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CONTENTS.

INDUSTRIAL WASTES AS MANURE.

I. AJOWAN AND MOHUA CAKES AS FERTILISERS.

II. UTILISATION OF REFUSE.

BY

R. D. Rege.



DR. M. O. FORSTER, F.R.S., CHAIRMAN OF EDITORIAL BOARD.

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CONTENTS.

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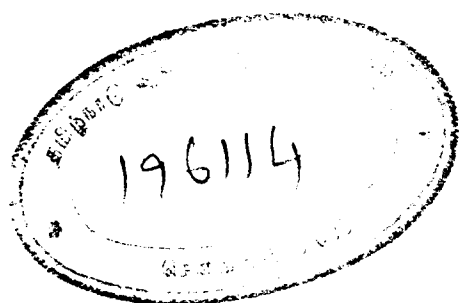
I. AJOWAN AND MOHUA CAKES AS FERTILISERS.

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BY

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THE USE OF AJOWAN¹ AND MOHUA² CAKES AS FERTILISERS.

Through the kindness of the Wardle Engineering Co., Hyderabad (Deccan), samples were obtained of *ajowan* and *mohua* cakes which are industrial waste-products in many districts. These have been examined for their value as fertilisers.

PART I.—AJOWAN CAKE.

The ajowan cake was not completely free from thymol, the smell of which was quite perceptible, but attempts to extract this by steam distillation showed that the quantity was extremely small. As, however, even this insignificant quantity might have a deleterious effect on bacterial growth and activity, it was considered necessary to investigate this point. For this purpose, two tubes of sterile water inoculated with 1 gram of soil and to one of which 1 gram of the powdered cake was added, were kept at ordinary temperature for two days, by which time a luxuriant bacterial growth was evident in both. A count of the organisms present, made by plating out on synthetic agar, gave the following results:—

Soil control		2900 colonies per gram.
Soil + ajowan cake		3800 ,,

The results conclusively prove that the amount of thymol present in the cake was quite insufficient to check bacterial activity in the soil.

Analysis of the cake for nitrogen, potash and phosphoric acid, the three chief ingredients of manurial value, gave the following results:—

Nitrogen		0.862 per cent.
P ₂ O ₅		0.856 ,,
K ₂ O		2.02 ,,
Ash		12.7 ,,

Nitrogen was determined by the Kjeldhal method, the phosphate estimated as Mg-pyrophosphate and potash by the perchloric acid method.

These results show that the cake is of only moderate manurial value as it contains comparatively little nitrogen and phosphoric acid

¹ Ajowan, *Carum copticum*, the seed of which yields about 1 per cent. of thymol.

² Mohua, *Bassia longifolia*.

though potash is present in appreciable amount. The material might, however, be of use if the nitrogen could readily be made available. It was considered that this point might be tested by the use of 'activated sludge,' the latter containing all bacteria necessary for the purpose.

Ten grams of ajowan powder were accordingly thoroughly mixed in a mortar with 100 c.c. 'activated sludge' and the total volume made up to 300 c.c.; 100 c.c. were taken for immediate analysis while 100 c.c. were aerated continuously for six days. At the end of this period the experiment was stopped, the samples filtered and the nitrogen determined.

TABLE I.

	Control. Ajowan powder + activated sludge 5-7-1923.	Aeration stopped after six days 10-7-1923.
Nitrogen (free ammonia) ...	0.84 mgms.	4.2 mgms.
" (albuminoid ammonia)...	6.0 "	3.36 "
" (nitrate and nitrite) ...	1.0 "	1.44 "
Total nitrogen in extract ...	21.84 "	26.88 "
" in residue ...	97.94 "	99.87 "
Grand total ...	119.78 "	126.75 "
Active insoluble nitrogen ...	19.8 per cent.	26.45 per cent.
Dry weight of the residue ...	3.0756 mgms.	2.5704 mgms.
Percentage loss of dry weight ...		16.43

These results lead us to conclude that in spite of its low nitrogen-content the cake might be of use, much of the protein nitrogen appearing to be in a labile form. Experiments on the nitrification value of this cake in soil confirm the above results.

In view of the fact that the total nitrogen in the experiment which was stirred for six days exceeded that in the control, it was thought that the cake might assist nitrogen-fixation. To test this, flasks containing 20 grams of sand (washed with hydrochloric acid), 1 gram of CaCO_3 , 5 grams of ajowan powder and 100 c.c. of water were sterilised in the autoclave. In another series CaCO_3 was replaced by MgCO_3 in view of the statements made by some workers that the latter is more efficient than CaCO_3 in helping nitrogen-fixation. The flasks were all inoculated with 1 c.c. pea soil. The control-pots were then again sterilised and all were incubated for six weeks, after which period the mixtures were analysed for total nitrogen by the Gunning method. The results are given below :—

	Gain or loss	
i. Control	101.09 mgms. nitrogen.	
ii. With CaCO_3	102.76 "	1.75 mgms. nitrogen (gain)
iii. With MgCO_3	86.2 "	14.89 " (loss)

While with CaCO_3 there was practically no change in the nitrogen-content, MgCO_3 involved a distinct loss amounting to 14.89 mgms. There is therefore no experimental evidence to support the view that the ajowan cake stimulates nitrogen-fixation.

As only small quantities of the cake were available no further experiments such as pot or plot-cultures could be made; but the experiments mentioned above clearly prove that the cake does not diminish the activity of soil micro-organisms, while its nitrogen appears to be in a form readily available to the plant, at any rate after adequate treatment. In districts where it is obtainable at a low cost it might, therefore, be used as a fertiliser.

PART II.—MOHUA CAKE.

Analysis of mohua cake gave the following results :—

Oil		8.06	per cent.
Nitrogen		2.72	"
P_2O_5		0.12	"
K_2O		2.03	"
Ash		7.56	"

It will be seen that the cake obtained on pressing the mohua seeds retains a large quantity of oil. It is also moderately rich in nitrogen and might therefore be expected to give useful results as a manure. Hutchinson,¹ however, has shown that on adding this cake to the soil there is no evidence of nitrification even after several weeks. The nitrogen of the cake must therefore either be present in a very resistant form, or the cake contains some substance which inhibits the action of nitrifying bacteria. Treatment of the cake with 'activated sludge' was therefore carried out in order to ascertain how readily the protein matter could be rendered available.

Experiment I.—10 gms. of mohua cake were thoroughly mixed with 100 c.c. 'activated sludge' in a mortar and the volume made up to 300 c.c.; 100 c.c. were taken for aeration while another 100 c.c. were used for preliminary analysis. During aeration, however, great difficulty was experienced from frothing as even with a slow current of air this took place and the material was liable to be carried over from the experimental vessel. The experiments were however continued for six days, at the end of which period the mixture was filtered and analysed as usual. The analyses are given in Table II.

¹ *Agri. Res. Inst. Pusa Sci. Reports*, 1920-21, 28.

TABLE II.

		Mohua cake + activated sludge (control)		Mohua cake + activated sludge. Aeration continued for six days	
Nitrogen (free ammonia) ...	...	0.384	mgms.	0.24	mgms.
„ (albuminoid ammonia) ...	...	1.44	„	1.55	„
„ (nitrite and nitrate) ...	...	0.10	„	0.24	„
Total nitrogen in extract ...	...	7.14	„	5.46	„
„ in residue ...	...	123.30	„	124.70	„
Grand total nitrogen ...	...	130.44	„	130.16	„
Loss in weight per cent. ...	...	4.496			

The figures show that no decomposition of the protein of the mohua cake has occurred. It seemed possible that this might be due, partly at any rate, to the high oil-content of the material. The oil was, therefore, extracted by petroleum-ether in a Soxhlet apparatus and the oil-free cake mixed as before with activated sludge and aerated. Frothing still occurred and was even more marked than before, making it impossible to continue the experiment. The oil is, therefore, not responsible for this phenomenon which is, in fact, due to a saponin.

Experiment II.—Attempts were made to extract the saponin and examine its effect on nitrification. The following method¹ was used.

The finely powdered material was extracted with petroleum ether to remove oil. The residue was then treated with boiling alcohol (60 per cent.), the extract filtered, the alcohol distilled off and the residue evaporated on the water-bath with calcined magnesia. The resulting paste was extracted with boiling alcohol (80 per cent.), filtered and concentrated by distillation. Ether failed to remove anything from the residue which on evaporation gave an amorphous, bitter and very hygroscopic powder, forming an acid solution which frothed greatly and gave the following reactions :—

- (1) A flesh coloration with aqueous sodium nitroprusside.
- (2) A yellow colour with solid diphenylamine.
- (3) A dark purple colour with concentrated sulphuric acid, forming gradually.
- (4) Reduction only on continued boiling with Fehling's solution. After hydrolysis a heavy precipitate of CuO was obtained.
- (5) The solid material when dissolved in sulphuric acid (3 per cent.) and hydrolysed in the autoclave at 105°C during one hour gave a

¹ *Compt. rend.*, 1912, 155, 844.

heavy precipitate of sapogenin and a solution which reduced Fehling's solution.

(6) Among the sugars in the solution after hydrolysis, glucose and xylose were identified by their osazones. Maltose and arabinose may also be present.

These reactions indicate the compound to be a saponin belonging to the acid group.

To see whether saponin had any effect on nitrification, the following solutions were prepared :—

- (I) Ashby's medium with CaCO₃ Control I.
- (II) „ „ With 4 c.c. of 1 per cent. saponin per 100 c.c. solution.
- (III) „ without CaCO₃ Do.
- (IV) „ „ Without saponin ; Control II.

100 c.c. of medium were used in each case with 1 per cent. CaCO₃ where necessary, and duplicates were run. The flasks were inoculated with equal quantities of a good nitrifying soil and were incubated for a week, after which period the nitrogen present as nitrite and nitrate was estimated in accordance with the standard methods advocated for water analysis (A.O.A.C.). The results are given in Table III. They are calculated on 1 c.c. of the liquid.

TABLE III.

Series	I	II	III	IV
Nitrite nitrogen (mgm.)	0.009	Trace.	0.001	0.01
Nitric nitrogen „	0.3	0.027	0.04	0.2

It will be seen that in series II and III where saponin was added there has been but little formation of nitrate.

The total nitrogen was also determined in each series with the following results :—

Series I		13.46 mgms. nitrogen.
„ II		13.44 „ „
„ III		5.53 „ „
„ IV		11.2 „ „

It will be noted that there has been a marked loss of nitrogen in series III which contained saponin but no calcium carbonate, probably due to denitrification being set up.

To confirm the inference that the saponin is responsible for the low availability of the cake the following experiment was arranged :—

- | | |
|-----|---|
| (1) | 100 gms. of soil + 3.5 gms. raw mohua cake. |
| (2) | " " fat-free mohua cake. |
| (3) | " " saponin-free mohua cake. |

The moisture was adjusted at 60 per cent. saturation and the experiments were continued in duplicate for three months. At the end of this period nitrate was determined by the phenoldisulphonic acid method. The results are given below :—

Nitric Nitrogen.

Control			0.37 mgms. per 100 grms. of soil.
Raw mohua cake		0.224	" "
Fat-free mohua cake		0.32	" "
Saponin-free mohua cake.	1.92	" "	" "

These experiments clearly prove that the low availability of the cake is due to presence of saponin which inhibits the formation of nitrates. In these circumstances the use of the cake as a fertiliser is hardly practicable.

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INDUSTRIAL WASTES AS MANURE.

II. The Utilisation of Refuse.

By R. D. Rege.

Refuse consists in general of street sweepings and household waste-material. The former, in addition to mineral matter, contains a varying quantity of leaves and similar organic matter according to the season of the year, while the household refuse is made up of ashes, food waste, e.g., vegetable trimmings, bones, fat, paper, tins and other containers. The composition is thus extremely variable; its disposal, and still more its profitable utilisation, raise many difficult problems.

The simplest method of disposal, and one formerly frequently employed, consists merely in dumping the material in low-lying land on the outskirts of the town, the sites thus filled being utilised sooner or later for building purposes. This system is, however, now generally recognised as objectionable and likely to be detrimental to health, such collections of rubbish forming breeding grounds for flies, while the decomposition of the organic matter contained in the refuse leads to unpleasant odours.

In the interests of sanitation, therefore, dumping has been succeeded by incineration and various systems have been employed to make this as economic a process as possible. In more advanced communities the refuse may receive a preliminary treatment before incineration, usually with the object of recovering the fat which is of considerable value. The material is then burnt, the heat produced in the process being utilised for the production of steam, water-gas or electricity. The ash left after incineration may again be used in the making of bricks, concrete, etc., so that very little real waste need occur. In smaller places, however, where less elaborate methods have to be employed, no such use is made of the process which then becomes essentially wasteful.

It is evident from its composition that under favourable conditions, refuse may be possessed of a not inconsiderable value as a manure, and in India where organic matter rapidly undergoes decomposition, it is quite commonly used for this purpose. The method of employment, however, varies. In some cases, the dry refuse, usually containing a considerable quantity of leaves, straw, etc., is spread on the fields and then burnt just before the land is ploughed. In other districts the material in a fresh and more or less undecomposed condition, is ploughed into the soil without being burnt. In a third method the material is composted in pits or heaps for some months before use whereby the organic matter undergoes considerable change and becomes much more available.

Considering these three methods it will be seen that in the first, only the mineral material of the manure is finally incorporated in the soil and while the actual process of incineration may in some cases have a beneficial effect on the soil, this does not compensate for the total loss of the nitrogen and the organic matter.

The second method may be actually injurious as the presence of considerable amounts of undecomposed carbohydrate material may set up denitrification and lead to a loss of crop.

The third method, if properly carried out, is undoubtedly the best. The organic matter is brought into a proper state of decomposition before use while retaining the bulk which has so beneficial an effect on most Indian soils, and a considerable portion of the nitrogen of the manure is conserved.

Unfortunately, however, no care is generally paid to the composting process, the material being merely exposed in heaps to sun and rain with the result that a very considerable proportion of the nitrogen and much of the soluble mineral matter is lost, and the fertilising value of the manure much reduced.

It is obvious, therefore, that it would be an advantage if methods could be devised whereby such waste material could be rapidly converted into an available condition without undue loss of nitrogen or other valuable constituents. It might then be possible in the larger towns, at any rate, where many tons of such refuse are collected daily, to convert this at a low cost into a useful manure.

The present paper contains an account of an investigation having as its objects the study of the decomposition of refuse under various conditions.

The material employed has consisted of the mixed refuse collected at the Indian Institute of Science, Bangalore. The average composition of this is shown in the table below :—

TABLE I.
Composition of mixed refuse.

		Period of collection	
		October to March	March to October
		Per cent.	Per cent.
Nitrogen	...	1.5 to 1.74	0.84 to 1.35
P ₂ O ₅	...	0.32 „ 0.64	0.06 „ 0.10
K ₂ O	...	1.74 „ 2.62	0.60 „ 0.89
CaO	...	4.37	0.1
Total ash	...	14.6 „ 22.6	10.34 „ 13.6

It will be seen that the refuse collected during the period October–March is much the richer, this being due to the large proportion of fallen leaves and other vegetable matter it contains at this time of the year. It is, in fact, richer than the cattle manure collected on the estate which gave the following figures on analysis :— Nitrogen, 0.73 to 1.13 per cent., K₂O, 0.7 per cent., CaO, 1.33 per cent., and Ash, 14.66 per cent.

The nitrogen in the refuse is, however, in the fresh state much less available than the nitrogen of cattle manure, as is shown by the proportion of active insoluble nitrogen in each which was as follows :—

	Active insoluble nitrogen per cent.	
Refuse		17.25
Cattle manure		24.89

The time required, therefore, for the decomposition of the refuse is considerably greater than is the case with cattle manure. This is not surprising in view of the far larger bacterial population of the latter material, a count yielding the following figures :—

Cattle manure		1,800,000 to 1,900,000 bacteria per gm.
Refuse		30,000 „ 70,000 „ „

Two conditions, in fact, would appear to be necessary for the more rapid decomposition of the refuse : (1) reduction of the material to a fine state of division and (2) inoculation with a suitable bacterial population.

The latter might be provided by the addition of ‘activated sludge.’ While the ‘activated sludge’ process of sewage disposal is at present used in but a few places in India, it is likely to be adopted in many of the larger towns as the present primitive methods are abandoned. It is in such areas that the greatest quantities of refuse also will be available. The utilisation of the sludge to render the refuse more valuable as a fertiliser would, if practicable, solve two problems, increasing the value of the refuse and facilitating the disposal of the sludge.

Preparation of Liquid Manures.—In the first place attempts were made to prepare a liquid manure containing all the nitrogen of the refuse.

The dried refuse was powdered, passed through a sieve and 22 gms. of the fine material mixed with 100 c.c. activated sludge and made up to 400 c.c. which was then divided into four equal portions.

two months. The resistant nature of this nitrogenous material is also indicated by the following experiment :—

5 gms. of the refuse powder were mixed with 100 c.c. activated sludge and aerated for three days. The liquid was then filtered off and the residue, with an equal volume of water, again aerated. This was repeated twice, when the extract was colourless and contained a negligible quantity of nitrogen. At this stage a further quantity of fresh activated sludge (100 c.c.) was added to the residue and the mixture again aerated for two days. The distribution of the nitrogen was then examined with the following result :—

Total nitrogen in extract		8.40 mgms.
„ „ in residue		133.28 „

It is evident, therefore, that after the comparatively rapid decomposition of about 25 per cent. of the nitrogenous material, the balance remains resistant to further change and undergoes decomposition at a very slow rate.

It was thought that somewhat better results might be obtained if care were taken to ensure thorough mixing of the sludge and refuse throughout the period of aeration and hence the following experiment was carried out :—

Two tubes were set up each containing 5 grms. refuse (leaf powder) + 100 c.c. activated sludge. In No. I arrangements were made for thorough mixture of the constituents while in No. II no special precautions were taken. Aeration was carried out for four days, the solutions were then filtered and the nitrogen in the extracts determined while the residues were submitted to further aeration after the original volume had been made up by water. The analytical results are given in Table III. The composition of the original mixture before aeration is also shown.

The results indicate, however, that the amount of nitrogen found in the extract is practically the same in either case. In both there is a loss of nitrogen but this is greater in No. II where no precautions were taken to ensure thorough mixing.

This loss in nitrogen probably arises from the refuse and not from the activated sludge, for the latter contains nitrogen-fixing organisms and if aerated under suitable conditions exhibits a gain in total nitrogen as the following experiment shows :—

100 c.c. of activated sludge were mixed with one gram mannite and one gram calcium carbonate and the mixture aerated for ten days,

TABLE III.

	Control			Tube No. I			Tube No. II		
	Activated Sludge Mgms.	Refuse powder Mgms.	Total Mgms.	1st aeration Mgms.	2nd aeration Mgms.	Total Mgms.	1st aeration Mgms.	2nd aeration Mgms.	Total Mgms.
Nitrogen (free ammonia)	0.144	0.072	0.216	0.448	0.096	0.544	0.300	0.036	0.336
„ (albuminoid ammonia)	0.288	1.500	1.788	1.925	0.324	2.259	0.900	1.380	2.280
„ (nitrite and nitrate)	0.355	0.108	0.462	0.280	0.048	0.328	0.060	0.060	0.120
Total nitrogen in extract	7.980	3.980	11.970	12.054	4.620	16.674	12.600	3.192	15.792
„ „ in solids	47.320	86.270	133.590	...	124.20	124.20	..	117.400	117.400
Total nitrogen	55.300	90.260	145.560	...	...	140.814	...	...	133.192
Dry weight of solids	0.814 grms.	4.540 grms.	5.354 grms.	...	...	4.780 grms.	...	...	4.780 grms.
Percentage loss in dry weight	...	...	...	...	...	10.8	...	...	10.8

the experiment being carried out in duplicate. Determination of total nitrogen before and after aeration gave the following results :—

		I	II
		Mgms.	Mgms.
Total nitrogen before aeration		70·91	70·91
„ after aeration		77·28	77·54
Gain		<u>6·37</u>	<u>6·63</u>

In view of the comparatively small amount of nitrogen rendered soluble in the above experiments and of the inconvenience in dealing with liquid manures, attempts were now made to obtain solid manures from mixtures of activated sludge and refuse and to increase the availability of such manures by different methods of treatment.

Solid Manures.—Fresh activated sludge is difficult to filter and takes a long time to dry. By stirring into the sludge a sufficient quantity of refuse, however, a pasty mass can be obtained which does not require filtration and which dries fairly rapidly.

The obvious advantage of such a preparation is that the liquid portion of the sludge, containing a considerable portion of the most easily available nitrogen, is absorbed and loss is thereby reduced.

Preparation I.—As aeration was considered advisable and the consistency of the mixture rendered bubbling ineffective, mechanical agitation was used whereby the whole of the mixture was well stirred and frequently exposed to the air. 500 c.c. of sludge were used and in all 361 gms. of refuse powder were added in the course of two days, the stirring being then continued for another day. As some difficulty was experienced in maintaining sufficient agitation by the mechanical stirrer, hand-stirring was occasionally employed. After three days the material was poured out on to trays and dried in the sun. When completely dry it was powdered and analysed. In this experiment stirring was not continuous, being stopped during the night.

Preparation II.—In this case mechanical stirring was more efficient and was continuous throughout. Otherwise the procedure was the same as in the case of I. The sludge used in this experiment was in a more active condition than in I, where it exhibited the phenomenon known as ‘ bulking.’

Analysis.—10 gms. of the manures thus prepared were soaked in water for 30 minutes and filtered, the residue being washed with water till the washings gave no reaction for ammonia. The extract thus

obtained was analysed for free and albuminoid ammonia, nitrite and nitrate-nitrogen and total nitrogen. The residue was analysed for total and for insoluble active nitrogen. The latter figure was determined by the alkaline permanganate method of the A.O.A.C. Similar analyses were made of the original sludge and refuse.

In addition to the chemical analysis, the rate of nitrification of the different samples was determined in the usual manner. The nitrate in the soil extracts in such cases was estimated by the phenoldisulphonic acid method after clarification of the extract with alumina.

TABLE IV.
Analyses of Preparations I and II.

	I		II	
	Original materials	Finished product	Original materials	Finished product
Nitrogen (free ammonia) ...	3·99 mgms.	5·36 mgms.	2·22 mgms.	3·18 mgms.
„ (albuminoid ammonia)...	36·32 „	23·44 „	34·33 „	116·10 „
„ (nitrite and nitrate) ...	0·22 „	nil	0·44 „	nil
Total nitrogen extract ...	95·11 „	70·33 „	123·12 „	155·5 „
„ „ solids before extraction ...	2·20 grms.	2·47 grms.	2·03 grms.	2·18 grms.
Percentage insoluble active nitrogen in residue ...	...	20·13	...	24·6
Proportion of sludge to refuse ...	1 : 24		1 : 30	

TABLE V.
Nitrification test.

			Nitric nitrogen produced per 100 grms. soil
Control, soil alone			0·256 mgms.
Refuse powder			0·320 „
Manure I			0·786 „
Manure II			0·450 „

From the chemical analysis one would expect preparation II to be the better, if equal quantities of nitrogen be considered, for a much larger percentage of this nitrogen is in a soluble condition. In the nitrification test, however, the results obtained were in direct opposition to this, I being nitrified more readily than II. Some pot-cultures

carried out with these materials also gave better yields with I, but much stress is not laid on these figures owing to the small scale of the experiment.

A possible explanation of this behaviour can be formed if the carbon-nitrogen ratio be considered. This ratio is higher in II than in I, and it has been shown by Clark and Adams¹ that a high ratio tends to check nitrification. In their experiments this effect was noticeable when the ratio reached 12 : 1. As in both the manures described above the ratio was much higher still, it might be expected that nitrification would be hindered and that this would be especially the case in II. Similar results have been obtained by Sievers² who, when working with straw, found nitrification was diminished when the C/N ratio exceeded 13 : 1.

In order to investigate this point more fully a further series of preparation was made. In each case 100 c. c. of sludge were used and diminishing quantities of refuse (straw powder) were incorporated, the mixture being stirred for 24 hours. After drying the manure, the nitrification test was carried out, 1 gram. of the manure being used for 100 grms. of soil and the mixture kept under suitable conditions for two weeks.

In no case where the proportion of refuse exceeded 10 grms. per 100 c. c. sludge was there any evidence of nitrification taking place. The activated sludge contained approximately 4 per cent. solid matter from which it is evident that the ratio of refuse to dry sludge should not exceed 2.5 to 1 if the method of preparation described above be employed. It should, however, be possible, materially to increase this ratio if a longer time of aeration be employed. In this case there would be a proportionately greater breaking down of carbohydrate matter and the final C/N ratio would again be reduced. The disadvantage of thus prolonging the fermentation is, however, to increase the risk of loss of nitrogen, and the process finally employed will evidently have to be a compromise between the requirements of these two sides of the question.

Fixation of Soluble Nitrogen Compounds by Refuse.

In the activated sludge process of sewage disposal, while the conservation of nitrogen is greater than in any other system, much of the nitrogen passes into the effluent and thereby goes to waste. Richards³ has shown, however, that a large proportion of this nitrogen

¹ *J. Ind. Eng. Chem.*, 1912, 4, 272.

² *Am. Chem. Abs.*, 1923, 17, 2161.

³ Richards and Weekes, Proceedings of the Engineering Conference of the Institution of Civil Engineers, 1921.

can be recovered by passing the effluent over beds of straw, the nitrogen being thereby fixed while the straw is converted into a substance of considerable manurial value.

Experiments have therefore been carried out to investigate to what extent other waste materials might exert a similar action in fixing soluble nitrogen. The work described earlier in the paper has indicated that not infrequently nitrates and other soluble nitrogen compounds are removed from solution without any loss in total nitrogen and the problem was, therefore, to ascertain the conditions most favourable to this action.

100 c. c. of activated sludge were mixed with 5 grms. of powdered corn-cobs, 0.5 gm. of ammonium sulphate was added and the volume made up to 200 c. c. The experiment was carried out in duplicate, the contents of one flask being stirred for 24 hours and in the case of the second for 48 hours. The mixtures were then filtered and the residue in each case washed until the washings were free from ammonia.

The various forms of nitrogen in the filtrate were then determined: also the total nitrogen and the insoluble active nitrogen in the residues. The quantity of activated sludge used contained 2.23 grms. dry matter, so that the ratio of dry sludge to refuse in this experiment was 1 : 2.2.

A similar experiment was carried out in which the weight of corn-cob refuse was increased to 7.5 grms., the ratio of dry activated sludge to refuse being therefore 1 : 3.36.

The analytical figures obtained in the two experiments are shown in Table VI below:—

TABLE VI.

	Expt. I, 5 grms. refuse			Expt. II, 7.5 grms. refuse		
	Control Mgms.	Stirred for 24 hrs. Mgms.	Stirred for 48 hrs. Mgms.	Control Mgms.	Stirred for 24 hrs. Mgms.	Stirred for 48 hrs. Mgms.
Nitrogen (free ammonia) ...	131.04	109.20	114.66	128.50	105.84	121.38
" (albuminoid ammonia) ...	16.80	5.50	9.20	12.60	11.76	15.12
" (nitrite and nitrate) ...	0.40	3.60	3.60	0.29	0.96	0.18
Total nitrogen in extract ...	155.80	126.00	137.30	145.32	130.20	139.02
Percentage soluble nitrogen taken up						
in solids ...	...	17.9	11.6	...	8.6	0.7
Nitrogen in solids ...	89.37	117.40	107.50	120.40	132.90	121.50
Total nitrogen ...	245.19	243.40	243.80	265.72	263.1	260.52
Loss in " ...	...	1.79	1.35	...	2.62	5.20
Insoluble active nitrogen ...	19.57	26.19	29.97	25.30	29.31	39.64
Percentage insoluble active nitrogen ...	21.83	22.31	27.87	21.01	22.06	32.64

From these figures it will be seen that in Experiment I the effect of stirring in 5 gms. of refuse powder for 24 hours has been to withdraw from solution and fix in the solid portion of the manure 28.03 mgms. N, or 17.9 per cent. of the nitrogen originally present in the extract. Continuation of the stirring for a further period is, however, injurious, nitrogen again passing from the solids to the extract, doubtless due to the action of proteolytic bacteria.

In Experiment II where the percentage of carbohydrate material added was higher, the results were less favourable, only 8.6 per cent. of the soluble nitrogen being fixed in 24 hours while practically the whole of this was again rendered soluble when the experiment was continued for a further period of 24 hours. Experiment II is, however, of considerable value as indicating that the removal of soluble nitrogen is probably not merely a physical process of absorption, for had this been the case the phenomena would be expected to be more marked in Experiment II which contained the greater quantity of refuse powder. Since the reverse is the case a chemical change is suggested. In Experiment I both free and albuminoid ammonia have been removed from solution; in Experiment II (24 hours), however, the albuminoid ammonia is practically unchanged and the loss of soluble nitrogen is almost entirely at the expense of the free ammonia. The loss in total nitrogen is negligible in Experiment I, but tends to increase in Experiment II which contains the higher carbon-nitrogen ratio, as might perhaps be expected.

In order to trace, if possible, the stages through which the changes described above take place, the following experiments were then made:—

100 c. c. portions of activated sludge were measured out in two bottles and to each was added 0.1 gm. ammonium sulphate and 1 gm. CaCO_3 . One bottle was kept as a control while the other received an addition of 0.5 gm. refuse (grass-powder). The volume in each case was made up to 200 c. c. and the mixtures were then stirred for 24 hours. The solids were allowed to settle and a small quantity of the supernatant liquid was removed for the determination of ammoniacal nitrogen, nitrite and nitrate with the following results:—

		A Activated sludge and refuse	B Activated sludge alone (control)
Ammoniacal nitrogen		0.2 mgms.	nil
Nitrous	..	0.1 ..	..
Nitric	..	19.2 ..	20.0 mgms.

These figures show that practically all the ammonium sulphate added had been converted into nitrate. No withdrawal of soluble nitrate

had, however, taken place at this stage. The experiment was therefore continued, nitrogen estimations being carried out daily. It was found that whereas in B (control) the nitrate figure remained unchanged, in A the nitrate rapidly diminished, being reduced to 10.4 mgms. on the second day, 8 mgms. on the third, while on the fourth it had completely disappeared.

From this point repeated alternate additions of ammonium sulphate and of refuse powder were made when it was found that disappearance of nitrate from the solution took place more and more rapidly with successive additions. Finally the additions of ammonium sulphate and refuse powder were made simultaneously and at this stage the whole process of nitrate-formation and disappearance took place within 24 hours.

Analysis of the solutions for total nitrogen indicated that there had been practically no loss of nitrogen throughout the course of the experiment and the whole of the nitrogen added to the solution in the form of ammonium sulphate must, therefore, have been converted into an insoluble form.

As it was likely that the nature of the refuse used might have an important bearing on the rate of removal of soluble nitrogen, a similar experiment was next carried out with three different varieties of refuse, namely (1) grass-powder, (2) paper and (3) vegetable garbage. The method of procedure was similar to that described above, 100 c.c. of activated sludge being used with 0.5 gm. of the refuse and calcium carbonate. Additions of 0.1 gm. ammonium sulphate and of refuse were made on alternate days.

The experiment was continued for 12 days when the nitrogen was determined in both the filtrates and the solid residue. The results are shown in Table VII.

TABLE VII.

	I Grass powder Mgms.	II Paper Mgms.	III Vegetable garbage Mgms.
<i>A. Nitrogen Added.</i>			
In sludge	... 78.01	78.01	78.01
In ammonium sulphate	... 94.44	94.44	74.24
In refuse	... 20.91	nil	12.34
Total supplied	... 193.36	172.45	164.59
<i>B. Nitrogen at end of Experiment.</i>			
In filtrate	... 11.76	17.34	20.20
In residue	... 172.10	152.60	149.80
Total found	... 183.86	169.94	170.00
Percentage loss or gain	... 4.91 loss	1.44 loss	3.29 gain

In all three cases there has been the same removal of soluble nitrogen and fixation of this in the solid residue, grass-powder giving the best results and garbage the worst. In the latter case however there has been no loss of nitrogen, but on the contrary a slight gain, the conditions (carbon-ratio, etc.) possibly favouring fixation of atmospheric nitrogen.

These results indicating the possibility of withdrawing the soluble nitrogen from solution suggest a new method of sewage treatment, the raw sewage being aerated or mechanically shaken with sludge and refuse material. Preliminary small scale experiments on these lines have already been carried out, but further work is required before the results can be usefully discussed. It is obvious that such a method, if practicable on a large scale, would be of the greatest importance from the point of view of nitrogen conservation.

Modification of Activated Sludge.

It will be seen from the experiments already described that when the proportion of carbonaceous matter was high, there was a distinct tendency for loss of nitrogen to occur. Moreover the breaking down of the carbohydrates was in no case very complete. It therefore seemed desirable to ascertain whether it might not be possible to modify the sludge in such a way that it might acquire a more active power of disintegrating cellulose while at the same time retaining the activity of the nitrifying organisms present. In the case of the grass powder, e.g., it was found that while the pectinous matter and the hemi-celluloses were rapidly disintegrated, the cellulose fibres remained more or less intact for a very considerable period.

The effect of adding to the sludge an extract from freshly decomposed refuse, which contained vigorous cellulose-fermenting organisms, was investigated as a preliminary experiment.

Two 100 c.c. portions of activated sludge were taken and to one was added 100 c.c. of manure extract, prepared by the addition of 300 c.c. tap-water to 100 grms. of a mixed manure which had been composting for eight months. The volume in the control having been brought up to the same figure, 0.5 gm. grass-powder was added in each case and both mixtures were stirred for 24 hours. A second addition of 0.5 gm. grass-powder was then made and stirring continued for another 48 hours. The solid matter remaining in each case was then determined with the following results:—

	Dry weight at beginning Grms.	Dry weight at the end Grms.	Percentage loss of dry weight
A. Control. Activated sludge + refuse ...	3.120	2.942	5.71
B. Activated sludge + manure extract + refuse ...	3.245	2.8112	13.37

Considering the short duration of the experiment, the proportion of carbohydrate material broken down is not unsatisfactory and this is obviously very much improved by the addition of the manure extract.

Experiments on a much larger scale to ascertain how far this process can be carried without loss of nitrogen have been instituted and will form the subject of a future communication.

In conclusion it is my pleasing duty to express my sincere thanks to Professor Gilbert J. Fowler for his constant interest and valuable guidance during the course of this work. My thanks are also due to Mr. Y. N. Kotwal for assistance in some of the analytical work.

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