

CXV. STUDIES ON THE ANAEROBIC DECOMPOSITION OF PLANT MATERIALS.

II. SOME FACTORS INFLUENCING THE ANAEROBIC DECOMPOSITION OF RICE STRAW (*ORYZA SATIVA*).

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IN a previous communication dealing with the anaerobic decomposition of rice straw [Acharya, 1935], it was noted that several factors such as temperature and reaction of medium greatly influenced the rate and course of the decomposition. An examination of some of these factors is reported in the present paper.

The experimental procedure and methods of analysis adopted were the same as used previously [1935], except for the changes and modifications noted in the experiments described below.

Exp. 1. Influence of temperature. To 20 g. portions of rice straw were added a mixture of 2 g. KHCO_3 and 2 g. NH_4HCO_3 in order to neutralise the acids formed, 0.2 g. K_2HPO_4 , 0.05 g. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 10 ml. of a 2% extract of a rice soil and water to make up 200 ml. The bottles were stoppered and paraffined and after evacuation were incubated at the desired temperatures. At weekly intervals the accumulated gaseous products were tapped off into a Hempel's burette, measured and samples taken for analysis. The liquid and solid residues in the bottles were analysed at the end of 6 months, according to the procedure already described. The data obtained, calculated per 100 g. straw taken, are given in Table I.

Table I. *Decomposition of rice straw at different temperatures.*

Per 100 g. straw	...	20°	25°	30°	35°	40°	45°
Total gas production (CO_2 not corrected for carbonates)		ml.	ml.	ml.	ml.	ml.	ml.
At the end of 1 month, CO_2		Nil	605	2705	3010	2810	1110
" " 1 " CH_4		Nil	Nil	20	35	40	65
" " 2 months, CO_2		1005	2050	3390	4125	3065	2090
" " 2 " CH_4		225	305	410	495	320	185
" " 3 " CO_2		2385	3770	6220	6980	4980	2985
" " 3 " CH_4		705	1460	3950	4350	2095	640
" " 6 " total CO_2		4940	6640	10290	10685	7180	3515
At the end of 6 months, net CO_2 (corrected for carbonates)		1262	4775	9830	9847	5530	1232
At the end of 6 months, CH_4		1205	4530	9585	9710	5310	1090
At the end of 6 months		g.	g.	g.	g.	g.	g.
Loss of dry matter less ash		17.3	23.1	35.2	35.8	24.1	15.4
Net CO_2 liberated corrected for carbonates		2.49	9.44	19.44	19.46	10.93	2.44
Net CH_4 liberated		0.86	3.24	6.87	6.96	3.81	0.78
Acetic acid present		8.57	4.43	1.07	1.92	3.86	5.42
Butyric acid present		1.88	0.83	0.23	0.48	0.82	1.02
Total weight of products		13.80	17.94	27.61	28.82	19.42	9.66

As the aim of the present investigation was to extend it to a study of anaerobic decomposition in the soil, the examination of the changes was confined to those taking place between 20° and 45°. The data show that the optimum amount of decomposition, as judged by loss of dry matter and total weight of products obtained, occurs between 30° and 35°. Outside this range there is an accumulation of acids and an increasing retardation in gas production. Hence in the experiments described below the temperature was maintained within this range. The figures given in Table I also show that though the amount of decomposition varies considerably at the different temperatures, the proportion of the net volume of CO₂ evolved, after correction for that evolved from carbonates, to that of methane produced is roughly unity.

Exp. 2. Influence of reaction of medium. Ammonium carbonate equivalent to 1% N on the straw and K₂HPO₄, MgSO₄, 7H₂O, soil extract and water in the quantities specified in the previous experiment were added to 20 g. portions of rice straw. The initial reaction of the medium was adjusted to the desired level by additions of H₂SO₄ or NaOH. All *p_H* determinations were made colorimetrically with the Hellige apparatus. Twice a week in the initial stages and at weekly intervals later, the accumulated gases were tapped off and 0.5 ml. of the reaction liquid was taken out for analysis, without the admission of air, by fitting a capillary of known volume into the exit tube, inverting the bottle and carefully unscrewing the pinchcock till the capillary was filled. The sample taken was diluted and titrated colorimetrically from a micro-burette to the desired *p_H* and the equivalent amount of acid or alkali was added to the contents of the bottle. On account of the buffering action of the organic acids formed, the method was found to give a reliable control of the *p_H* at the time of operation correct to ±0.1. Owing to the rapid formation of acids in the early stages, however, difficulty was experienced in maintaining the *p_H* at the desired figure over a number of days, though an effort was made to keep to the approximate level by frequent checks and additions of alkali when necessary. It has already been noted that the use of buffers was rendered impossible on account of the high concentration of acids formed.

The data obtained (Table II) show that the maximum loss of dry matter occurs at about *p_H* 8, at which level there is also the maximum evolution of methane and the minimum accumulation of acids. Above and below this *p_H*

Table II. *Decomposition of rice straw at different p_H values.*

<i>p_H</i>	g. per 100 g. of straw						
	...	5	6	7	8	9	10
At the end of 6 months:							
Loss of dry matter less ash		15.2	25.3	35.5	56.6	50.5	48.2
CO ₂ evolved and in solution		1.61	8.27	13.40	22.87	14.12	12.92
CH ₄ evolved and in solution		0.14	2.43	3.75	6.94	3.67	1.39
Acetic acid present		5.35	3.57	3.13	2.11	10.85	16.05
Butyric acid present		1.89	1.02	0.85	Nil	1.76	3.21
Total weight of products		8.99	15.29	21.13	31.92	30.40	33.57

there is an increasing tendency for an accumulation of acids and a retardation of methane production, showing that the initial stage of acid formation is much more tolerant of changes in the reaction of medium than is the second stage of methane production. Very little methane production takes place below *p_H* 6 and above *p_H* 9; the rate is optimum at *p_H* 8 and falls rapidly on either side of this value. The accumulation of acids at *p_H* 9–10 suggests a method for the conversion of straw into acids, mainly acetic and butyric, by anaerobic digestion

at this range. For gas production a range of p_H 7.5 to 8 seems to be optimum. The concentration of CO_2 in solution is high at the higher p_H levels which may be due to the chemical action of alkali at high concentrations on rice straw.

Exp. 3. Influence of different proportions of water. According to the hypothesis [Symons and Buswell, 1933; Boruff and Buswell, 1934; Tarvin and Buswell, 1934] that anaerobic degradation is an oxidation-reduction reaction involving the addition of water and scission of the molecule into simpler compounds, a higher proportion of water would probably be necessary than if water played no part in the decomposition. Langwell and Lymn [1932] noted that a mobile liquid medium was necessary for successful thermophilic digestion of plant materials. A concentration of 8–10 % solids has been found optimum for the anaerobic digestion of sewage solids and garbage [Keefer and Kratz, 1934]. It was therefore thought interesting to examine the effect of adding different proportions of water on the rate of decomposition of rice straw.

The experimental details were the same as in Exps. 1 and 2, except that the ratio of straw to water was varied to give the following proportions: 1 : 4, 1 : 6, 1 : 8, 1 : 10, 1 : 15 and 1 : 20.

The results obtained (Table III) show that gas production is much impeded below a straw : water ratio of 1 : 8. The optimum concentration appears to be about 1 : 10, above which further additions of water are of no advantage. On

Table III. *Decomposition of rice straw with different proportions of water.*

	Per 100 g. straw					
	Straw : water ratio					
	1 : 4	1 : 6	1 : 8	1 : 10	1 : 15	1 : 20
Total evolution of gas (CO_2 not corrected for carbonates)	ml.	ml.	ml.	ml.	ml.	ml.
At the end of 1 month, CO_2	Nil	Nil	175	1080	1890	1585
" " 1 " CH_4	Nil	Nil	35	980	1105	925
" " 2 months, CO_2	545	1245	3625	5585	4865	3860
" " 2 " CH_4	105	350	3900	5225	4670	3695
" " 3 " CO_2	1830	3590	6095	7220	6930	5620
" " 3 " CH_4	135	1520	5775	7030	6855	5335
" " 6 " total CO_2	3865	8340	10525	11020	10985	10785
" " 6 " net CO_2	461	7266	10126	11020	10985	9833
(corrected for carbonates)						
At the end of 6 months, CH_4	325	6975	9945	11185	11210	10015
At the end of 6 months:	g.	g.	g.	g.	g.	g.
Loss in dry matter less ash	16.2	28.4	35.8	37.6	38.2	38.6
Net CO_2 (corrected for carbonates)	0.91	14.36	20.01	21.79	21.72	19.44
Net CH_4	0.24	5.00	7.13	8.02	8.04	7.18
Acetic acid present	7.56	2.29	1.07	Nil	Nil	2.06
Butyric acid present	2.30	0.86	Nil	Nil	Nil	0.72
Total weight of products	11.01	22.51	28.21	29.81	29.76	29.40

the other hand, at a concentration of 1 : 20 there is actually a decrease in gas production. The optimum proportion of straw to water of 1 : 10 may be compared with the proportion of 1 : 4 or 5 generally found to be favourable for aerobic decomposition.

Exp. 4. Effect of inoculation with soil. The object of this experiment was to examine the differences if any between the rate and course of decomposition in presence of soil extract and in its absence. It is well known that natural products, e.g. straw, carry a microflora able to bring about their rapid decomposition. The experimental details were the same as before but no soil extract was added.

The data (Table IV) show that the rate and course of fermentation are the same both in presence and absence of soil inoculant, proving that the straw itself carries the micro-organisms necessary to bring about its decomposition.

Exp. 5. Effect of addition of phosphate. The present sample of straw contained 0.42 % P_2O_5 and the question as to whether addition of phosphate assisted anaerobic decomposition was examined by fermenting samples with and without the addition of phosphate (1 g. K_2HPO_4 per 100 g. straw).

The results (Table IV) show that the phosphate requirement for anaerobic digestion is quite small and is apparently satisfied by the phosphate present in

Table IV. *Exps. 4, 5, 6 and 7.*

	Results in g. per 100 g. straw.				
	Control	Exp. 4 Omission of soil inoculum	Exp. 5 Omission of phos- phate	Exp. 6 Powdered straw	Exp. 7 Inocula- tion with vigorous culture
After 6 months:					
Loss of dry matter less ash	35.2	36.8	35.6	33.1	37.6
Net CO_2 (corrected for carbonates)	19.44	19.72	18.78	16.86	19.98
Net CH_4 evolved	6.87	7.10	6.52	5.82	7.32
Butyric acid present	0.23	Nil	0.86	0.98	Nil
Acetic acid present	1.07	0.83	1.82	3.22	0.42
Total weight of products	27.61	27.65	27.98	26.88	27.72
Preliminary lag period (days)	12	10	12	14	12

the straw itself. Additions of phosphate, *e.g.* K_2HPO_4 , do not exert any stimulating effect except by way of improving the reaction of the medium where the initial reaction is unfavourable for anaerobic digestion. Excess of phosphate at concentrations of 5 % and above on the straw taken was found greatly to impede the decomposition.

Exp. 6. Influence of powdering the straw (Table IV). This was tested by using 20 g. portions of straw ground so as to pass a 60-mesh sieve. The powdered sample in presence of excess of water formed a compact layer at the bottom, which did not ferment so readily as when chaffed straw was used. The total amount of decomposition is of the same order as with chaffed straw, but more acid is present. The amount of methane evolved is 8110 ml. as compared with 9585 ml. in the control.

Exp. 7. Effect of inoculation with an old culture (Table IV). A period of preliminary incubation or lag period is generally associated with anaerobic digestion and is attributed to the time taken for the development of the specific microflora necessary for effecting decomposition. The object of the present experiment was to see whether the lag period could be shortened by the addition to the medium of a small quantity of an inoculant from a vigorously growing culture. To 20 g. portions of rice straw, 5 ml. of the inoculant from an actively fermenting culture were added in place of the soil extract. The results show that the addition of such a small quantity of inoculant has no appreciable effect in shortening the period of incubation or hastening the rate of decomposition. It has been found that a fairly high proportion of seed is necessary to carry on successful digestion of fresh sewage solids [Whitehead and O'Shaughnessy, 1931].

Exp. 8. Use of different neutralising agents (Table V). Attention has already been drawn to the large amount of acids formed and the difficulty found in maintaining an alkaline reaction by the use of buffers. As the aim was to keep the reaction at about p_H 7.5 to 8, various weak neutralising agents were tried.

Table V. *Decomposition of rice straw in presence of different neutralising agents.*

g. per 100 g. straw after 6 months.

Neutralising agent	Loss of dry matter less ash	Net CO ₂	Net CH ₄	Acetic acid	Butyric acid	Total weight of products
1. CaCO ₃ , no N added	18.6	1.15	0.22	8.62	2.82	12.81
2. Ammonium carbonate	35.2	19.44	6.87	1.07	0.23	27.61
3. NaNO ₃	31.8	16.01	4.19	3.64	Nil	23.84
4. NaHCO ₃ , no N added	26.2	13.62	4.96	1.32	Nil	19.90
5. KHCO ₃ , no N added	33.4	17.82	6.21	2.46	0.82	27.31
6. NH ₄ HCO ₃	34.6	18.62	6.46	2.26	0.84	28.18
7. CaCO ₃ + NaHCO ₃ , no N added	30.4	15.42	5.41	2.92	1.04	24.79
8. CaCO ₃ + NH ₄ HCO ₃	31.8	16.26	5.61	3.40	1.12	26.39
9. NH ₄ HCO ₃ + KHCO ₃	34.6	19.16	6.42	1.92	0.60	28.10
10. NH ₄ HCO ₃ + NaHCO ₃	36.1	17.66	6.24	2.86	0.96	27.72

The figures show that CaCO₃ alone is not a satisfactory neutralising agent but it may give good results in combination with other reagents such as NH₄HCO₃, KHCO₃ or NaHCO₃. By itself NaHCO₃ is probably too alkaline (p_H 8.4–8.5) and impedes decomposition; it is not definitely known, however, whether the effect is due to the p_H of the system or to the high concentration of the salt. A mixture of NH₄HCO₃ and NaHCO₃ has given good results, as has also a mixture of NH₄HCO₃ and KHCO₃. The use of ammonium carbonate and sodium nitrate as neutralising agents has been examined in a previous communication [1935]. The degree of decomposition obtained by the use of KHCO₃, NaHCO₃ and NaHCO₃ + CaCO₃, in each case without the addition of nitrogen, further confirms the low nitrogen requirement for an anaerobic digestion.

The neutralising agents described above are useful in that they can be added in advance along with the material in sufficient amount to neutralise the acids formed and so lead to successful decomposition without having recourse to frequent adjustments of the reaction of the medium. The acids formed may of course be neutralised from time to time as already described in Exp. 2. The degree of decomposition and the products formed are about the same in both cases.

Exp. 9. Use of different sources of nitrogen (Table VI). The parts played by ammonium carbonate and sodium nitrate in influencing anaerobic decomposition have previously been considered. The aim of the present experiment was to

Table VI. *Decomposition of rice straw in presence of different forms of nitrogen.*

g. per 100 g. straw after 6 months.

	Ammonium carbonate	NaNO ₃	Urea	Cyana- mide	Egg- albumin	Blood- albumin	Casein- ogen
Loss of dry matter less ash	35.2	31.8	32.8	29.6	23.8	22.4	18.2
Net CO ₂ evolved	19.44	16.01	15.61	13.46	3.46	2.58	1.22
Net CH ₄ evolved	6.87	4.19	5.32	4.52	1.12	0.84	0.38
Acetic acid present	1.07	3.64	3.12	4.24	7.29	7.52	4.72
Butyric acid present	0.23	Nil	0.96	1.12	6.60	5.66	8.18
Total weight of products	27.61	23.84	25.01	23.34	18.47	16.60	14.50

examine whether other nitrogenous compounds, easily reducible to ammonia under anaerobic conditions, could replace ammonium carbonate as neutralising agents. For this purpose, urea, calcium cyanamide, caseinogen, blood-albumin and egg-albumin were tried. 2 g. of each were added to 20 g. of straw, no other neutralising agent being added. The other experimental details were as before.

The figures show that urea and cyanamide are in a different category from the proteins and more closely resemble ammonium carbonate in their ability to neutralise the acids formed from straw.

An interesting point is the high proportion of butyric acid to acetic acid in cultures containing proteins. Possibly the butyric acid is derived from the anaerobic degradation of the added protein. The proportion of butyric acid to acetic acid is comparatively low where straw is digested by itself or in presence of ammonium carbonate, sodium nitrate, urea or cyanamide.

Expt. 10. Influence of the volume of the culture vessel (Table VII). The volume of the available free space above an anaerobic culture medium may sometimes markedly influence the rate of decomposition, *e.g.* a small vessel may produce high concentrations of CO₂ which may be directly toxic to the bacterial population [Kliewe and Kindhäuser, 1933] or may indirectly impede their activity by lowering the *p*_H. The use of culture vessels of different capacities may, in the present case, also throw some light on the mechanism of formation of methane.

In order to examine these points samples of 20 g. of rice straw were packed in bottles of different sizes as specified in Table VII and the decomposition was allowed to proceed for a period of 6 months.

In series I, one of the exit tubes of the bottle (300 ml. capacity) was connected to a receiver containing a known amount of sodium hydroxide to absorb the CO₂ formed as soon as liberated. The gas accumulating in the system was therefore almost wholly methane and was tapped off once every week. In series II, 400 ml. bottles were used and the gaseous products were removed weekly but the sodium hydroxide attachment was omitted and hence the CO₂ evolved was in contact with the culture medium for a week. In series III, with 1000 ml. bottles, the accumulated gases were removed monthly. 4-litre bottles were used in series IV and all the gases evolved were allowed to remain until the end. A mixture of 2 g. NH₄HCO₃ and 2 g. KHCO₃ per 20 g. straw, was added to all the bottles so as to neutralise the acids formed and maintain an alkaline reaction throughout the experiment.

Table VII. *Decomposition of rice straw in bottles of different sizes.*

	g. per 100 g. straw after 6 months.			
	Series I 300 ml. bottles with NaOH receiver	Series II 400 ml. bottles	Series III 1000 ml. bottles	Series IV 4000 ml. bottles
Loss in dry matter less ash	34.2	35.4	36.1	36.8
Net CO ₂ evolved	18.94	19.70	20.40	21.52
Net CH ₄ evolved	6.71	7.15	7.46	7.61
Acetic acid present	1.87	1.62	1.52	0.92
Butyric acid present	0.82	0.58	0.26	0.24
Total weight of products	28.34	29.05	29.64	30.29
Volume of CO ₂ evolved corrected for carbonates	ml. 9580	ml. 9965	ml. 10320	ml. 10725
Volume of CH ₄ evolved	9365	10115	10410	10610

The results obtained show that the size of the interacting vessel has comparatively little influence on the course of the decomposition, provided the reaction of the medium is properly maintained. In all cases about 10,000 ml. of methane and an equal amount of CO₂ are evolved, confirming the observation made in a previous communication that a particular sample of straw produces an

approximately constant quantity of methane. This is ascribed to the formation of a definite amount of acids from straw which are later decomposed into CO_2 and CH_4 . It is worth pointing out that in all the series the proportion of CO_2 to CH_4 is roughly unity even though in some cases the CO_2 was removed as soon as it was liberated while in others it was allowed to be in contact with the medium for as long as 6 months. This lends additional support to the above hypothesis regarding the formation of methane and is opposed to the view that the methane is formed by reduction of carbon dioxide.

DISCUSSION.

An examination of the above results shows that the mechanism of anaerobic degradation of straw offers several points in marked contrast to aerobic fermentation. The former is much more restricted in its tolerance of changes in reaction than the latter. This may be explained as being due to the differences in the micro-flora in the two cases. The decomposition under aerobic conditions is brought about by a wide group of different species of microorganisms including fungi, bacteria and actinomycetes, which exert their optimum activity at different p_{H} levels. Under anaerobic conditions, however, the population is probably wholly bacterial and is possibly restricted to a few types operating within a narrow range.

Of the two stages in the decomposition of straw, *viz.* the first of acid formation and the second of gas production, it is noteworthy that the former is more tolerant to changes in reaction than the latter. The production of methane is active only in the range of p_{H} 7.5–8.5, whereas acid formation can go on between p_{H} 6 and 10. Anaerobic digestion at p_{H} 9–10 results in a rapid formation of organic acids which are stored up as salts and hence this might form a convenient method for the conversion of straw into acids, principally acetic. As the speed of acid formation is much more rapid than of gas production and since it is possible to calculate from the amount of acids formed the potential methane-producing capacity of straw, digestion at the above p_{H} range might also act indirectly as a method for the rapid determination of the methane-producing capacity of raw materials like straw.

Another interesting point of difference between aerobic and anaerobic decomposition of straw lies in the much larger amount of water required for successful decomposition in the latter case. Whereas a proportion of straw to water of 1 : 4 or 1 : 5 is sufficient for aerobic decomposition, over twice this amount is necessary for successful anaerobic decomposition. Part of the extra water required in the latter case is probably used up in the chemical reactions involving the degradation of straw material into acids and gases, but part of it may serve also to dilute the acids and other products of the fermentation which at high concentrations impede the progress of the decomposition.

The low nitrogen requirement for anaerobic digestion as compared with aerobic decomposition has been examined in detail in a previous communication [1935].

The presence of an active microflora on the straw itself and the absence of the need for extraneous inoculation, such as soil extract, confirms the observations of previous workers [Fowler and Joshie, 1920; Langwell and Lynn, 1932; Boruff and Buswell, 1929]. The absence of any response to the addition of a small amount of inoculum from a vigorously fermenting culture does not apparently support the hypothesis that the initial lag period is due to the need for the development of the specific microflora necessary to start the decomposition. Further work on this point is required before any conclusion can be drawn.

The low requirement for phosphate in anaerobic digestion could, as in the case of the low nitrogen requirement, be explained by assuming that a portion of the phosphorus in the straw dissolves and undergoes a continuous cycle of bacterial synthesis and disintegration sufficient to meet the needs of the bacterial population; thus the phosphorus would not be locked up in comparatively unavailable forms as it is under aerobic conditions.

SUMMARY.

1. For the anaerobic digestion of rice straw by mesophilic organisms, a temperature range of 30 to 35° is found to be optimum.

2. The p_H of the medium exerts a controlling influence on the course of the fermentation. The preliminary stage of acid formation is more tolerant to changes of reaction, but gas formation is greatly impeded outside the range of p_H 7.5–8.

3. The water requirements for anaerobic digestion are much higher than for aerobic decomposition. A proportion of straw to water of 1 : 10 is found optimum for the former.

4. The organisms required to decompose the straw are present on the straw itself and further inoculation is unnecessary and does not shorten the preliminary lag period.

5. The phosphorus requirement of anaerobic digestion is satisfied by the amount contained in the straw itself (0.42 %). Additions of phosphate have no appreciable stimulating effect. Excess of phosphate actually depresses the rate of decomposition.

6. Finely ground straw does not ferment more quickly than chaffed straw; the rate of gas production is in fact slower.

7. Of the different reagents tried for neutralising the acids formed during anaerobic digestion, NH_4HCO_3 and $KHCO_3$ are the best; they could be used singly or better still in admixture. It is possible also to control the reaction of the medium by additions of alkali or acid from time to time. Urea or cyanamide can replace ammonium carbonate but proteins cannot.

8. The amounts of CO_2 and CH_4 produced are approximately the same irrespective of the volume of free space above the culture medium, provided the accumulated gases are removed from time to time so as not to produce high concentrations of CO_2 or lower the p_H of the medium.

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