

LXI. STUDIES ON THE ANAEROBIC DECOMPOSITION OF PLANT MATERIALS.

I. THE ANAEROBIC DECOMPOSITION OF RICE STRAW (*ORYZA SATIVA*).

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(Received December 30th, 1934.)

THE aerobic decomposition of plant materials has in recent years attracted much attention, on account of its importance in the formation of synthetic manure, in the decomposition of soil organic matter, in the purification of sewage and trade wastes, in the decay of wood *etc.*, but comparatively little is known regarding the course and rate of decomposition under anaerobic and partially aerobic conditions. Tenney and Waksman [1930] have reported some data in this connection and a certain amount of work has been done in regard to the changes in organic matter in marshy and peaty soils, generally associated with such conditions. While the agricultural importance of such soils is comparatively limited, in other cases anaerobic and semi-aerobic conditions have played a significant rôle in increasing crop production and have been objects specially aimed at in the system of cultivation, *e.g.* in the production of rice under swamp or water-logged conditions as practised in most parts of India and China and to an increasing extent in Burma. The biochemistry of the changes in organic matter and other constituents in such soils has been examined by Nagaoka [1905], Daikuhara and Imaseki [1907], Harrison and Iyer [1913; 1914; 1915; 1916; 1919; 1920], Panganiban [1923], Subrahmanyam [1927; 1929; Subrahmanyam and Sreenivasan, 1934], Itano and Arakawa [1927; 1932], Metzger [1929; Metzger and Janssen, 1928], Joachim and Kandiah [1929], Misu and Shimohira [1929], Bartholomew [1929], Robinson [1930], Osugi *et al.* [1931; 1932] and others. But their work has been done mostly under the semi-aerobic conditions of the water-logged soil and no detailed study of the decomposition of organic matter under strictly anaerobic conditions has been reported. Data under such conditions, it was thought, might serve not only for purposes of comparison, but might also help in the interpretation of the results obtained already under water-logged conditions.

The object of the experiments reported in this and succeeding papers was to examine in detail the course of anaerobic decomposition of a variety of plant materials by themselves and in admixture with soil, as compared with the course of aerobic decomposition on the one hand and with the decomposition under water-logged or swamp soil conditions, on the other.

Recently much work has been reported on the anaerobic digestion of sewage and trade wastes, with special reference to securing the maximum evolution of combustible gases [*cf.* Whitehead and O'Shaughnessy, 1931; Adeney, 1932; Heukelekian, 1933; Rudolfs, 1932; Rudolfs and Heukelekian, 1932; Rudolfs and Setter, 1932; Buswell and Boruff, 1932; Larson *et al.* 1934]. But it should be noted that the conditions influencing the decomposition of trade wastes or sewage, where the concentration of the dissolved or suspended matter is comparatively low, may vary markedly from those controlling the decomposition of

plant materials in much higher concentrations and in presence of soil. Comparisons of data obtained under the two sets of conditions may, however, be made with advantage and will be discussed later.

A different aim has guided several workers [Richards and Amoore, 1920; Fowler and Joshie, 1920; Langwell and Lymn, 1923; 1932; Boruff and Buswell, 1929], who have examined the anaerobic or semi-aerobic decomposition of plant materials on the technical scale with a view to obtaining the maximum yield of gaseous products. As their results bear close comparison with some data obtained during the course of the present experiments, their work may be considered in somewhat greater detail. Fowler and Joshie [1920] fermented various cellulosic materials and found that maximum gas evolution occurred at 30°, the mixed gases evolved having an average composition of 80–90 % CH₄, 4–12 % H₂ and 3–7 % CO₂. Hemicelluloses gave maximum gas production, and an acidity of more than 1 % retarded further fermentation. Langwell and Lymn [1923; 1932] adopted thermophilic digestion at 60° and observed that celluloses from different sources, hemicelluloses, starches and sugars could be easily fermented while the so-called ligno- and cuto-celluloses were unfermentable. Cereal straws were intermediate between true and ligno-celluloses and left an unfermentable residue of cellulose, apparently in combination. The addition of phosphorus and nitrogen was found to promote the fermentation. Aeration of the medium increased the amount of ethyl alcohol produced and a portion of the methane was replaced by hydrogen. Richards and Amoore [1920] working with straw and Nile sudd found that the optimum conditions for the production of methane were (1) a temperature between 35 and 40°; (2) exclusion of air; (3) ample water supply; (4) presence of some available nitrogen compound and (5) absence of acidity, which could be secured by addition of CaCO₃.

The methane fermentation of simple organic compounds and of sewage sludge has been followed by Buswell and co-workers [Buswell and Boruff, 1932; Symons and Buswell, 1933; Tarvin and Buswell, 1934; Boruff and Buswell, 1934]. This type of fermentation is held to be an oxidation-reduction reaction under anaerobic conditions, catalysed by bacteria and resulting in the production of almost theoretical quantities of CH₄ and CO₂: the primary products appear to be CO₂ and H₂, which react to form methane. Decarboxylation, deamination and hydrolysis were the principal means of decomposition, while the lower fatty acids were the chief intermediate compounds produced. A larger weight of substance was found as gas than was decomposed as organic matter, owing to the water present taking part in the reactions.

TECHNIQUE.

Rice straw from the Government Central Farm, Coimbatore, India, was chaffed into pieces about an inch long, and weighed quantities were packed into bottles of known volume fitted with rubber stoppers carrying exit tubes and pinch-cocks. Mineral nutrients, soil inoculant and distilled water in the quantities specified in the experiments described below were added, the contents well mixed and the stoppers secured by wiring with copper wire and then coating with paraffin wax. The enclosed air was removed by suction and the bottles were incubated at the required temperature. At specified intervals, generally once a week, the accumulated gases were tapped off through the exit tube into a Hempel's burette, measured and analysed for CO₂, CH₄, H₂ and N₂. At the end of the period of incubation, usually determined by the stoppage of gas evolution, or at intermediate intervals, samples were opened and the contents analysed. The liquid portion was separated from the solid matter by filtration through a

Büchner funnel and washing. The residue was dried, weighed and analysed for cellulose, lignin, furfuraldehyde-yielding compounds, protein and ash. The filtrate was made up to a known volume and aliquots were taken for the determination of volatile and non-volatile acids, total, ammoniacal, protein-, nitrate- and amino-nitrogen and carbonates.

Cellulose was determined by the method of Norman and Jenkins [1933]. Furfuraldehyde was estimated by Kröber's phloroglucide method, with final treatment of the phloroglucide with boiling alcohol to remove non-pentosan derivatives. The total furfuraldehyde yield and that obtained from the cellulose fraction are expressed in the accompanying tables in terms of xylan, for purposes of comparison of the hemicellulose fractions associated with cellulose and not so associated. It is realised that a portion of the total furfuraldehyde yield may be due to uronic acid derivatives, and that the hemicelluloses in rice straw may not all be in the form of xylan. Norman [1929] found that in oat and rye straws, the furfuraldehyde contributed by uronic acids was about 6-7 % of the total furfuraldehyde yield and it is probable that the correction necessary in the present case may be of the same order.

Lignin determinations were made by the 72 % sulphuric acid method (15 hours, below 15°) both on the material without any treatment (referred to in the tables as "lignin direct") and after hydrolysing it with 5 % sulphuric acid (referred to in the tables as "lignin after hydrolysis") [cf. Norman and Jenkins, 1933, p. 826]. It was found that the latter method gave uniformly lower results than the former, the difference varying from 10 to 30 % of the "lignin-direct" figure. In both cases, the ash content and nitrogen calculated as protein were deducted before arriving at the lignin figures. The allowance for nitrogen on the basis of its protein value is made in the absence of any definite knowledge of the form in which nitrogen is associated with lignin, though recent evidence [Norman and Jenkins, 1934] tends to show that the correction "factor" for nitrogen is liable to considerable variation depending on the nature of other substances present, especially furfuraldehyde-yielding compounds.

Volatile acids were estimated by steam-distillation, and the proportion of the constituents in the distillate was determined by the method of Virtanen and Pulkki [1928]. The fractional distillation constants obtained by this method for successive 25 ml. fractions agreed with the equations for a mixture of acetic and butyric acids. Qualitative tests on the distillate showed that it was almost wholly a mixture of acetic and butyric acids; there were only traces of formic, propionic and valeric acids.

Lactic acid was determined on the non-volatile liquid residue and, for comparison, also on an aliquot of the original solution, by the permanganate oxidation method of Friedmann and Graeser [1933]. Only negligible amounts of lactic acid could be detected at any stage.

Amino-nitrogen was estimated by Foreman's formaldehyde titration method as outlined by Brown [1923].

Determinations of the total, ammoniacal, nitrate- and protein-nitrogen were made by the methods recommended by the Association of Official Agricultural Chemists, U.S.A. [1930]. Volumes of gases have been given in the Tables after reduction to N.T.P.

EXPERIMENTAL.

Preliminary experiments showed that temperature and reaction exerted a profound influence on the rate and course of anaerobic fermentation. The extent of this influence and that due to other factors will be examined in detail in a later

communication, but it may be noted here that a range of 30–35° and a slightly alkaline reaction (p_{H} 7.5–8.0) were found optimum for the progress of the decomposition. The maintenance of an alkaline reaction offered special difficulties on account of the large amount of organic acids formed rapidly in the early stages of the decomposition. The use of buffers at the high concentrations necessary to maintain constancy of the reaction, effectively inhibited bacterial activity as shown by absence of decomposition. Calcium carbonate, generally recommended in bacterial technique for use with gas-forming anaerobes was found (*cf.* Exp. 2 below) ineffective for maintaining a neutral reaction, especially in presence of CO_2 under pressures higher than atmospheric, and gas production was greatly inhibited even though there was excess of CaCO_3 present. It was found that the addition of a sufficient amount of ammonium carbonate (to the extent of 2 % nitrogen on the rice straw) served to maintain the p_{H} on the alkaline side. Sodium nitrate, when added to straw at the rate of 2 % nitrogen, also produced, after denitrification, sufficient alkali to neutralise the acids formed. In a few cases, however, the above proportion of sodium nitrate was found to result in too great an alkalinity in the early stages, while the amount of ammonium carbonate at 2 % nitrogen on straw taken was found insufficient to neutralise the acids formed; in these cases gas production was impeded. So in later experiments, attempts were made to maintain the p_{H} by an addition of other neutralising agents like KHCO_3 , NaHCO_3 , NH_4HCO_3 *etc.* and also by the addition of alkali at regular intervals. A discussion of these experiments will be reported later.

Exp. 1. Course of decomposition of rice straw without the addition of nitrogen or calcium carbonate. To 20 g. portions of rice straw were added 10 ml. of an aqueous extract of a garden soil (1 g. soil to 50 ml. water), 0.5 g. K_2HPO_4 , 0.05 g. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and enough distilled water to make up 200 ml. in all. The bottles were evacuated, sealed and incubated as described above. At the end of stated intervals one of the bottles was opened and the contents analysed. There was no gas evolution from any of the bottles though a certain amount of CO_2 was found in solution. The results of analysis, calculated per 100 g. original straw taken, are given in Table I.

It will be noted that the p_{H} of the medium falls at the end of the decomposition to 4.4. The accumulation of acids amounts to about 6.6 g. of which the major portion is acetic (about 4.0 g.) and the remainder butyric. About 20 % of the total hemicelluloses expressed as xylan, 8–9 % of cellulose and 5–7 % of lignin, as well as 35–37 % of the proteins, have been decomposed. The almost stationary state of the decomposition figures after the first month, during which most of the acid formation takes place (Table I), indicates that the progress of the decomposition is inhibited by the accumulation of acids and consequent lowering of p_{H} (*cf.* Exps. 4–6).

As regards nitrogen distribution, it is interesting to note that the major portion of the nitrogen which goes into solution remains in the form of protein and only a small portion is liberated as ammonia. Of the total nitrogen originally present in the straw, about 65 % remains in the solid residue, about 25 % is present in solution in the form of protein and only about 8–10 % is converted into ammonia. A similar kind of nitrogen distribution is also to be found in the succeeding experiments.

Exp. 2. Decomposition of rice straw without the addition of nitrogen but with CaCO_3 added as neutralising agent. To 20 g. portions of rice straw, 4 g. of CaCO_3 were added to neutralise the acids formed. The other experimental details were the same as in Exp. 1.

The results of analysis are shown in Table II. They show that in the presence

Table I. *Decomposition of rice straw without the addition of nitrogen or any neutralising agent.*

	100 g. original straw g.	After			
		1 month g.	2 months g.	3 months g.	4 months g.
(A) Losses of constituents.					
Dry matter less ash	81.15	13.85	14.65	14.85	14.95
Straw cellulose (including xylan)	45.45	3.31	3.69	3.47	4.09
Xylan in cellulose	11.97	0.79	0.77	0.95	1.11
Xylan in polyuronides	9.50	3.90	3.01	3.71	3.01
Total xylyans	21.47	4.69	3.78	4.66	4.11
Lignin (direct)	10.89	0.47	0.43	0.36	0.57
Lignin (after hydrolysis)	9.31	0.40	0.64	0.66	0.88
Protein	2.94	1.08	1.10	0.83	1.06
(B) Products formed.					
CO ₂ in solution	—	0.79	0.77	0.81	0.84
Acetic acid	—	3.35	3.97	4.07	3.75
Butyric acid	—	1.89	1.95	2.52	2.83
Total weight of products	—	6.03	6.69	7.40	7.42
p _H of medium	6.4	4.8	4.6	4.4	4.4
(C) Nitrogen distribution.					
Initial N content	0.469	—	—	—	—
Protein-N in residue	—	0.296	0.294	0.335	0.299
Protein-N in aqueous extract	—	0.105	0.112	0.088	0.115
NH ₃ -N in aqueous extract	—	0.033	0.038	0.038	0.050

of CaCO₃ the reaction proceeds a stage further than in Exp. 1, both as judged by the amount of acids and gases formed as well as by the percentage losses of the straw constituents. Larger amounts of cellulose (20–23 %) and xylan (25–26 %) have been decomposed, and the acids formed amount to about 9.0 g. of which about 6.1 g. are acetic and the rest butyric.

Table II. *Decomposition of rice straw in presence of CaCO₃ (no nitrogen added).*

	100 g. original straw g.	After			
		1 month g.	2 months g.	3 months g.	4 months g.
(A) Losses of constituents.					
Dry matter less ash	81.15	13.45	14.25	15.64	16.25
Straw cellulose (including xylan)	45.45	7.95	8.25	8.88	9.65
Xylan in cellulose	11.97	1.65	1.68	1.70	1.72
Xylan in polyuronides	9.50	2.40	2.84	3.46	3.93
Total xylyans	21.47	4.05	4.52	5.16	5.65
Lignin (direct)	10.89	0.27	0.46	0.72	0.81
Lignin (after hydrolysis)	9.31	0.71	0.81	0.85	0.87
Protein	2.94	0.61	0.65	0.67	0.68
(B) Products formed.					
Gases (volumes in ml.):					
CO ₂	—	785	820	890	990
CH ₄	—	40	50	55	65
Acetic acid	—	5.68	5.81	5.92	6.12
Butyric acid	—	2.45	2.50	2.62	2.83
p _H of medium	7.0	5.0	4.9	4.8	4.8
(C) Nitrogen distribution.					
N content of original straw	0.469	—	—	—	—
Protein-N in residue	—	0.373	0.380	0.370	0.361
Protein-N in aqueous extract	—	0.070	0.065	0.070	0.080
NH ₃ -N in aqueous extract	—	0.020	0.025	0.025	0.025

It will be noted that even in presence of CaCO_3 the p_{H} of the medium falls below 5.0. This is in agreement with the p_{H} of bicarbonate mixtures in presence of CO_2 , especially under pressures higher than atmospheric [Banta and Pomeroy, 1934]. As a result of the fall in p_{H} , further decomposition and gas production have been arrested, and only 1055 ml. of gas, mostly CO_2 , have been evolved. The proportions of protein-nitrogen going into solution (16–18 %) and of ammoniacal nitrogen (about 5 %) are lower in presence of CaCO_3 than without it.

Exp. 3. Decomposition of rice straw in presence of CaCO_3 and ammonium carbonate. 5 g. CaCO_3 and ammonium carbonate to the extent of 1 % nitrogen on the straw taken were added to 20 g. portions of rice straw. The other experimental details were the same as in the previous experiments. The results are shown in Table III.

Table III. *Decomposition of rice straw in presence of CaCO_3 and ammonium carbonate (1 % N).*

	100 g. original straw g.	After				
		1 month g.	2 months g.	3 months g.	4 months g.	6 months g.
(A) Losses of constituents.						
Dry matter less ash	81.15	14.45	16.90	20.15	24.20	28.50
Straw cellulose (including xylan)	45.45	5.90	9.67	15.06	17.77	20.60
Xylan in cellulose	11.97	1.22	2.26	3.85	4.05	4.86
Xylan in polyuronides	9.50	3.67	4.79	5.22	6.11	6.56
Total xylns	21.47	4.89	7.05	9.07	10.16	11.42
Lignin (direct)	10.89	0.95	1.21	1.41	1.52	1.61
Lignin (after hydrolysis)	9.31	1.02	1.10	1.26	1.33	1.35
(B) Products formed.						
Gases (volumes in ml.):						
Total CO_2 evolved	—	685	1510	3240	3525	3735
Correction for CO_2 evolved from carbonates	—	630	1330	2955	3060	3225
Net CO_2 evolved	—	55	180	285	465	510
CH_4 evolved	—	35	165	245	430	475
H_2 evolved	—	5	15	20	25	30
Total volume of gases	—	95	360	550	920	1015
Gases (weights in g.):						
CO_2	—	0.11	0.36	0.57	0.92	1.01
CH_4	—	0.03	0.12	0.18	0.31	0.34
Acetic acid	—	8.88	10.43	11.03	12.83	14.23
Butyric acid	—	1.45	1.76	3.30	4.33	5.97
Total weight of products	—	10.47	12.67	15.08	18.39	21.55
p_{H} of medium	7.8	5.4	5.2	5.0	5.0	4.8

They show that a mixture of CaCO_3 and ammonium carbonate at 1 % N on straw is satisfactory from the point of view of losses of the different constituents, about 45–50 % of cellulose, 45–47 % of the total xylan and 14–15 % of the lignin being decomposed, but fails to keep the medium from turning acid, in spite of excess of CaCO_3 , being present at the end of the reaction. This result is probably due to insufficiency of the ammonium carbonate added to neutralise the acids formed and the inability of CaCO_3 , as already noted under Exp. 2, to prevent the fall in p_{H} . The p_{H} of the medium falls to 4.8 and the acids accumulate to 20.2 g. of which 14.23 g. are acetic and the remainder butyric. 3735 ml. of CO_2 and 475 ml. of CH_4 are evolved, most of the CO_2 coming from the interaction of the organic acids formed with the added CaCO_3 . By comparison with the

results of Exps. 4 and 6, the small amount of CH_4 evidently results from the fall in p_{H} and consequent inhibition of the decomposition of organic acids formed into gaseous products.

Exp. 4. Decomposition of rice straw in presence of ammonium carbonate: CaCO_3 absent. To 20 g. portions of rice straw was added ammonium carbonate at the rate of 2 % nitrogen on the straw taken, but no CaCO_3 . Other experimental details were as before. The initial p_{H} was 7.8–7.9. In a few cases, the progress of the reaction was hindered by too rapid an accumulation of acids in the early stages and a corresponding decrease in the rate of gas production; but in others the two reactions proceeded at comparable rates and the p_{H} was maintained at about 7.4–7.8. This improved considerably the yield of combustible gases.

The results of the experiment are given in Table IV A. A comparison of these figures with those obtained in Exp. 3 using CaCO_3 and ammonium carbonate at

Table IV A. *Decomposition of rice straw in presence of ammonium carbonate (no CaCO_3 added).*

	100 g. original straw g.	After				
		1 month g.	2 months g.	3 months g.	4 months g.	6 months g.
(A) Losses of constituents.						
Dry matter less ash	81.15	15.15	17.55	22.95	26.65	35.20
Straw cellulose (including xylan)	45.45	4.38	6.63	10.47	13.67	21.26
Xylan in cellulose	11.97	1.54	2.35	3.25	3.73	6.36
Xylan in polyuronides	9.50	4.10	4.72	5.53	6.23	6.43
Total xylans	21.47	5.64	7.07	8.78	9.96	12.79
Lignin (direct)	10.89	0.87	1.15	1.47	2.02	3.04
Lignin (after hydrolysis)	9.31	1.20	1.43	1.99	2.37	2.60
Protein	2.94	0.87	0.86	0.87	0.87	0.85
(B) Products formed.						
Gases (volumes in ml.):						
Total CO_2 evolved	—	2705	3390	6220	7,320	10,290
Correction for CO_2 from carbonates by action of acids present	—	2700	3040	2430	2,225	460
Net amount of CO_2 evolved	—	5	350	3790	5,095	9,830
CH_4 evolved	—	20	360	3950	4,855	9,585
H_2 evolved	—	—	5	25	50	90
Total volume of gases	—	25	715	7765	10,000	19,505
Gases (weights in g.):						
CO_2	—	0.02	0.69	7.49	10.07	19.44
CH_4	—	0.02	0.26	2.83	3.48	6.87
Acetic acid	—	6.65	6.43	4.90	4.51	1.07
Butyric acid	—	0.85	2.52	2.36	2.13	0.23
Total weight of products	—	7.54	9.90	17.58	20.19	27.61
p_{H} of medium	7.9	6.6	6.4	7.0	7.2	7.8
(C) Nitrogen relationships.						
N in original straw	0.469	—	—	—	—	—
Protein-N in residue	—	0.329	0.322	0.333	0.348	0.333
Protein-N in aqueous extract	—	0.153	0.138	0.138	0.188	0.237
Total protein-N after fermentation	—	0.482	0.460	0.471	0.536	0.570
N immobilised per 100 g. straw or nitrogen factor	—	0.013	nil	0.002	0.067	0.101
Nitrogen equivalent	—	0.086	nil	0.009	0.219	0.252

1 % nitrogen on the straw shows that the essential difference lies in the degree of gas production in the two cases. Whereas in Exp. 3 the acids formed accumulated to 20.2 g. and the p_{H} fell to 4.8, the total acids formed in the present experiment gave a maximum value of 8.95 g. at the end of 2 months and then decreased steadily to 1.3 g. at the end. Corresponding with this variation in the amount of acids, the p_{H} of the medium first showed a fall from 7.9 to 6.4 at the end of 2 months after which it registered a steady rise to 7.8. Evolution of appreciable amounts of combustible gases, principally methane, began at the end of the second month and showed a rapid increase in that period when the acids were rapidly falling.

The gas evolution, corrected for CO_2 evolved by the interaction of added carbonates with the organic acids formed, amounted in the present experiment to 19,505 ml. containing 9585 ml. methane. In Exp. 3 the corresponding figures were 1015 and 475. That the much larger amount of methane produced in the present experiment has in all probability resulted from the decomposition of the organic acids which accumulated in Exp. 3 is shown by a comparison of the total possible methane production in the two cases, given in Table IV B.

Table IV B.

	Exp. 3	Exp. 4
Acetic acid present	14.23 g.	1.07 g.
Butyric acid present	5.97 g.	0.23 g.
CH_4 evolved	475 ml.	9585 ml.
CH_4 equivalent to acetic acid present	5311 ml.	399 ml.
CH_4 equivalent to butyric acid present	4588 ml.	176 ml.
Total capacity for methane production	10374 ml.	10,160 ml.

The similarity of the total volumes of methane derivable from the organic acids produced in the two experiments supports the hypothesis that the lower organic acids are the chief intermediate compounds formed in the breakdown of the constituents of straw, and that the organic acids are further split up into CH_4 and CO_2 [*cf.* Buswell and Boruff, 1932; Tarvin and Buswell, 1934]. The evidence obtained in these experiments does not support the theory that CO_2 and H_2 are the first products of decomposition and that a portion of the CO_2 is reduced by hydrogen to CH_4 and organic acids.

It is worth noting that if the total yield of CO_2 in both Exps. 3 and 4 is corrected for that portion evolved from added carbonates by the action of acids formed, the CO_2/CH_4 ratio is almost unity at successive stages of decomposition. Such a constant ratio may not be expected on the basis of a partial reduction of CO_2 by H_2 but agrees well with the decomposition of organic acids into equivalent amounts of CH_4 and CO_2 .

The total yield of gases is about 19,500 ml. per 100 g. of straw, and allowing for the residual 1.3 g. of acid present, the yield will rise to about 20,500 ml., containing about 50 % methane. This corresponds to a yield of about 7200 cb. ft. of gas per ton of straw.

Another interesting point to be noted from Table IV is the low nitrogen requirement for anaerobic digestion. Rege [1927] and Richards and Norman [1931] found that for aerobic decomposition the "nitrogen factor" (the amount of nitrogen converted from the mineral into protein form per 100 g. of material taken) ranged from 0.7 to 0.9 for rice straw, whereas the highest factor obtained in the present experiment was about 0.1. The above authors found a nitrogen equivalent (the amount of nitrogen immobilised per 100 g. of material

destroyed) of about 2 for rice straw. For anaerobic decomposition, the nitrogen equivalent varies from nil to 0.25.

Exp. 5. Decomposition of rice straw in presence of varying amounts of sodium nitrate. It is well known that under anaerobic conditions nitrates are rapidly denitrified in presence of organic matter, leaving an alkaline residue which serves the purpose of a neutralising agent. To 20 g. portions of rice straw was added sodium nitrate at the rate of 0.5, 1, 2 and 3 % nitrogen on the straw. No CaCO_3 was added and other experimental details were as before.

The data obtained are given in Table V. They show that by the use of sodium nitrate in proper amounts, successful decomposition of rice straw could be

Table V. *Decomposition of rice straw with different doses of sodium nitrate.*

	100 g. original straw g.	NaNO_3 at 0.5 % N g.	NaNO_3 at 1 % N g.	NaNO_3 at 2 % N g.	NaNO_3 at 3 % N g.
(A) Losses of constituents.					
Dry matter less ash	81.15	15.02	20.42	28.26	18.34
Straw cellulose (including xylan)	45.45	7.02	11.83	12.90	8.38
Xylan in cellulose	11.97	2.81	4.05	4.41	2.98
Xylan in polyuronides	9.50	5.27	5.38	5.98	5.25
Total xylans	21.47	8.08	9.43	10.39	8.23
Lignin (direct)	10.89	2.18	2.29	2.39	2.31
Lignin (after hydrolysis)	9.31	1.90	2.02	2.41	2.57
Protein	2.94	0.86	0.94	1.10	1.12
(B) Products formed.					
Gases (volumes in ml.):					
CO_2 evolved and in solution	—	1860	3310	6260	3885
CH_4	—	210	410	2630	150
H_2	—	40	65	105	30
N_2	—	225	440	920	280
Total volume of gases	—	2335	4225	9915	4345
Gases (weights in g.):					
CO_2	—	3.68	6.54	12.38	7.68
CH_4	—	0.15	0.29	1.89	0.11
H_2	—	—	—	0.01	—
N_2	—	0.29	0.53	1.24	0.54
Total weight of gases	—	4.12	7.36	15.52	8.33
Acetic acid	—	4.05	7.41	9.10	7.32
Butyric acid	—	0.54	1.70	1.76	0.84
Total weight of products	—	8.71	16.47	26.38	16.49
p_{H} of medium	—	5.0	6.2	7.4	8.6

brought about which would compare favourably with the decomposition accomplished by the use of ammonium carbonate. The amount of decomposition increases with increasing doses of NaNO_3 up to an amount representing 2 % N on the straw, after which the reaction becomes presumably too alkaline to permit favourable decomposition.

The action of sodium nitrate is evidently two-fold, the first stage being denitrification of nitrate (accompanied by evolution of free nitrogen) and oxidation of organic matter (with evolution of CO_2) and the second being the usual type of anaerobic decomposition with formation of organic acids and evolution of combustible gases met with in the experiments above described. No methane or hydrogen could be detected till all the nitrate had been reduced and the evolution of free nitrogen had ceased. It is interesting to compare in this connection the observations of Elma *et al.* [1934] who found that the oxidation-reduction

potential of a nitrate or nitrite medium undergoing denitrification by *Micrococcus denitrificans* was maintained at a steady level depending on the p_H of the medium till all the nitrite or nitrate had been reduced, after which it showed an immediate and great fall. It is suggested that this stage corresponds in the present experiment to the starting of the second stage of anaerobic decomposition accompanied by the formation of acids and evolution of combustible gases; but the point needs further examination.

It would appear from Table V that sodium nitrate at the rate of 2% N on the straw would, after denitrification, yield enough alkaline residue to neutralise the acids formed in the second stage of decomposition and maintain the alkaline reaction so essential for the further progress of the reaction. With a view to examining the course of the decomposition in more detail, the experiments with sodium nitrate were repeated at the above concentration, samples being examined after definite time intervals.

Exp. 6. Decomposition of rice straw in presence of sodium nitrate equivalent to 2% N on straw. A number of bottles were set up with 20 g. portions of rice straw and sodium nitrate at the rate of 2% N on the straw, and samples were taken out for analysis at intervals. The results obtained are given in Table VI.

Table VI. *Decomposition of rice straw by NaNO₃ equivalent to 2% N on straw.*

	100 g. original straw g.	After			
		1 month g.	2 months g.	4 months g.	6 months g.
(A) Losses of constituents.					
Dry matter less ash	81.15	15.15	19.65	30.25	31.95
Straw cellulose (including xylan)	45.45	6.13	9.58	15.60	16.66
Xylan in cellulose	11.97	1.81	2.27	4.47	4.72
Xylan in polyuronides	9.50	4.95	5.39	5.67	6.13
Total xylians	21.47	6.76	7.66	10.14	10.85
Lignin (direct)	10.89	1.74	1.97	2.31	2.89
Lignin (after hydrolysis)	9.31	1.84	1.89	1.92	2.15
Protein	2.94	1.08	1.08	1.18	1.09
(B) Products formed.					
Gases (volumes in ml.):					
CO ₂ evolved and in solution	—	2575	2815	5925	8095
CH ₄ evolved and in solution	—	—	45	2655	5850
H ₂	—	—	25	50	90
N ₂	—	710	700	815	720
Total volume of gases	—	3285	3585	9445	14,755
Gases (weights in g.):					
CO ₂	—	5.09	5.57	11.71	16.01
CH ₄	—	—	0.03	1.90	4.19
H ₂	—	—	—	—	0.01
N ₂	—	8.88	8.76	10.20	9.00
Total weight of gases	—	13.97	14.36	23.81	29.21
Acetic acid	—	2.79	6.00	5.36	3.64
Butyric acid	—	—	0.63	0.79	—
Total weight of products	—	16.76	20.99	29.96	32.85
p_H of medium	—	7.2	7.4	7.8	8.2
(C) Nitrogen distribution.					
N in original straw	0.469	—	—	—	—
N added as NaNO ₃	2.000	—	—	—	—
Protein-N in residual straw	—	0.297	0.297	0.281	0.295
Protein-N in aqueous extract	—	0.163	0.150	0.175	0.175
NH ₃ -N in aqueous extract	—	0.050	0.025	0.038	0.025
Total protein-N and NH ₃ -N	—	0.510	0.472	0.494	0.495
N fixed from added nitrate	—	0.041	0.003	0.025	0.026

A comparison of the results obtained in Exps. 6 and 4, using sodium nitrate and ammonium carbonate respectively, shows essential differences in the course of the two reactions, especially in the earlier stages. With sodium nitrate denitrification of nitrate and oxidation of the organic matter proceed quite rapidly. At the end of one month the sodium nitrate culture has oxidised about 13 % of cellulose and 31 % of total xylans, as compared with 8.4 and 26 % respectively in the ammonium carbonate culture. However, with sodium nitrate only 2.8 g. of organic acid are present at the end of one month while 7.5 g. are present after the same period when ammonium carbonate is used. After the denitrification stage is completed the usual type of anaerobic degradation begins, as evidenced by the accumulation of organic acids and evolution of CH_4 .

It is interesting to note from Table VI that practically the whole of the added nitrate is lost, so far as the non-gaseous portion is concerned, the actual amount of nitrate-nitrogen converted into protein or ammoniacal form being negligible. In spite of the comparatively large amount of protein in solution, the level of ammoniacal nitrogen is quite low, there being only 5–10 mg. in a volume of 200 ml. This low level of ammoniacal nitrogen, even in cultures where decomposition has been active, confirms what has already been noted, namely, that a small supply of available nitrogen is enough to sustain anaerobic decomposition and that there is no advantage gained by adding larger amounts of ammonium salts than may be used to neutralise the organic acids formed.

DISCUSSION.

The experiments outlined above show that the first phase of anaerobic decomposition is the rapid formation of organic acids and considerable loss of organic matter in the early stages. The evolution of combustible gases, *e.g.* methane, appears to be a secondary reaction, and from the relative amounts of acids and gases produced under differing conditions it seems that the organic acids first formed are decomposed further into CO_2 and CH_4 , though at a rate much slower than the speed of acid formation. This second stage of gas production appears to be more sensitive to the influence of p_{H} than the first and proceeds best at a slightly alkaline reaction (about p_{H} 7.5). The rapid progress of anaerobic decomposition therefore depends primarily on the maintenance of a favourable p_{H} range, which is much narrower than that for aerobic decomposition.

A second point brought out by these experiments is the very low nitrogen requirement for anaerobic decomposition. Hutchinson and Richards [1921; 1925] have shown that for rapid conversion of waste materials into manure under aerobic conditions, the addition of nitrogen at the rate of about 0.7 % on the material taken is necessary. With anaerobic decomposition, however, additions of nitrogen above a low minimum value appear to exert no direct beneficial influence. Using rice straw, about 30 % of the total protein goes into solution even in the early stages and about 5–10 % is converted into ammonia. The insoluble residue at the end of the decomposition contains only about 70 % of the original nitrogen present in the straw, even though large additions of ammonia in the form of ammonium carbonate are made. This no doubt is explicable on the assumption that anaerobic decomposition is one of reduction to simpler products and hence very little synthesis of added ammonia into protein takes place under these conditions. The bacterial population is conceivably of a limited nature and there is probably a continuous cycle of microbial synthesis and disintegration, making the nitrogen requirement much less than under aerobic conditions. It has been noted under Exp. 4 that even under the most favourable conditions, the

nitrogen factor [Rege, 1927] is only of the order of 0.1, while the nitrogen equivalent [Richards and Norman, 1931] is about 0.2–0.25, as compared with the values of 0.7–0.9 and 1.8–2.0 respectively obtained for aerobic decomposition. In several cases the values have been negative, showing that under anaerobic conditions the net tendency is towards hydrolysis rather than towards synthesis of nitrogen compounds.

The apparent stimulating effect of additions of ammonium carbonate seen in Exps. 3 and 4 therefore seem to be due to its neutralising effect on the acids formed and the maintenance of an alkaline reaction rather than to the supply of available nitrogen.

The products obtained as a result of anaerobic decomposition have been of a simple character, the organic acids formed being mainly acetic and butyric and the gases evolved being mostly CO_2 and methane. Only negligible amounts of hydrogen are evolved at any stage. Where carbonates are used to neutralise the acids formed, nearly the whole of the gas first evolved is CO_2 . Later, when the decomposition of organic acids into CO_2 and CH_4 occurs, larger and increasing proportions of CH_4 are formed, rising to 70–80 % of the gases evolved. When, however, allowance is made for the amount of CO_2 evolved from added carbonates and that retained in solution, the CH_4/CO_2 ratio is nearly unity at successive stages of the decomposition.

As regards the extent of decomposition, under optimum conditions 40–45 % of ash-free dry matter, about 45 % of cellulose and 60 % of the hemicelluloses, in terms of furfuraldehyde yield, are decomposed. The hemicelluloses are more rapidly attacked in the early stages, but at the end of the reaction there is a larger total amount of cellulose decomposed than of hemicelluloses. In this respect the course of decomposition is similar to that under aerobic conditions, though the relative percentages are lower [*cf.* Rege, 1927; Tenney and Waksman, 1929]. Under conditions favourable to the accumulation of acids it is possible to obtain as much as 20 g. of acids per 100 g. of straw taken. Under other conditions which favour gas production, over 20 litres of gas containing about 50 % methane could be obtained from the same amount of straw. This corresponds to a yield of about 7200 cb. ft. gas per ton of straw and is comparable with the yields of 4400 cb. ft. per ton of wheat straw and 9360 cb. ft. per ton of Nile sudd obtained by Richards and Amoore [1920].

A significant point brought out by these experiments is the degree to which lignin appears to have been decomposed. About 25–30 % of the lignin, as determined both directly on the material as well as after a preliminary hydrolysis with 5 % H_2SO_4 [Norman and Jenkins, 1933], has undergone decomposition. This is in marked contrast to the difficulty with which lignin is attacked under aerobic conditions. Boruff and Buswell [1934] have also found a similar considerable loss of lignin content, in some cases amounting to 50 % of the total lignin, when corn stalks were decomposed under anaerobic conditions. It should be noted however that much confusion exists at present in methods adopted for lignin determination and errors and differences of a serious nature may be obtained, due to differences in technique or the presence of interfering substances [Phillips, 1934; Norman and Jenkins, 1934].

SUMMARY.

1. The course of anaerobic decomposition of rice straw has been followed with reference to losses of its major constituents, such as cellulose, furfuraldehyde-yielding compounds, lignin, proteins *etc.*, and the formation of organic acids and gases.

2. Of the straw constituents about 60 % of the hemicelluloses, 45 % of the cellulose and 25–30 % of lignin are decomposed. The ultimate loss of ash-free dry matter is about 40–45 %.

3. Acetic and butyric acids, CO₂ and methane are the main products formed. From 100 g. of straw, either about 20 g. of organic acids or over 20 litres of gases containing about 50 % methane are obtainable. This is equivalent to about 7200 cb. ft. of gas yield per ton of straw.

4. The decomposition appears to proceed in two distinct stages, the first involving the formation of organic acids and the second the conversion of these into gaseous products. A slightly alkaline reaction, a p_H of about 7.5, is important for the progress of the second stage of decomposition. The uses of CaCO₃, NaNO₃ and ammonium carbonate for this purpose have been compared. Ammonium carbonate in sufficient amount to neutralise the acids formed was found most suitable.

5. The reaction with NaNO₃ proceeds in two stages, the first being denitrification of the nitrate and oxidation of the organic matter; the alkaline residue left after denitrification serves to neutralise the organic acids formed in the second stage, which is the usual type of anaerobic fermentation accompanied by the evolution of combustible gases.

6. While the supply of available nitrogen is of paramount importance for the aerobic decomposition of plant substances, it is relatively insignificant in anaerobic decomposition. 100 g. of rice straw require about 0.7 part of nitrogen for decomposition aerobically but only 0.1 part or even less under anaerobic conditions.

The author's thanks are due to Sir E. J. Russell for placing the facilities of the Rothamsted Experimental Station at his disposal, to Mr E. H. Richards for guidance and to Drs. S. H. Jenkins and A. G. Norman for helpful criticism.

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