	—	0·727	0·829	0·921	0·997	0·465	0·473	0·492	0·530	0·753	0·785	0·807	0·856	0·726	0·791
N immobilised or nitrogen factor	—	0·266	0·368	0·460	0·536	0·004	0·012	0·031	0·069	0·292	0·324	0·346	0·395	0·265	0·330
Nitrogen equivalent	—	1·10	1·03	1·02	1·11	0·039	0·079	0·135	0·196	1·604	1·322	1·074	0·961	0·701	0·932

Table II.

Per 100 g. straw	Anaerobic ml.	Mild aeration ml.	Strong aeration ml.
CO_2 evolved	10,850	17,405	32,500
CH_4 evolved	10,570	6,880	2,130
H_2 evolved	105	5,625	925
N_2 evolved	85	12,155	14,205

Whereas under anaerobic and water-logged conditions increasing amounts of protein dissolve with increasing periods of fermentation, in the aerobic system there is a continued accumulation of protein in the residue, due to the synthesis of insoluble microbial tissue.

The water-soluble protein-nitrogen is at a minimum under aerobic conditions (0·125 g. per 100 g. straw), as compared with anaerobic (0·207 g.) and water-logged systems (0·425 g.). The high value for soluble protein-nitrogen under water-logged conditions is noteworthy since most of it is synthesised from the ammonia originally added. It is an index of the great microbial activity under these conditions and is in marked contrast to the poor nitrogen immobilisation under anaerobic conditions.

The figures for nitrogen factor [Rege, 1927] and nitrogen equivalent [Richards and Norman, 1931] also show striking differences between the three sets of conditions. They are highest under aerobic conditions being 0·536 and 1·11 respectively, followed by water-logged conditions (0·395 and 0·961 respectively), while the anaerobic system shows the minimum values (0·069 and 0·169 respectively). Even "mild aeration" favours protein synthesis as shown by the jump of the nitrogen factor from 0·069 to 0·265, while "strong aeration" increases it to 0·330.

Discussion.

The results indicate that partially aerated systems show the characteristics of both the aerobic and anaerobic types of decomposition of straw and that the degree of aeration decides which type predominates. Thus "mild aeration" resembles the anaerobic system because of the organic acids and methane formed, with the important difference that under suitable conditions a large amount of hydrogen is also liberated. More CO_2 is evolved than under anaerobic conditions. With "stronger aeration," conditions approach the aerobic system as shown by the small amount of organic acids, CH_4 and H_2 produced and the large amount of CO_2 evolved. Water-logged conditions occupy an intermediate position between the above two systems.

The water-logged system resembles the anaerobic system in some respects, *viz.* in the formation of organic acids, liberation of methane and formation of soluble protein, but in others it shows differences, *e.g.* in the relative losses of cellulose, hemicelluloses and lignin and in the synthesis of protein from ammonia. These differences could be explained by the presence of a wider variety of micro-organisms in the water-logged system able to attack cellulose and lignin. Moreover, the lower reducing power under water-logged conditions, as compared with the anaerobic system, permits an accumulation of proteins synthesised as a result of microbial activity. In these respects the water-logged system more closely resembles the aerobic system.

The admission of even limited amounts of air at weekly intervals alters the nitrogen relationships of the anaerobic system, as shown by the synthesis of protein from ammonia and by the rapid increase in the values for nitrogen factor and nitrogen equivalent.

Summary.

1. The relative rates of decomposition of rice straw in decreasing order are: aerobic, water-logged and anaerobic fermentations. Cellulose and lignin are more easily attacked under water-logged conditions than under anaerobic conditions.

2. Partially aerobic conditions resemble anaerobic conditions in regard to the nature of the products obtained. Smaller amounts of organic acids and methane are, however, formed and there is an increase in the amount of CO_2 evolved. Under conditions of mild aeration much larger quantities of hydrogen are obtained than under anaerobic conditions.

3. The nitrogen factor and nitrogen equivalent decrease in the order: aerobic (0·536; 1·11), water-logged (0·395; 0·961) and anaerobic (0·069; 0·169), showing the degree of protein accumulation in the three cases. The protein formed under aerobic conditions is mostly insoluble in water, while under water-logged conditions it remains in solution.

4. In most respects the decomposition of rice straw under water-logged conditions gives results intermediate between aerobic and anaerobic decompositions.

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