

# CLXXVI. STUDIES ON THE ANAEROBIC DECOMPOSITION OF PLANT MATERIALS.

## IV. THE DECOMPOSITION OF PLANT SUBSTANCES OF VARYING COMPOSITION.

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THE course of anaerobic decomposition of rice straw in relation to losses of straw constituents, products formed and nitrogen transformations, has been examined in some detail in earlier communications [Acharya, 1935, 1, 2, 3]. The present paper reports the results of the anaerobic digestion of oat, wheat and barley straws, bracken leaves, young grass (lawn mowings) and rape seed cake in comparison with the decomposition of rice straw.

In one set of experiments 20 g. portions of the materials were digested without the addition of nitrogen, but with 3 g.  $\text{KHCO}_3$  added to neutralise the acids formed. Wherever this amount of bicarbonate was not enough to keep the reaction alkaline, and as a result stoppage of gas evolution occurred, additions of NaOH were made from time to time. In a parallel set of experiments, ammonium carbonate equivalent to 1 % N on the material taken was added. The other experimental details and analytical methods adopted were the same as those previously described [1935, 1].

Besides the above, a few digestions were also made of the cellulose, hemicellulose and lignin fractions isolated from oat straw.

The results of anaerobic decomposition, as shown by losses of plant constituents, were also compared with the losses brought about by 3 different types of acid treatment: (1) aqueous acid, 5 %  $\text{H}_2\text{SO}_4$ ; (2) alcoholic acid, a solution of

Table I. *Analysis of materials used for anaerobic digestion.*

Constituents	Weights in g. per 100 g. of original material.						
	Rice straw	Oat straw	Wheat straw	Barley straw	Bracken leaves	Grass (lawn mowings)	Rape seed cake
1. Cellulose (deducting xylan contained)	32.82	33.70	35.57	36.37	17.98	24.08	15.52
2. Xylan associated with cellulose	11.73	15.72	16.71	15.03	3.43	7.29	2.44
3. Xylan not associated with cellulose	9.31	8.92	8.48	7.81	1.49	3.01	4.16
4. Lignin (direct)	10.67	16.70	17.04	16.08	30.68	12.13	8.46
5. Protein	2.88	2.51	2.31	3.56	11.67	22.90	35.30
6. Ash	17.50	6.60	5.62	4.59	5.05	10.74	8.34
7. Alcohol-benzene extractives	3.05	5.88	3.52	5.47	17.50	9.54	12.01
8. Moisture	8.40	7.51	8.04	10.02	10.10	9.89	11.16
Total	96.36	97.54	97.29	98.93	97.90	99.58	97.39
9. Lignin (after hydrolysis)	9.12	13.31	12.60	11.88	21.72	10.46	7.47
10. Ash-free organic matter soluble in water	10.07	9.75	7.62	9.97	24.83	18.01	20.46
11. Dry matter less ash	74.10	85.89	86.34	85.39	85.55	74.37	80.50

5 % HCl in 95 % alcohol; and (3) an oxidising acid agent, 2 % and 5 %  $\text{HNO}_3$ . The aim of the comparison was to examine whether any of the treatments could be used as a rapid method for forecasting the degree of anaerobic digestion and the probable amount of acid or gas obtainable as a result of such digestion; ordinarily several months elapse before gas evolution stops.

Data for the composition of the seven materials under examination, given in Tables I and II, show that whilst rice, oat, wheat and barley straws resemble one another in general composition, bracken leaves, grass and rape cake differ from these and among themselves. Bracken leaves are poor in hemicelluloses (as measured by furfuraldehyde yield) and cellulose, but rich in lignin and fairly rich in protein. Grass contains a good proportion of protein and cellulose, while rape seed cake is poor in hemicelluloses but rich in proteins.

The losses of constituents as a result of anaerobic digestion are given in Table II and the products formed in Table III.

### 1. *Losses of constituents.*

It is noteworthy that a considerable proportion of the total decomposition can be brought about without the addition of nitrogen if the  $p_{\text{H}}$  and other factors influencing anaerobic digestion [Acharya, 1935, 2] are maintained at the optimum. Still, the addition of nitrogen in the form of ammonium carbonate

Table II. *Losses of constituents.*

Weights in g. per 100 g. of original material.

Constituents	Loss by fermenting for 6 months		Loss by boiling for 1 hour with			
	Without N addition	With N added	5 % $\text{H}_2\text{SO}_4$	5 % alc. HCl	2 % $\text{HNO}_3$	5 % $\text{HNO}_3$
1. Rice straw.						
Dry residue less ash	31.41	35.24	29.46	27.12	34.55	37.44
Cellulose (including xylan)	19.18	21.26	15.74	10.47	14.47	11.59
Xylan in cellulose	5.72	6.36	7.75	8.36	8.65	9.08
Xylan not in cellulose	6.22	6.43	8.12	1.81	7.18	7.84
Total xylans	11.94	12.79	15.87	10.17	15.83	16.92
Lignin (direct)	2.92	3.04	1.55	3.24	5.68	7.02
Lignin (after hydrolysis)	2.51	2.60	—	—	—	—
Protein	1.02	0.87	1.46	1.42	1.34	2.14
2. Oat straw.						
Dry residue less ash	37.79	41.14	34.12	35.14	39.85	45.53
Cellulose (including xylan)	20.62	23.06	16.39	12.85	12.94	12.18
Xylan in cellulose	6.92	8.67	9.68	9.83	10.75	11.73
Xylan not in cellulose	5.52	5.42	8.81	2.38	7.07	8.90
Total xylans	12.44	14.09	18.49	12.21	17.82	20.63
Lignin (direct)	4.55	5.04	3.39	5.46	12.11	14.37
Lignin (after hydrolysis)	3.11	3.51	—	—	—	—
Protein	1.11	1.01	1.53	1.54	Nil	1.08
3. Wheat straw.						
Dry residue less ash	29.12	36.84	33.80	37.80	39.78	44.89
Cellulose (including xylan)	16.44	20.58	16.63	14.78	15.11	17.27
Xylan in cellulose	5.31	6.91	10.87	10.32	11.56	12.43
Xylan not in cellulose	5.71	5.88	7.25	3.16	7.58	8.24
Total xylans	11.02	12.79	18.12	13.48	19.14	20.67
Lignin (direct)	3.06	4.04	4.44	6.26	12.43	14.96
Lignin (after hydrolysis)	1.94	2.90	—	—	—	—
Protein	0.91	0.51	1.31	1.21	Nil	0.84

Table II (*cont.*).

Constituents	Loss by fermenting for 6 months		Loss by boiling for 1 hour with			
	Without N addition	With N added	5 % H <sub>2</sub> SO <sub>4</sub>	5 % alc. HCl	2 % HNO <sub>3</sub>	5 % HNO <sub>3</sub>
4. Barley straw.						
Dry residue less ash	35.12	40.06	36.19	35.35	40.41	45.64
Cellulose (including xylan)	23.20	26.54	18.68	15.79	15.36	16.39
Xylan in cellulose	6.97	7.77	10.22	10.31	10.37	11.63
Xylan not in cellulose	4.21	4.69	5.92	2.68	6.44	6.93
Total xylans	11.18	12.46	16.14	12.99	16.81	18.56
Lignin (direct)	3.10	3.27	4.20	5.16	11.11	12.85
Lignin (after hydrolysis)	1.38	1.93	—	—	—	—
Protein	1.91	1.41	2.13	2.20	0.58	1.60
5. Bracken leaves.						
Dry residue less ash	29.10	29.80	35.87	44.30	36.94	57.36
Cellulose (including xylan)	4.52	4.84	4.49	5.14	3.40	5.74
Xylan in cellulose	0.98	1.06	2.06	1.64	1.45	2.35
Xylan not in cellulose	0.60	0.96	1.06	0.12	0.88	0.74
Total xylans	1.58	2.02	3.12	1.76	2.33	3.09
Lignin (direct)	7.78	7.82	8.96	12.82	5.68	23.02
Lignin (after hydrolysis)	0.84	0.88	—	—	—	—
Protein	1.25	0.88	5.28	5.14	2.19	6.09
6. Grass (lawn mowings).						
Dry residue less ash	45.52	46.20	40.41	38.37	38.65	47.18
Cellulose (including xylan)	20.85	21.12	12.13	11.60	11.21	10.74
Xylan in cellulose	4.77	4.94	4.82	5.70	5.93	6.33
Xylan not in cellulose	1.85	2.20	2.42	0.07	2.06	2.12
Total xylans	6.62	7.14	7.24	5.77	7.99	8.45
Lignin (direct)	1.63	1.92	1.67	3.64	4.66	6.86
Lignin (after hydrolysis)	1.06	1.23	—	—	—	—
Protein	16.58	15.42	15.95	12.64	13.16	16.74
7. Rape seed cake.						
Dry residue less ash	55.02	55.40	53.60	42.07	49.15	59.50
Cellulose (including xylan)	13.72	13.91	9.65	7.22	6.41	7.26
Xylan in cellulose	1.74	1.89	1.79	1.96	1.90	2.02
Xylan not in cellulose	3.08	3.06	3.03	1.85	2.89	2.63
Total xylans	4.82	4.95	4.82	3.81	4.79	4.65
Lignin (direct)	0.60	0.62	0.99	2.22	1.37	4.55
Lignin (after hydrolysis)	0.56	0.56	—	—	—	—
Protein	30.85	29.75	28.73	19.93	24.20	27.92

stimulates the decomposition, as measured by losses of plant constituents and by-products formed, in the case of materials like straws poor in nitrogen. The effect of addition of nitrogen, however, is much less than that noted for aerobic decomposition [Hutchinson and Richards, 1921].

The degree of decomposition is approximately the same in the case of the 4 straws, about 45–50 % of cellulose, 55–60 % of hemicellulose and 25–30 % of lignin, calculated on the amounts originally present, being destroyed. With bracken, only 22 % of cellulose and 41 % of hemicellulose are decomposed and the destruction of protein is also low. This partial digestion is associated with the high lignin content of the material which, it is suggested, prejudicially affects the decomposition of the other constituents. Levine *et al.* [1935] recently found that additions of lignin to actively fermenting corn stalk flour or packing house (anaerobic) sludge inhibited gas formation, attributing this to chemical combination of lignin with the material under decomposition and formation of complexes resistant to microbial decomposition. In grass and rape seed cake cellulose and

hemicelluloses suffer considerable loss; the decomposition of the proteins is also high. This rapid destruction is associated with the comparatively low content of lignin in the two materials.

## 2. Products formed.

The general nature of the by-products is the same in the seven cases,  $\text{CO}_2$ ,  $\text{CH}_4$ , acetic and butyric acids being the chief substances formed. The relative amounts of acids and gases present at the end of 6 months vary, however, with the different materials, probably owing to the differing relative rates of the two

Table III. *Products formed by anaerobic fermentation for 6 months*

Per 100 g. of material	Volume of $\text{CO}_2$ (corrected for car- bonates)	Volume of $\text{CH}_4$ evolved	Wt. of $\text{CO}_2$	Wt. of $\text{CH}_4$	Acetic acid present	Butyric acid present	Total pro- ducts
	ml.	ml.	g.	g.	g.	g.	g.
1. Rice straw, without N addition	8010	8660	15.84	6.21	2.46	0.82	25.33
Rice straw, with N addition	10870	10570	21.49	7.58	1.03	0.27	30.37
2. Oat straw, without N addition	7040	7720	13.92	5.54	7.57	1.52	28.55
Oat straw with N addition	6050	6960	11.96	4.99	11.43	4.13	32.51
3. Wheat straw, without N addition	4010	4960	7.93	3.56	10.14	3.52	25.15
Wheat straw, with N addition	9420	10150	18.62	7.27	4.29	1.63	31.81
4. Barley straw, without N addition	5240	6120	10.36	4.39	7.36	3.08	25.19
Barley straw, with N addition	7110	7980	14.06	5.72	7.22	2.52	29.52
5. Bracken leaves, without N addition	4850	6240	9.59	4.47	4.28	4.24	22.58
Bracken leaves, with N addition	5120	5910	10.13	4.23	6.23	2.83	23.42
6. Grass (lawn mowings), without N addition	9220	13240	18.23	9.49	2.52	4.26	34.50
Grass (lawn mowings), with N addition	9670	13720	19.12	9.83	1.32	4.86	35.13
7. Rape seed cake, without N addition	1920	3075	3.80	2.20	16.85	18.05	40.90
Rape seed cake, with N addition	1630	2880	3.22	2.07	17.57	18.55	41.41

stages of anaerobic decomposition, *viz.* acid formation and gas production [Acharya, 1935, 1, 2]. The gas yield is very low with rape seed cake, on account of the rapid accumulation of acids and the lack of enough  $\text{KHCO}_3$  to maintain the  $p_{\text{H}}$  in the range favourable for gas formation.

The largest amount of products per 100 g. of material is obtained from rape seed cake (41 g.) followed by grass (35 g.), oat straw (33 g.), wheat straw (32 g.), rice straw (30 g.), barley straw (29 g.) and bracken (23 g.).

Whereas with straws the relative proportions of  $\text{CO}_2$  and  $\text{CH}_4$  evolved (after correcting  $\text{CO}_2$  for the fraction evolved from carbonates) are of the same order, in the case of grass and rape cake the amount of  $\text{CH}_4$  formed is much higher than that of  $\text{CO}_2$ . This difference is associated with a relatively low amount of butyric acid present in the former group and higher amounts in the latter. The bulk of the butyric acid seems to originate from the protein fraction in the case of rape seed cake, for the amount present cannot be accounted for by the losses of other constituents. It has already been noted [1935, 2] that when proteins, such as caseinogen, blood albumin and egg albumin, were added to decomposing rice straw, the proportion of butyric acid formed showed a rapid rise.

The increase in the amount of  $\text{CH}_4$  formed and its relationship to the  $\text{CO}_2$  produced and the butyric acid present lend support to the view advanced by Symons and Buswell [1933] that anaerobic digestion consists of a series of oxidation-reduction reactions in which water plays an important part.

3. *Nitrogen transformations.*

The nitrogen transformations during anaerobic digestion of the different materials are shown in Table IV. Positive values for nitrogen factor and nitrogen equivalent carry meanings assigned to them by Rege [1927] and Richards and Norman [1931], while negative values represent the net amount of nitrogen mineralised or the proportion of original protein reduced to ammonia at the end of the decomposition.

Table IV. *Nitrogen transformations in anaerobic decomposition.*

Plant material	N in 100 g. of original material g.	After fermentation for 6 months				
		Protein- N in residue g.	Protein- N in aqueous extract g.	Total protein- N g.	N immo- bilised or N factor g.	Nitrogen equivalent g.
1. Rice straw, without N addition	0.461	0.297	0.129	0.426	-0.035	-0.112
Rice straw, with N addition	0.461	0.323	0.207	0.530	+0.069	+0.196
2. Oat straw, without N addition	0.401	0.205	0.163	0.368	-0.033	-0.088
Oat straw, with N addition	0.401	0.237	0.305	0.542	+0.141	+0.343
3. Wheat straw, without N addition	0.369	0.225	0.095	0.320	-0.049	-0.168
Wheat straw, with N addition	0.369	0.305	0.230	0.535	+0.166	+0.451
4. Barley straw, without N addition	0.570	0.265	0.225	0.490	-0.080	-0.228
Barley straw, with N addition	0.570	0.344	0.420	0.764	+0.194	+0.485
5. Bracken leaves, without N addition	1.867	1.665	0.125	1.790	-0.077	-0.265
Bracken leaves, with N addition	1.867	1.727	0.155	1.882	+0.015	+0.050
6. Grass, without N addition	3.664	1.011	0.425	1.436	-2.228	-4.895
Grass, with N addition	3.664	1.197	0.505	1.702	-1.962	-4.247
7. Rape seed cake, without N addition	5.646	0.712	0.675	1.387	-4.259	-7.739
Rape seed cake, with N addition	5.646	0.888	0.510	1.398	-4.248	-7.668

The data show that when no additional nitrogen is added, about 10 % of the original protein is converted into ammonia with the straws, while the proportion is much higher with grass (60.8 %) and rape seed cake (75.4 %). Though bracken contains a comparatively high percentage of nitrogen (1.867 %) only 4.1 % of it is converted into ammonia. This may be due either to the difference in the nature of the proteins present or to the protective action exercised by the high lignin content in the bracken.

The distinct, though slight, stimulating action exercised by addition of available nitrogen on the degree of decomposition and the amount of decomposition products, has been referred to above. Nitrogen supply appears also to modify the course of nitrogen transformations in the anaerobic system, as shown by the values for nitrogen factor and equivalent. With materials poor in nitrogen, *e.g.* straws, these values are negative in the absence of added nitrogen and positive in its presence. The positive values represent a net gain of synthesised protein during the period of digestion. They are, however, much lower than the figures given for aerobic decomposition [Richards and Norman, 1931]. With materials rich in anaerobically available nitrogen, *e.g.* rape seed cake or grass, the nitrogen factor and equivalent give high negative values, both in presence and absence of added nitrogen, showing that the net result in either case at the end of the digestion is the reduction of a considerable portion of the original protein to ammonia. Bracken, however, differs in showing a low negative value in the absence of nitrogen addition and a small positive value in its presence, thus resembling the straws poor in nitrogen. This behaviour of bracken could be explained if it be assumed that for purposes of anaerobic digestion the protein is not available.

4. *Comparison of the losses during anaerobic digestion with those during acid hydrolysis.*

10 g. portions of the materials were boiled for one hour with 200 ml. of (1) aqueous 5 %  $\text{H}_2\text{SO}_4$ ; (2) a solution of 5 %  $\text{HCl}$  in 95 % alcohol; (3) aqueous 2 %  $\text{HNO}_3$ ; (4) aqueous 5 %  $\text{HNO}_3$ . The residues were weighed and analysed for cellulose, furfuraldehyde yield, lignin and protein. The losses of constituents by the different treatments, calculated on 100 g. of original material, are presented in Table II.

It will be noted that none of the acid treatments gives the same results as those obtained by anaerobic digestion, the difference being most marked in the case of cellulose. A considerable proportion of cellulose, varying from 40–60 % of the amount originally present, is lost by anaerobic digestion, whilst the losses by acid treatment amount to 25–30 % only. The higher concentration of nitric acid (5 %) was used to oxidise the cellulose, if possible, and so secure a greater loss; but the attempt was unsuccessful.

A second difference between the two kinds of decomposition lies in the relative proportions of cellulose to xylan, associated with cellulose, left after decomposition. In anaerobic fermentation the amount of cellulose which disappears is roughly proportional to the loss of xylan associated with it. But acid treatments disturb this ratio by removing a much higher proportion of the associated xylan than of cellulose.

The differential removal of the straw constituents by the four acid treatments is worth noting. While 5 %  $\text{H}_2\text{SO}_4$ , 2 %  $\text{HNO}_3$  and 5 %  $\text{HNO}_3$  remove most of the xylan not associated with cellulose and also a large portion of the xylan so associated, 5 % alcoholic  $\text{HCl}$  acts mostly on the xylan fraction associated with cellulose and much less on the fraction not so associated.

Nitric acid at 2 % as well as 5 % concentration oxidises and dissolves large proportions of the lignin originally present, ranging from 60 to 80 %, and would therefore be unsuitable for purposes of comparison with anaerobic treatment, where the losses average about 25–30 % only. With bracken leaves the loss of lignin is low with 2 %  $\text{HNO}_3$ ; this is associated with a smaller loss of protein as compared with the other acid treatments. It is probable that a portion of the protein might have been oxidised by the nitric acid with the formation of "apparent" lignin [Norman and Jenkins, 1934] which may mask the loss of lignin originally present.

With straws the losses in protein are higher during acid treatment than during anaerobic digestion, but there is good agreement in the case of materials richer in nitrogen, *e.g.* grass and rape seed cake. Bracken, however, is an exception since its protein is less soluble under anaerobic conditions than with acid treatment. This low solubility under biological action may be due, as already observed, to the protective action of lignin. The nitrogen losses on treatment with nitric acid are irregular, owing possibly to the nitrating action of the acid on the constituents.

Of the four acid treatments tried, that with 5 %  $\text{H}_2\text{SO}_4$  seems to come closer to the results of anaerobic digestion than the others. The attack on cellulose is no doubt distinctly lower than occurs in the biological action, but this is to a certain extent made up by the greater attack on the xylans. Considering the widely varying composition of the straws on the one hand and of bracken, grass and rape cake on the other, the agreement may be considered satisfactory. Bearing in mind the remarks made above in regard to the attack on cellulose and xylans, the method can be put forward as one which could rapidly give some idea of the

anaerobic decomposability of a plant material and the approximate amount of products, either acid or gas, which could be obtained from it. More work with a larger variety of substances is necessary before any definite recommendations in the matter can be made.

##### 5. *Decomposition of constituents isolated from oat straw.*

A comparative study of the anaerobic decomposition of the different constituents of oat straw was made using preparations of lignin, hemicellulose and cellulose isolated from it. The sample of lignin was obtained by treatment with 72 %  $\text{H}_2\text{SO}_4$ , of hemicellulose by boiling with 4 %  $\text{NaOH}$  and subsequent precipitation with acetic acid and alcohol, and of straw cellulose by the method of Norman and Jenkins [1933]. A portion of the cellulose was treated with boiling 5 %  $\text{H}_2\text{SO}_4$  for one hour to remove a part of the xylan contained and was also fermented for purposes of comparison. The sample of crude hemicellulose gave a furfuraldehyde yield equivalent to 76.8 % xylan. The sample of oat cellulose and that treated with acid contained 30.81 and 19.64 % respectively of xylan.

To 5 g. portions of the substances 2 g.  $\text{KHCO}_3$ , 2 g.  $\text{NH}_4\text{HCO}_3$ , 0.2 g.  $\text{K}_2\text{HPO}_4$ , 0.05 g.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 10 ml. of an extract from a rice soil, 10 g. of asbestos to allow for the surface development of a bacterial film and 100 ml. of water were added, the other experimental details being as usual. Gas evolution began earliest with the hemicellulose preparation, but in all cases there was a rapid accumulation of acids with consequent lowering of  $p_{\text{H}}$  and fall in gas yield.

The data given in Table V show that "acid" lignin does not undergo any appreciable decomposition and yields no gases or acids. Hemicellulose is more

Table V. *Decomposition of constituents isolated from oat straw.*

g. per 100 g. after 6 months' fermentation.

	Acid lignin	Crude hemi- cellulose	Oat cellulose	Purified oat cellulose
Unfermented insoluble residue	97.2	8.2	17.2	18.1
Volume of $\text{CO}_2$ (corrected) for carbonates (ml.)	Nil	11,120	3,820	3,340
Volume of $\text{CH}_4$ evolved (ml.)	"	12,830	4,410	3,860
Weight of $\text{CO}_2$	"	21.99	7.55	6.60
Weight of $\text{CH}_4$	"	9.20	3.16	2.77
Acetic acid present	"	31.32	50.56	53.58
Butyric acid present	"	8.27	13.34	14.70
Total products	"	70.78	74.61	77.65
Nitrogen immobilised or nitrogen factor	"	0.280	0.320	0.340
Nitrogen equivalent	"	0.305	0.387	0.415

rapidly decomposed than cellulose as shown by the larger yield of gases and smaller accumulation of acids at the end of 6 months. There is no appreciable difference between the rates of fermentation of oat cellulose and that treated with 5 %  $\text{H}_2\text{SO}_4$  for one hour.

It is interesting to note that though the amounts of gases and acids formed vary considerably between the hemicellulose and cellulose preparations used in the present experiment, the proportions of acetic to butyric acids and of  $\text{CO}_2$  to  $\text{CH}_4$  in the two cases appear to be similar. Thus with both the ratio of acetic to butyric is roughly 4 : 1 and that of  $\text{CO}_2$  to  $\text{CH}_4$  by volume, roughly 7 : 8. The bearing of this relationship on the decomposition of straws, where cellulose and hemicellulose are the chief constituents decomposed, will be discussed later.

The hemicellulose and cellulose preparations also show similar values for nitrogen factor and nitrogen equivalent. The values for nitrogen factor are higher than with straws, probably owing to more complete utilisation of the

extracted constituents than of the plant material as a whole. The nitrogen equivalent values, representing the nitrogen immobilised per 100 g. of material actually decomposed, are of the same order for straw and for its isolated constituents.

#### DISCUSSION.

In the aerobic decomposition of plant materials it has been observed that lignin inhibits the decomposition of pentosans and cellulose; Rege [1927] proposed a pentosan/lignin factor as a means of predicting decomposability of substances [cf. Norman, 1929]. The present experiments indicate that lignin may act as a still more powerful inhibitor in anaerobic decomposition, not only of hemicelluloses and cellulose but also of proteins as well.

In all the plant residues examined there are appreciable fractions of cellulose, hemicelluloses and proteins which are left undecomposed. This may be due either to the inhibitory action of lignin or to the above fractions being encrusted on the cell wall so as not to be easily accessible for microbial attack. It should be noted that a large proportion of the cellulose and appreciable proportions of hemicelluloses and proteins remain also unattacked by weak acids.

The experiments on the nitrogen relationships confirm what has already been described for rice straw regarding the low nitrogen requirements of anaerobic digestion and the absence of any considerable synthesis of proteins from ammonia.

The products obtained by anaerobic decomposition of the cellulose and hemicellulose fractions isolated from oat straw show an interesting similarity both as to the qualitative and quantitative nature of the components. Thus the proportion of acetic to butyric acids is roughly 4 : 1. The approximate constancy of this proportion leads us to expect a similar ratio with straws, where loss of cellulose and hemicellulose accounts for the greater part of the organic matter which disappears. It is proposed to extend this work to an examination of the products obtained by anaerobic digestion, under conditions favouring acid and gas production respectively, of a large variety of cellulose, hemicellulose, protein and other fractions isolated from different plant materials and to compare the data obtained with those for plant materials pretreated so as to remove most of certain constituents.

The absence of gas or acid production from "acid" lignin confirms the work of others [Levine *et al.*, 1935], but it must be noted that the susceptibility of lignin in the natural state to anaerobic attack may be different from that of the fraction isolated by acid or alkali treatment. Recently Levine *et al.* [1935] have suggested that much of the loss of lignin hitherto reported in anaerobic digestion [cf. Boruff and Buswell, 1930] may be due to the mechanical solution of lignin rather than to its chemical decomposition. But in the experiment on "acid" lignin there was an almost complete recovery of added lignin, indicating that at the  $p_H$  maintained the solution of "acid" lignin was not appreciable. However, further work is necessary before this point can be satisfactorily settled.

The results of acid treatment show marked differences from those obtained by anaerobic digestion. During the course of decomposition by micro-organisms, cellulose is destroyed more rapidly than with mineral acids and the losses in cellulose keep pace with those in the xylan fraction associated with it. The preferential extraction by alcoholic acid of the xylan fraction associated with cellulose is also noteworthy. The use of an alcoholic solution of sulphuric acid and a correction factor applied for the cellulose actually decomposed, might perhaps give results which approximate to the losses found under anaerobic conditions.



## SUMMARY.

1. The anaerobic decomposition of oat, wheat, rice and barley straws, bracken leaves, young grass (lawn mowings) and rape seed cake has been studied with reference to losses of constituents, products formed and nitrogen transformations.

2. A good proportion of the total decomposition could be brought about by digesting the materials without the addition of nitrogen, under conditions of optimum temperature and reaction. With materials poor in available nitrogen, however, *e.g.* straws, the addition of nitrogen exerts a distinct, though slight, stimulating effect.

3. The chief products obtained are the same in all the cases: *viz.* acetic and butyric acids,  $\text{CO}_2$  and  $\text{CH}_4$ .

4. Whilst the straws yield a large amount of acetic and small amounts of butyric acid, the reverse is the case with materials rich in available protein, *e.g.* grass and cake. The  $\text{CO}_2 : \text{CH}_4$  ratio is roughly unity in the former case, while in the latter the proportion of  $\text{CH}_4$  is much higher.

5. The losses of plant constituents by anaerobic fermentation have been compared with those brought about by boiling for one hour with (1) aqueous 5 %  $\text{H}_2\text{SO}_4$ ; (2) alcoholic 5 %  $\text{HCl}$ ; (3) aqueous 2 % and 5 %  $\text{HNO}_3$ . The cellulose fraction destroyed by acid treatment was smaller and the hemicelluloses decomposed greater than by biological agency. It is suggested that the losses on treatment with 5 %  $\text{H}_2\text{SO}_4$  for one hour, with a correction for the cellulose decomposed, might be used to forecast the anaerobic decomposability of a plant material and the probable yield of acid and gaseous products.

6. The decomposition of lignin, hemicellulose and cellulose fractions isolated from oat straw has been studied. Lignin is not fermented. Hemicellulose and cellulose fractions are fermented, the former more readily than the latter, yielding acetic and butyric acids in the proportion of 4 : 1 (approx.) and  $\text{CO}_2$  and  $\text{CH}_4$  in the ratio of 7 : 8 (approx.).

7. The presence of a high lignin content markedly inhibits the anaerobic decomposition of protein and other constituents.

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