

THE CONSERVATION OF NITROGEN WITH SPECIAL REFERENCE TO ACTIVATED SLUDGE.

by Gilbert J. Fowler, D. sc., F. I. c.

PRELIMINARY NOTE.

The object of the following paper is to bring together in a convenient form the chief data with regard to the * nitrogen question * and in particular to review the evidence both from practical experience and from purely scientific investigation of the possibility of recovering a greater proportion of the value of the nitrogen in sewage for the benefit of agriculture than has hitherto been the case.

The utilisation of the nitrogen in sewage in this way has been the ultimate aim of the writer's scientific activity during some twenty years prior to his present appointment in Bangalore.

Experimental work in connection with this subject is now being carried out under his direction at the Indian Institute of Science, Bangalore, at Jamshedpur and at Shanghai and he is in touch with developments in other parts of the world.

It is hoped that the paper may serve as an introduction and stimulus to research work in these various centres and also be of interest to the general reader, to whom the subject may appeal in its social and economic aspect.

The contents of the paper may be summarised as follows :—

- I. Introduction.
- II. Sources of Nitrogen.
- III. Methods of Sewage Treatment.
- IV. Chemical changes in Nitrogenous organic matter.
- V. Nitrogen Fixation.
- VI. The Nitrogen in Activated Sludge.
- VII. Green manures.
- VIII. General conclusions and problems for research.
- IX. Summary of references.

I. Introduction.

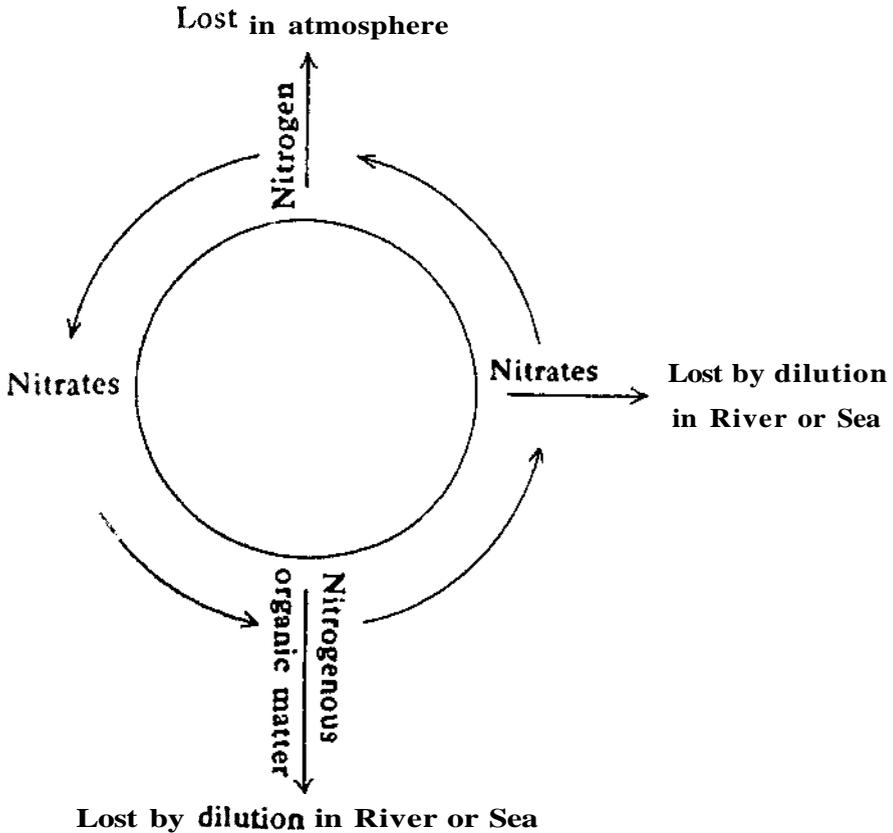
The conservation of nitrogen may be said to be the most important problem confronting the human race. Without an adequate supply of nitrogen the soil loses its fertility and food ceases to be produced. Long before this complete exhaustion takes place however, limited supply will become evident in poverty and in stress of competition which may explode in war.

The whole fabric of intelligent and harmonious human intercourse known as civilisation breaks down completely in absence of adequate food production. The importance of the nitrogen question was brought vividly before the world in an address by Sir William Crookes in 1898 who showed that unless the yield of wheat per acre were increased by suitable fertilisers a time would come when there would be grave difficulty in producing sufficient bread for the bread-eating peoples. The necessity for conserving every form of nitrogenous material capable of being used as manure was thus evident, as well as the great waste of nitrogen generally involved in the water carriage system of sewage disposal.

The present paper is mainly concerned with a discussion of the possibilities of usefully recovering the nitrogen lost in this way. Incidentally, in order to give a more complete picture of the problem under discussion, other methods of nitrogen conservation are referred to, as well as the problems concerned with what is known as "nitrogen fixation".

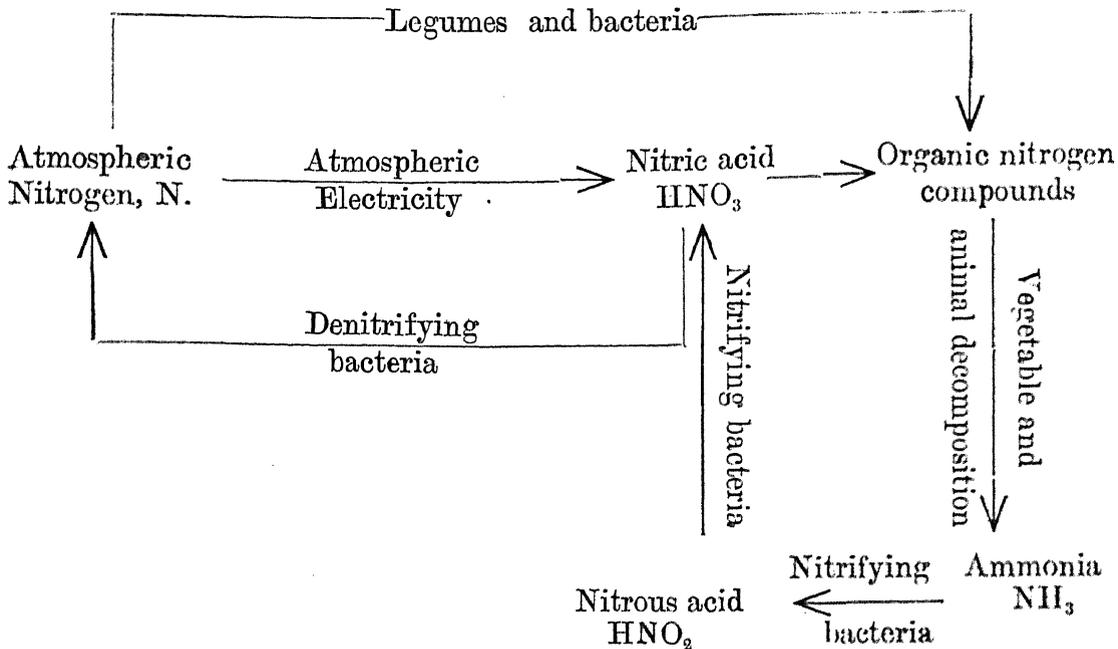
The term nitrogen fixation refers to the conversion of inert nitrogen into useful and available solid products and is the most important link in the chain of transformations known as the *nitrogen cycle* the harmonious operation of which is comprised in the larger problem of the *conservation of nitrogen*.

The possible losses of nitrogen are indicated in the following diagram from which it may be seen that of the nitrogen originally added to the soil as nitrate some may escape ultimately as *sewage matter* passing into river or sea, as nitrate present in sewage effluents and lost in the same way, and as *nitrogen* passing into the atmosphere.



This cycle may quite properly be termed the wheel of life for it will be seen that if the whole of the nitrogen passes off at the periphery life will cease, life is retained in proportion as the nitrogen is retained and is more intense in proportion as the cycle of changes takes place rapidly or slowly or as it might be expressed as the wheel revolves with greater or less rapidity. This appears in practice in the fact that land which is intensively cultivated will support a larger population per acre than where primitive methods prevail. The problem of the conservation of nitrogen will be solved when the whole cycle is so far under control that adequate food supply can be assured for any desired population.

The actual processes by which these broad changes are brought about and the chief methods by which escape of nitrogen can be prevented or by which it can be recovered even if it has escaped are indicated more specifically in diagram No. 2.*



It is difficult to think of a subject of study fuller of interest and importance than the cycle of changes set out in this diagram. The problems it presents are of the most varied description comprising virtually all the biochemical activities of animals and plants and bacteria and the comparison of these with purely chemical processes for bringing about some of the same transformations. The goal of a thorough understanding of the nitrogen cycle is unlimited food supply and peace and plenty for a race of men who no longer should need to earn their bread in "the sweat of their brow".

II. Sources of Nitrogen.

In diagram 2 it will be seen that there are three main forms in which nitrogen occurs in nature, others being intermediate products. These main forms are :—

1. Atmospheric nitrogen.

*Professor P. H. Guye quoted on p. 11 of 'Utilization of Atmospheric Nitrogen' by Thomas H. Norton.

2. Nitrates.
3. Organic nitrogen or nitrogen combined in complex organic substances the immediate products of animal or vegetable life.

These various sources of nitrogen may now be considered from the point of view of their quantity and availability. The maximum world consumption of fixed nitrogen up to the present may be taken at 1,300,000 tons per annum, this being a war time figure.

1. ATMOSPHERIC NITROGEN.

The volume of air over each square yard of the earth's surface contains 5·8 tons of nitrogen and the nitrogen above 1 sq. mile of land amounts therefore to 20,000,000 tons or enough to supply the world's present requirements for 20 years. Of this amount only a very small fraction *viz.* about 0·000002 is actually in circulation among animals, plants and bacteria.

It is obvious therefore that in the atmosphere, there is a reservoir of nitrogen of unlimited extent provided it can be economically brought back into the nitrogen cycle.

2. NITRATES.

In 1917 the annual output of nitrogen as nitrate of soda (Chili salt-petre) from the Chilian deposits was 380,000 tons and it is believed that this could be increased to a maximum of 500,000 tons.

The annual production of nitrogen as Indian saltpetre (potassium nitrate) is only small *viz.* 2,800 tons in 1917 but its potash content makes it of special value as a fertiliser.

3. ORGANIC NITROGEN.

(a) *Nitrogen stored in coal, peat and river-ilt.*

The nitrogen of ancient vegetation either of past or recent geological periods can be recovered as ammonia by various processes of distillation from the above materials.

The nitrogen contained in the actual annual coal output of the world amounts to 11,000,000 tons, of this only 450,000 tons is recovered as ammonium sulphate, which emphasises the importance of by-product recovery from coal.

The recovery of ammonia from peat has not yet attained large proportions, though enough is present to justify

recovery. One ton of dry peat contains 1% nitrogen equivalent to 80 lbs ammonium sulphate per ton.

The mud of many rivers and estuaries contains much vegetable debris, such deposits in fact being comparable to some extent with those from which the coal measures were originally formed. Deposits of this kind occur in Germany and may contain from 2 to 4 % of nitrogen.

From the point of view of agriculture, nitrogen as sulphate of ammonia is practically almost as useful as nitrogen in the form of nitrate as there is evidence that nitrogen in the form of ammonia can be absorbed by plants and in any event it is converted into nitrate by the nitrifying organisms in well aerated arable soil.

For the production of nitrates for explosives, or other technical as distinct from agricultural uses, the ammonia can be readily oxidised to nitric acid by various catalytic processes to be referred to later, or even possibly by suitably developed bacterial action.

(b) *Slaughter house refuse.*

This is a highly nitrogenous material consisting mainly of the entrails of animals and is sometimes put on to land direct, or is first "rendered" down to recover fat and the residue worked up into forms convenient to handle. The bulk of the blood is recovered as such and either used for various food preparations, or dried down and used as a fertiliser. The general washing down liquors which are highly nitrogenous and liable to be very offensive are generally sent into the nearest sewer, if such exists and become part of the town sewage.

(c) *Fish meal.*

This is an important source of nitrogen as there is much waste fish material at most fishing centres which can be properly used in this way. Moreover as will be shown later the nitrogen which exists as nitrate in sewage effluents and which may readily become unavailable by discharge into river or sea can be specially conserved in the form of fish and so brought back into circulation. No doubt much of the nitrogen escaping into river and sea does actually come back in this way in the long run, but the process is capable of being controlled.

An average fish-meal contains the following percentages of important constituents :—

Nitrogen	9
Phosphoric acid P_2O_5	7
Oil	10

If fed to cattle the oil is utilised and the nitrogen and phosphates conserved in the dung or in the bones both of which can be used as a fertiliser.

(d) *Animal debris, hair, leather, hoofs, horns, &c.*

These are sometimes used directly as a fertiliser but some form of preliminary treatment to render them more “available” is to be recommended. Thus by the action of high pressure steam, horns and hoofs become friable and capable of being readily powdered and consequently easily mixed with the soil. Under such circumstances these otherwise intractable materials form a quite useful fertiliser.

(e) *Green manures.*

These will be referred to later when considering the subject of nitrogen fixation.

(f) *Oil Cake.*

The cake remaining after pressing the oil from the various oil seeds of commerce always contains an appreciable amount of nitrogen.

The figures for a number of Indian oil seeds are given below, the determinations having been made by Mr. S. R. Bhate, B. Sc. one of the writer's former students, partly at the Indian Institute of Science and partly at the Government Industrial Laboratory, Hyderabad, Deccan.

Description of oil seed.	Nature of cake.	Per cent nitrogen.
Bassia Latifolia	Pressed	3.20
Castor—Mahboobnagar	Pressed	5.60
” ”	Solvent extracted] 1.50
” Aurangabad	”	9.72
” Hyderabad	”	10.66
” Italian	”	9.76
Ground nut	Pressed	6.85
Safflower	”	2.40
	Solvent extracted	9.28

It will be seen that the percentage of nitrogen is appreciable in all cases and becomes considerable if the oil is thoroughly extracted from the cake by means of solvents.

It may sometimes however be found preferable to obtain the value of the oil as well as the other food material of the cake by feeding to cattle when the nitrogen will be also available, a large proportion of it at any rate, as solid and liquid manure.

Owing to the export of oil seeds from India, much of this nitrogen is lost to Indian agriculture, and the question is receiving the careful attention of Indian Agricultural advisers. It has been suggested that measures to limit the export of oil seeds would be advantageous.*

(g) *Animal manures.*

The proper conservation of animal manures, *i. e.*, the dung and urine of horses, cattle, sheep, and goats is one of the most important farming problems.

True economy would urge that all should go back to the soil, but in practice there is loss of nitrogen through improperly controlled fermentation in the manure heap and in many cases complete loss of the urine under the belief that it cannot be satisfactorily used for manurial purposes.

This belief has been shown to be erroneous by experiments by Prof. Kendrick of Aberdeen and by trials carried on at the College of Agriculture, Holmes Chapel Cheshire, by Mr. (later Major) and Mrs. Mumford and Messrs Gaul and Chamney, under the general supervision of the present writer (The effect of liquid manure on crops—Special reports to Board of Agriculture and Fisheries, 1915, 16 and 17.) The nitrogen in liquid manure exists mainly as hippuric acid and laboratory experiments showed that the free acid is incapable of nitrification. The calcium salt however nitrifies readily and consequently if lime is present in the soil or if it is added to the liquid manure in adequate but not excessive amount, this material yields very good crops and there is certainly no warrant for allowing it to run to waste.

In India the use of cattle manure is hampered by the frequent shortage of wood fuel which necessitates the burning of the cowdung instead of its return to the soil. The importance of the agriculturist having a ready access to cheap fuel wherever

* R. V. Norris—"Note on the Exhaustion of Indian Soils"—"Agricultural Journal of India" Vol. XV, July 1920, p. 433.

possible has been pointed out by Voelcker (Improvement of Indian Agriculture Chapter VII.)

Cowdung or other animal manure should be well rotted (fermented) and reduced to fine powder before use so as to increase the availability of the nitrogen and prevent its loss by prolonged storage of the manure in the soil. The common method of the Indian *mahli* (gardener) is to powder his manure, and make a heap of alternate layers of manure and soil, which is allowed to stand for some weeks before being brought into use, when the earth and manure are well mixed and brought to the place where they are to be used. This method is scientifically sound as the nitrogenous matter have time to decompose and even partially to nitrify before being actually brought in contact with the plant. Of course loss of soluble matter by rain has to be guarded against.

An enormous deposit of manure exists in the form of guano or bird excrement on certain islands belonging to Peru. The annual production amounts to about 100,000 tons of which 14,000 is nitrogen. A fresh deposit estimated at 10,000,000 tons has recently been discovered on an island opposite Campeche, Mexico.

(h) *Town sewage.*

In order properly to appreciate the nitrogen value of town sewage certain fundamental physiological data have to be considered,

Various data are given by different authorities.

Rubner gives 1.4 grams nitrogen per capita as faecal nitrogen. (Richet, Dictionnaire de Physiologie).

Metcalf and Eddy—(American Sewerage Practice, Vol. III, p. 153) give the following figures :—

Fæces. Dry solids 20.5 grams per capita per day.
Fæcal nitrogen. 0.9 gram per capita per day,
Urine nitrogen. 7.0 grams per day.

The total amount of material to be dealt with in grams per capita per day apart from water and general household waste, *i. e.*, on an efficient dry conservancy system is as follows :—

Fæces	...	90
Urine	...	1170
Paper	...	20

In American *sewage* the daily amount of nitrogen in per capita is as follows :—

As free ammonia	...	8·0
Other nitrogen	...	7·0

(Metcalf and Eddy table 52, p. 194, Vol. III).

Indian latrine *sewage* does not average more than approximately 2 grams nitrogen per capita daily but a large proportion of the urine fails to be included.

Taking Rubner's figure of 1·4 grams nitrogen per capita daily as *foecal nitrogen i. e.* rather more than the figure 0·9 given as foecal nitrogen by Metcalf and Eddy but less than that given by them for organic nitrogen ; it follows that a population of 1,000,000 people will produce 1·4 tons approximately per day or about 500 tons per annum.

The purely *foecal* nitrogen however if recovered amounts to a large proportion of the world's needs as is shown by the following table, the totals being arrived at by multiplying the millions of population in eight of the chief countries of the world by 500.

Name of Country.	Millions of inhabitants.	Tons of nitrogen produced per annum.
China	400	200,000
India	800	150,000
Great Britain	45	22,500
Japan	40	20,000
Germany	60	80,000
Austria	50	25,000
France	40	20,000
U. S. A.	100	50,000
		500,000

If to this the nitrogen in solution (urine nitrogen) is added we get a total of 4,000,000 tons or more than three times the total world consumption of nitrogen viz., 1,300,000 tons per annum.

It has been estimated that the total production of solid and liquid excreta by the world's population represents annually about 8,000,000 tons of nitrogen and Dr. E. J. Russell estimates

the annual value of the sewage nitrogen of Great Britain alone at £18,000,000.*

The chief cities of the world containing over 500,000 inhabitants represent a population of 35 millions which is equivalent to 17,600 tons nitrogen in the solid form and 122,500 tons in solution per annum,

In a recent paper on the Nitrogen in Sewage by Dr. McGowan (Surveyor—May 7, 1920, p. 405) published since these calculations were made, the following figures are given on the authority of Colonel M. Flack whose data are given in detail at the end of Dr. McGowan's paper.

Daily nitrogen in urine :—

Man	16	grams.
Woman	13	„
Child	8	„

Daily nitrogen in fœces :—

Average	2	grams.
---------	---	--------

According to these figures the average daily total nitrogen produced per million inhabitants in the United Kingdom is :—

Nitrogen in urine 12 tons

„ fœces 2 „

These figures are higher than those on which the calculations in the present paper are based, so that it may be taken that the error, if any, lies in under-estimating the value of the nitrogen in human excreta.

A striking proof of the value of this nitrogen is the fact that the city of Shanghai is paid £40,000 per annum by contractors for its nightsoil, which will include a part but not the whole of the urine of the population.

In addition to this large payment the contractors maintain an army of coolies and a fleet of nightsoil boats.

By the courtesy of Mr. C. H. Godfrey, M. Inst. O. I. Commissioner of Public Works, Shanghai, the author is able to

* The work of the Rothamsted Experimental Station from 1914 to 1919. (Journal of the Board of Agriculture Vol. XXVII No. 5 cf. also Agricultural Journal of India Vol. XV page 1 to 69.)

publish a series of photographs showing the methods employed for collecting nightsoil in Shanghai and applying it to agriculture. Briefly it is removed in buckets from the houses, dumped into barges and taken into the country and supplied to individual agriculturists who first allow a certain amount of fermentation to take place in large "kongs" before carefully applying the manure to the growing crop.

There can be no doubt that the fundamental economic stability of China depends on the universal use of human excreta for manure and the same is true in large measure of Japan.

The whole process however, though sound scientifically and economically, is of a very unsavoury description and the problem presented is* to obtain the same economic results without offensive accompaniments.

III. Methods of Sewage Treatment.

Before considering in detail the possible solution of this problem, the chief available methods for purifying ordinary water-carried sewage may for convenience be briefly indicated.

They are as follows:—

1. Direct treatment upon land.
2. Preliminary treatment by :—
 - (a) Sedimentation tanks
 - (b) Chemical precipitation tanks
 - (c) Septic tanks
 - (d) Slate beds.

followed by final treatment on land or artificial filters.

3. The Activated Sludge Process.

1. *Direct treatment upon land.* This is only successful where large areas of land of suitable quality are available and where rapidly growing crops can be cultivated. Even so there is great liability to nuisance owing to the difficulty in distributing such a liquid as raw sewage equally over the land. Local deposits of solid matter tend to form and to give rise to nuisance. As will be pointed out later there is likelihood of considerable loss of nitrogen when sewage is utilised under conditions so little under control as are afforded by direct land treatment.

*Of Farmers of Forty Centuries by F. H. King, D. Sc. published by Mr. F. H. King, Madison, Wis. 1911.



Plate I. A coolie collecting ordure from an alley way. The 'whisk' in his right hand is used to clearout the commode pans.



Plate II. A typical coolie with buckets slung from a bamboo.



Plate I. A coolie collecting ordure from an alley way. The 'whisk' in his right hand is used to clearout the commode pans.



Plate II. A typical coolie with buckets slung from a bamboo.

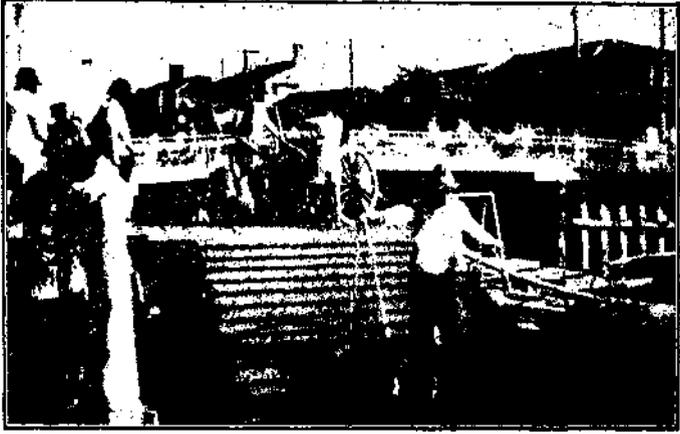


Plate III. In foreground a coolie bringing his buckets to the boats. The cart on wheels is a wooden tank used for the same purpose. It is just being tipped up for discharging.

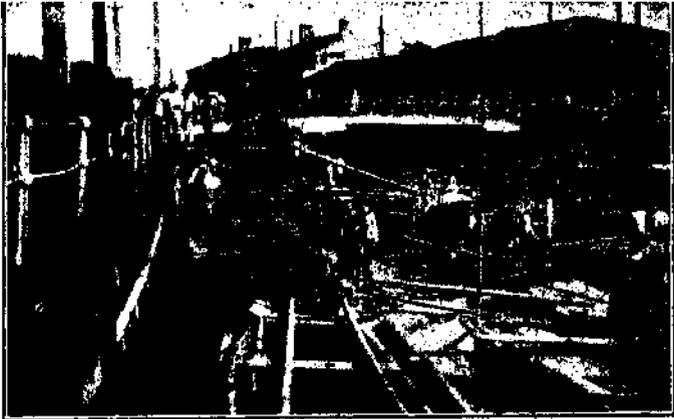


Plate IV, A typical loading Station.

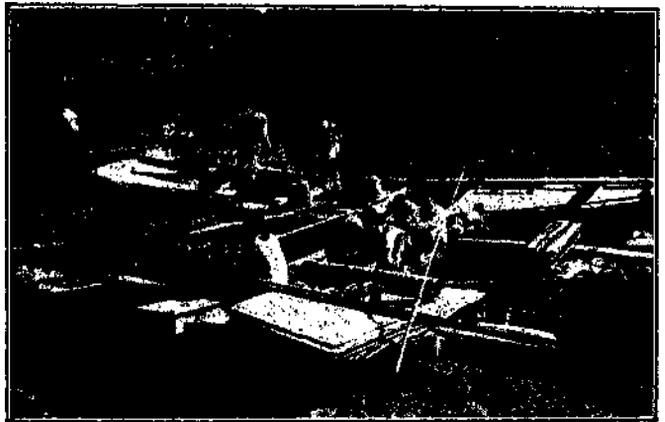


Plate V Coolies bringing buckets to the boats. Note the
& VI rice bowls set out on the middle boat. In the
second picture the four coolies are seen eating
their meal.

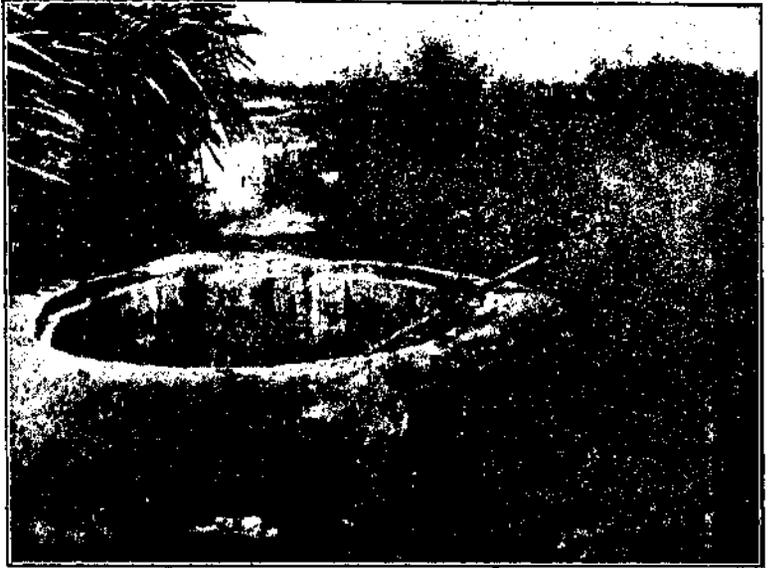


Plate VII. A "kong" or vat for storing nightsoil. It is kept until sufficiently ripe before being ladled out and applied to the crops.



Plate VIII. A typical garden showing position of "kong" (on extreme left) in relation to crops.



Plate IX, A coolie applying the fertilizer to a bed of young cabbages.

Moreover the sewage normally contains its manurial constituents in such highly dilute solution that in temperate or humid climates at any rate the addition of adequate manurial value involves the application of excessive quantities of water.

2. Preliminary treatment by any of the processes (a) (b), (c) and (d) results in an effluent containing greater or less amounts of suspended and colloidal matter and in which the nitrification of the organic matter in solution has not as a rule begun.

The *sedimentation* tank retains only the heavier matters in suspension.

Chemical treatment according to the quantity of chemicals used, and the care taken in their application, removes in addition a greater or less proportion of colloidal matter.

In the *septic tank* anaerobic fermentation takes place, cellulose material yields marsh gas and hydrogen, and albuminoid matter breaks down with production of ammonia compounds and under certain conditions with loss of gaseous nitrogen.

In none of these three processes is there usually more than 2 to 3 per cent of nitrogen in the sludge, as is seen in the following

TABLE,
Percentage of Nitrogen in various types of sewage sludge.
(Calculated on sludge dried at 100°C)

Source of sludge.	Percentage of Nitrogen.
<i>Sedimentation tank.</i>	
Manchester—England ...	3.32 ¹
Lawrence — Mass. ...	2.37 ¹
<i>Chemical precipitation.</i>	
Dorking — England ...	0.89 ²
Glasgow — Scotland ...	1.30 ³
<i>Septic tanks.</i>	
Manchester—England ...	2.3 ¹
Lawrence — Mass. ...	2.3 ¹

1. Fowler & Clifford, Residual Products from Sewage—J. C. S. I. Vol. 33, 1914 pp. 815—19.

2. Brenchley & Richards "The Fertilising Value of Sewage Sludges" J. C. S. I. Vol. 39, 1920, pp. 177—182 T.

3. From analysis of Globe Fertiliser quoted by Metcalf and Eddy, *loc cit*

Source of sludge.	Percentage of Nitrogen.
<i>Slate beds.</i>	
Harpden—England ...	2·6 ²
Devizes — „ ...	3·6 ¹
<i>Humus tanks.</i>	
Lawrence — Mass. ...	1·4 ¹
<i>Activated Sludge Process.</i>	
Withington—Manchester, (England).	7·09 ²

In the *slate bed* the sewage is held in contact with layers of slate some two inches apart on which the grosser suspended solids deposit. On discharging the sewage, the deposit is left exposed to the air, and numerous forms of organic life develop constituting what has been described as a “living earth” which transforms the organic deposit into inoffensive products, which have a fairly high nitrogen content and valuable manurial qualities.

The effluent as has been stated still requires final oxidation.

The most frequent method of finally purifying the effluents from the various preliminary tank treatments is by application to so called percolating or ‘trickling’ filters. These consist of heaps of material such as slag, gravel, clinker &c. suitably sized and underdrained over which the effluent is sprayed by various forms of sewage “distributors” .

In course of time growths of the requisite organisms form on the material of the filter and the soluble nitrogenous matter and ammonia still present in the effluent are finally oxidised to nitrates. Colloidal and other substances become changed to a granular deposit and are discharged as so called “humus” to be retained in final “humus tanks”. This material is inoffensive and has a certain value as manure though its nitrogen content is not high, seldom exceeding 2 per cent. As will be explained later much nitrogen is lost by various interactions taking place in the filters.

(3.) *The Activated Sludge Process.* The activated sludge process differs from any of the above methods in being practically

1. Fowler & Clifford, Residual Products from Sewage—J. C. S. I. Vol. 33, 1914 pp. 815—19.

2. Brenchley & Richards “The Fertilising Value of Sewage Sludges” J. C. S. I. Vol. 39, 1920, pp. 177—182 T.

a single operation process and one which entails no loss of nitrogen. The sewage is purified in specially constructed tanks by the direct injection of air in a finely divided state, in presence of so called "activated sludge".

The activated sludge process was originally suggested by the present writer and was developed by him in Manchester with the assistance of his scientific staff.

If air is allowed to bubble through a sample of sewage a number of changes progressively take place. The first to be noticed is the gradual conversion of the colloidal matter, which gives the turbid appearance to the sewage, into brown granular particles which readily settle leaving a sparklingly clear liquid. In course of time nothing is left in solution but mineral salts, viz chlorides and nitrates.

This complete oxidation of sewage by the action solely of air in conjunction with the bacteria originally present in the sample of sewage, occupies a considerable time, possibly many days, and for this reason purification of sewage by aeration alone has long been deemed impracticable. It has been found however that if the brown granular deposit which forms is allowed to settle out from the liquid and the latter decanted away and a further quantity of sewage aerated in contact with the brown deposit and this process repeated, the brown deposit being retained at the end of each operation, then as the quantity of the deposit increases the time required for the purification of the sewage decreases. Finally when the deposit has accumulated to the extent of about a quarter of the total volume of the sewage, the latter can be purified in a few hours time and the process becomes a practical proposition. The brown deposit has been termed 'activated' sludge.

For the practical carrying out of the activated sludge process forced aeration is of course necessary and the sewage needs to be freed from heavy detritus and from floating solids, that is to say, it, requires in general to be passed through a system of catch pits and screens before the aeration process.

Briefly the process is carried out in practice as follows :— The screened sewage passes into a long narrow aeration tank into which air is forced in a state of fine division. It has been found that this fine division is necessary for the sake of economy of air and it is effected by the use of what are known as *diffusers* of porous material through which the air is forced creating a fine emulsion of air and sewage. The effluent passing away at the end of the aeration tank is purified but contains, of

course, activated sludge in suspension which must be separated and returned to the inlet of the tank. The aeration tank is, therefore, followed by a *settlement tank* in which the activated sludge rapidly settles out, and from which the clear and purified effluent passes. The deposited activated sludge can be lifted from the bottom of the settlement tank by means of compressed air either back to the inlet of the aeration tank or out of the system altogether on to sludge drying beds, from which it can be removed and used as manure. Any surplus, over and above the 25 or 30 per cent of the volume of the tank which is necessary to effect purification, is thus removed from time to time. The whole installation occupies very little space. The sewage *e. g.* of 100,000 people can be purified in tanks holding a million and a half gallons, that is a space of 300 ft. by 100 ft. by 8 ft. In addition, of course, building? will be required to accommodate machinery for air compressing and possibly sludge pressing and drying.

To purify under European conditions the same quantity of sewage to an equal extent, by means *e. g.* of septic tanks and percolating filters, would require approximately double the tank space and in addition 6 acres of percolating filters 6 feet deep.

It has been found that not only is there no loss of nitrogen in the process but that there is an actual gain, as there are discovered to be present in the sludge not only *nitrifying* organisms converting nitrogenous matter into nitrates, but also *nitrogen fixing* bacteria which are able to catch and retain nitrogen from the air. The sludge contains 5 to 7 per cent of nitrogen as compared with 1 to 3 per cent from the sludge produced by the processes of purification already referred to. It thus affords the possibility of recovering and utilising the material value of sewage which is very often entirely lost, as has been stated; when the water carriage system is adopted.

The results obtained from the activated sludge process in operation at Withington, Manchester, dealing with a dilute and purely domestic sewage show a yield of about 500 tons per annum of nitrogen per million inhabitants a figure almost identical with that given earlier in the paper for the faecal nitrogen produced by that number of people.

It would appear therefore that the activated sludge process succeeds in recovering all the faecal nitrogen in the sewage. The urine nitrogen passes away without loss in the effluent. If the nitrogen in the effluent as well as in the sludge • can be profitably used for agriculture, it is evident that the world's nitrogen requirements, can be met without sacrifice of

sanitary or aesthetic requirements, the crowded populations of towns and cities producing manure for country districts, which in turn supply food for the towns.

Before deciding how far this claim is valid it will be necessary rather more closely to consider the chemical changes involved in this and other processes of sewage purification, and also to examine the needs of the growing crop and determine how far they are met from sources at present available.

We have next in fact to consider the lower right hand portion of Diagram II.

IV. Chemical changes in Nitrogenous Organic matter.

Organic nitrogenous manures when dug into the soil suffer a series of changes due to the activity of soil bacteria, resulting first in the breaking down of complex albuminoid substances with production of ammonia and the final oxidation of this to nitrous and nitric acids. The latter process is known as "nitrification" and is accomplished by two organisms one which converts ammonia into nitrous acid and another which finally oxidises the latter to nitric acid. In both cases it is necessary for a base such as lime to be present to neutralise the acids as they are formed. The nitrogen in an organic manure is said to be more or less "available" in proportion to the readiness with which it is converted into these simpler bodies. At the same time such complex nitrogenous manures yield a substance called "humus" to the soil which seems to be necessary for the satisfactory growth of plants.

Activated Sludge as Manure.

Activated sludge has been found by the pot experiments of Bartow and Hadfield at the University of Illinois to give better results with wheat than such nitrogenous fertilisers as (dried) Hood, nitrate of soda, sulphate of ammonia and gluten meal. These results have been broadly confirmed recently by field experiments by Axden in Manchester* working on lines suggested by Dr. Russell of Rothamsted, and by the pot experiments of Brenchley and Richards at Rothamsted. (*loc Cit.*)

In a paper read in 1918 before the Buffalo Convention and printed in the proceedings of the American Society for Municipal Improvements, Bartow and Hadfield recommend that activated sludge be put on land one month before seeding. They state that activated sludge is less toxic than dried blood and that $1\frac{1}{2}$ tons

* Journ. Soc. Chem. Ind. Vol. XXXIX No. 5, 1920 pp 60-63 T.

per acre is the least quantity to use. Experiments by Nasmith & McKay of Toronto have shown quite remarkable effects in increase of yield compared with ordinary farmyard manure, as is shown by the following table :—

Plant used.			Increase in yield over Standard barnyard manure. Per cent.
Radishes	40
Lettuce	103
Beans	77
Beets	138
Late radishes	316
Tomatos	291
Carrots	no increase.
Onions Spanish	191
Weatherfield	554
„ Danvers yellow globe	87

This table has been given in extenso as it shows the extraordinary variation in effect as between different crops. There is evidently an important field for study here. Why for example is there no effect upon the yield of carrots and why should different species of onions vary so greatly in responsiveness? Obviously if the yield of beans and beets could be increased to the same extent as of Weatherfield onions, it would mean a very important increase in valuable foodstuffs.

These various published results have been confirmed by numerous private observers and mention may be made of the excellent effect of activated sludge on certain flowering plants *e. g.* sweet peas, lilies and hydrangeas, the increase in the size of the plants and the quality of the blooms being very marked over control plants,

It is evident that activated sludge is a valuable manure in itself. If the nitrogen in sewage is to be fully utilised however means must be found for conserving the nitrogen dissolved in the effluent as ammonia and as nitrate. Of course if used for irrigation such an effluent would be of great value and the dissolved nitrogen would be readily taken up by plants. In dry tropical countries this will generally be found to be the best plan and as the effluent is clear and odourless, a sewage farm will run no risk of becoming a centre of nuisance. In countries where irrigation is not so necessary or practicable or during the rainy season in

tropical countries, some other means for conserving this nitrogen must be found. This can be attained through the medium of fish.

Fish-ponds. At the Berlin sewage farms there are large ponds into which partially purified sewage flows and in which blue carp are raised in large quantities and after being transferred to clean water are afterwards sold for food. At many centres in Austria the process of "manuring" fish ponds with sewage is frequently resorted to. According to King (*loc cit*) such fish culture is practised in China in both deep and shallow basins. The shallow basins are only used for fish in the rainy seasons, being drained and planted during the dry season. In these cases however the pond has to be formed of fresh water and only a limited quantity of sewage can be admitted *viz.* such as can be oxidised without a serious drain on the dissolved oxygen of the water or without the formation of appreciable deposit. Consequently a natural pond must be already in existence or the necessary dilution water must be provided from other sources. The effluent from a successfully operated activated sludge plant is perfectly clear and incapable of forming a deposit, can be easily saturated with dissolved oxygen and is almost free from sewage bacteria and consequently can be used directly for the production and maintenance of fish ponds or tanks. By careful cultivation of aquatic vegetation and accompanying microflora and fauna ideal conditions for fish life are created and fish growing and living under such conditions should be suitable for food if cooked, and in any event should be valuable sources of nitrogen and phosphorus for manure and even possibly of oil in the case of certain species.*

Intensive nitrification. The very rapid nitrification produced by activated sludge suggests the possibility of an economic conversion of the ammonium salts, produced by various processes from coal, into nitrate which can then serve as a source of the nitric acid necessary for manufacturing chemistry as apart from agriculture.

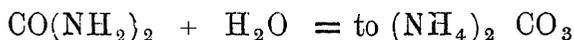
The experiments of Muntz and Lainé where nitrogenous matter was oxidised on a nitrifying bed of peat showed that the nitrifying organisms operating at a temperature of 30°C were active up to a concentration of 22% of nitrate although they will not survive in a concentration of more than 1% of ammonium salts. (Norton, Utilisation of atmosphere Nitrogen p. 44 -- 5.)

* It is evident that the scientific method for "precipitating the ammonia and nitrate nitrogen from sewage liquors" looked forward to on p. 312 of the Final Report of the Nitrogen Products Committee, recently published, is found in the operation of such a life-cycle.

Experiments are in progress at the Indian Institute of Science to determine what concentration of nitrate can be obtained by the use of activated sludge and if an economic reaction is indicated to decide how best it can be applied in practice.

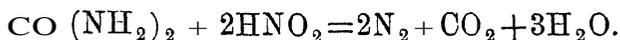
Loss of nitrogen. There are numerous ways in which nitrogen is lost *i e.* rendered unavailable in the course of the changes involved in the nitrogen cycle. In its application either in the form of organic nitrogen, ammonia or nitrate to agriculture loss of nitrogen occurs through various processes of reduction, known generally as *denitrification*.

There is evidence that under certain conditions free nitrogen may be evolved during the anaerobic decomposition of complex organic matter. The most frequent change however is a conversion of organic into ammoniacal nitrogen the simplest case of which is the ammoniacal fermentation of urea according to the following equation :—

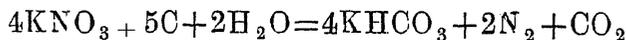


This and other analogous changes take place in the septic tank process of sewage treatment and result in a decreased percentage of nitrogen in the sludge and an increase of ammonia in the effluent. This is seldom converted quantitatively into nitrate even in very efficient percolating or oxidising filters owing to denitrification changes either of a purely chemical or of a biochemical character.

The first is represented by such an equation as the following :—



The biological changes may sometimes be due to direct reduction of nitrates by carbon containing substances according to the following general equation :—



but more probably as was shown by Hulme working in the Frankland laboratory in Manchester under the writer's direction (J. C. 8. 1914 Vol. 105, p. 623) with solutions containing dextrose, peptone and nitrate, nascent hydrogen is evolved by the organism acting on the carbohydrate, and the nascent hydrogen reduces nitrous acid to nitrogen according to the equation :—



It was found at any rate that so long as nitrate was present in solution nitrogen was evolved, but as soon as the nitrate was exhausted hydrogen only appeared. The initial reduction of nitrate to nitrite was shown to be partly chemical, due to the interaction of the nitrate and dextrose, and partly due to a bacterial reduction apparently through the intervention of a reducing substance of the nature of an extra cellular enzyme.

Similarly if sewage is applied direct to land much nitrogen is lost through a sequence of changes of this character.

Further research work is still to be done on the exact chemistry of these changes. It has been shown by Beesley working under the writer's direction (J. C. S. Trans. 1914, Vol. 105, p. 1014) that even in the apparently simple oxidation of ammonia to nitrous acid there is an intermediate stage in which hydroxylamine is formed. Nitrification is doubtless a process of progressive hydroxylation of ammonia.

The following substances were found by Beesley to nitrify practically at equal rates :—

Urea, uric acid, asparagine, glycine, methylamine, acetamide, ammonium oxalate, ammonium sulphate.

In these experiments Beesley employed dilute solutions of the various substances in presence of suitable mineral nutrients, inoculated with nitrifying organisms and allowed them to stand for many days, samples being taken at fairly long intervals, aeration was carried on for a few minutes only every week.

Much more rapid nitrification of these substances can be brought about by activated sludge and a number of experiments were made by Mrs. Mumford to trace the course of the changes taking place (Reports to Board of Agriculture *loc cit*).

For these and other laboratory experiments where the effect of activated sludge upon sewage on other substances has to be studied and fairly large volumes are employed the sludge is suspended in water contained in an earthenware cylinder with a porous false bottom through which air can be forced under pressure in small bubbles. Such a cylinder is known as a "diffuser" * The general appearance is seen in the diagram (facing p. 269) given to illustrate experiments with activated sludge and growing plants referred to later.

*The terra 'diffuser' is also applied to the porous plate and the iron box in which it is mounted, of which large numbers are used as a means of injecting finely divided air into activated sludge tanks.

To the liquid can be added any substances on which the effect of the sludge is to be examined. The sludge being kept in constant motion by the stream of air bubbles exposes a constantly changing surface and an intensive bacterial action results.

Urea, hydroxylamine, glycine, asparagin, uric acid and hippuric acid were all readily nitrified by activated sludge in presence of sufficient calcium carbonate to neutralise any acidity produced and in most cases no appreciable loss of nitrogen took place in so far that the greater part of the nitrogen originally present in solution was recovered as nitrate. An exception occurred in the case of hydroxylamine and in one case with ammonium sulphate loss of ammonia took place when an excess of calcium carbonate was added to the solution.

No analysis was made of the sludge before and after the experiments so that no conclusion can be drawn from them as to the possible simultaneous fixation of nitrogen referred to later (page 256 *et seq.*).

The experiments both of Beesley and Mrs. Mumford confirmed the work of Munro (J. C. S. 1885, 49 p. 632) as to the nitrifiability of most organic nitrogen compounds containing amino groups. It is curious however in view of the ready oxidation of urea, and of ammonium sulphocyanate (*cf* Fowler, Ardern and Lockett, J. S. C. I. Vol. 30, 1911, p. 176) that the experiments both of Beesley and Mumford showed that thio-urea was entirely unaffected under similar conditions.

The case of aniline is also of interest as indicating the possible course of oxidation of proteid decomposition products containing a benzene ring. Beesley found that although change took place under the influence of bacterial action, resulting in the production of ammonia no nitrification occurred during the period of the experiment. It was assumed that simple hydrolysis took place resulting in the production of ammonia and phenol but that the latter is destroyed by bacterial action as soon as formed†, as it could not be detected in the solution.

Where loss of nitrogen occurs in the course of such changes as were studied in the researches above described it is probable that unstable intermediate products are formed, which may interact with loss of free nitrogen. For the complete study of these reactions it is necessary that all possible intermediate products should be prepared in a pure state and their purely chemical

†: *Of* Fowler, Ardern and Lockett Proc. Roy. Soc. Series B. Vol. 83 194, p. 149,

interactions studied as well as their biological oxidation either directly or through the process of reduction of nitrates.

In any event, whatever the exact reactions may be there is undoubtedly a large percentage loss of nitrogen from manure heaps, from the operations of agriculture and from most artificial methods of sewage purification, with the exception, as the above experiments indicate, and as will be further shown later, of the activated sludge process.

V. Nitrogen Fixation.

In order to bring back this lost nitrogen into the cycle and render it once more available various processes of nitrogen fixation occur in nature or can be artificially induced or regulated. These may be roughly classified as:—

- (i) Electrical
- (ii) Thermo-electrical
- (iii) „ chemical
- (iv) Bio-chemical.

Only brief reference need be made for the sake of completeness to the first three types of process, although large industrial plants of great importance technically and commercially have been and are being constructed and operated.

The following data are taken from a paper on 'The Present Status of Nitrogen Fixation' by Alfred H. White in the *Journal of Industrial and Engineering Chemistry*, March 1919, p. 231.

I. *The Arc process.*

In this process the oxygen of the air is caused to combine with nitrogen in a high tension electric arc with formation of oxides of nitrogen which are afterwards absorbed by water to form nitric acid of 33% strength. There is some discussion as to whether this process should be classed as a purely thermal or as an electrical process, but the balance of experimental evidence appears to be in favour of considering it as mainly electrical.

It consumes 10·5 H. P. years of electrical energy per ton of nitrogen fixed as nitric acid per annum.

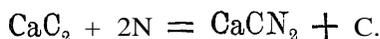
A certain amount of nitrogen is fixed in this way doubtless by lightning, and returned to earth in the rain during thunderstorms.

The following may be described as thermo-electrical processes as the high temperature they need is obtained by means of the electric furnace.

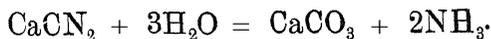
II. *The Cyanamide process.*

This process comprises the following stages:—

- (i) Calcium carbide is formed by heating lime and coke in an electric furnace.
- (ii) Calcium carbide and nitrogen at a red heat produce calcium cyanamide according to the following equation :—



- (iii). Calcium cyanamide is decomposed by steam under pressure to form ammonia thus :—



- (iv). The ammonia is oxidised by air and steam in presence of a catalyst to form nitric acid.

2·5 H. P. years are required per ton nitrogen converted to nitric acid per annum.

The Nitride process Aluminium nitride is first produced from alumina, carbon and nitrogen in the electric furnace at 1800°C. The nitride is then decomposed with steam yielding ammonia.

III. The following may be termed thermo-chemical processes as electric energy is not called for.

- (i). *The direct synthesis of ammonia* from nitrogen and hydrogen (the Haber process). In this process pure nitrogen and hydrogen are caused to combine in presence of a catalyst at 500°—600° (under pressure of at least 100 atmospheres). The ammonia is then oxidised catalytically to nitric acid.

The process requires 0·5 H. P. year per ton nitrogen fixed as nitric acid per annum.

- (ii). *The cyanide process.* In this process a mixture of sodium carbonate, carbon and finely divided iron are heated in nitrogen at 1000°C with production of sodium cyanide, which is decomposed with steam yielding ammonia.

In the foregoing processes the nitrogen is generally obtained from liquid air, hydrogen by various processes, *e. g.* electrolysis of water, the action of steam on iron, the interaction of carbon monoxide on steam, &c. Further research is needed in connection with the economical production of pure hydrogen.

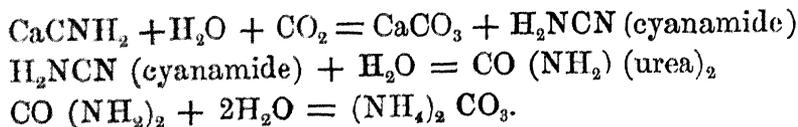
The ammonia produced is oxidised to nitric acid by passing along with oxygen over platinum gauze heated to a temperature of from 750° to 850°C when nitric oxide is produced which is passed down towers along with air and water yielding 50% nitric acid.

All the foregoing processes, it will be seen, require high temperatures with consequent expensive plant and high operation and maintenance charges.

The total world's annual production of nitrogen by these processes in 1917 was as follows:—

Arc process	...	31,000 tons
Cyanamide (Nitrolim process)	} ...	200,000 ,,
Haber process	...	114,000 ,,
Sundry	...	39,000 ,,
Total	...	384,000 ,,

It will be seen that this is only a very small proportion of the nitrogen in the solid and liquid excreta of the world's population (*viz.* 8,000,000 tons *cf.* p. 236). Moreover the right conditions are not always present for the successful use of calcium cyanamide or "nitrolim" which is believed to give rise to the following reactions in the soil:—



The biochemistry of these changes requires careful study, as the efficiency of nitrolim varies greatly under different conditions. (See Russell, Journal of Board of Agriculture Vol. XXVI No. 5, *loc cit.*)

The natural biochemical processes of nitrogen fixation go on silently at ordinary temperatures and pressures and in the

aggregate fix vastly more nitrogen than all the great installations operating the artificial processes.

The nature of these natural processes and the possibility of their utilisation and control may now be discussed.

IV. *Biochemical Processes of Nitrogen Fixation.*

The conversion of atmospheric nitrogen into organic nitrogen by the action either separately or in combination of bacteria and leguminous plants is one of the most fascinating biochemical processes in nature.*

It has been known for a long time that leguminous plants contained more nitrogen than others and that this nitrogen could be used as food for other crops when the nitrogen containing plant was used as a green manure.

How the plant came to have this nitrogen, and how it often thrives on a soil almost devoid of nitrogen was for a long time unexplained.

It was by no means generally agreed that the nitrogen came from the air although certain experiments of Priestley's appeared fairly decisive. Up to 1879 the general opinion of agriculturists including the Rothamsted authorities was against nitrogen fixation. In 1885 Berthelot suggested that soil and the bacteria therein played a part in such a process.

In 1898 Hellriegel and Wilforth showed definitely the part played by bacteria. They treated plants with (a) distilled water (b) an extract of soil sterilised by boiling (c) an extract of soil unsterilised. The growth was much more marked when the plant was treated with the unboiled extract, showing that in some way bacteria-assisted the growth of the plant.

They noted the presence of nodules or tubercles on the roots of peas and that these were more numerous in soil poor in nitrogen. They also showed that the nitrogen was not stored in the tubercles.

In 1897 Maze definitely studied the bacteria in the nodules and other workers showed that nitrogen fixing bacteria occurred in soil quite distinct from the nodule bacteria. There are thus two chief varieties of nitrogen-fixing organisms:—

Bacillus radicicola (occurring in nodules) with an optimum temperature of 15°C.

* "Green Manures and Manuring in the Tropics" by P. de Sorvay.

Azotobacter chroococcum with an optimum temperature of 28°C.

The medium generally used for developing these organisms has the following composition :—

Water	...	100.00	grams
Mannite	...	2.00	„
K ₂ HPO ₄	...	0.02	„

It is found that the fixation of one gram of nitrogen needs 165 grams of glucose, so that as an artificial process on a large scale it would seem likely to be costly. There are however many sources of waste carbohydrate material which could be used in this way, indeed molasses from the sugar factories is actually used in Mauritius as a manure to stimulate nitrogen fixation. It is likely that the scientific use of the waste from breweries and other processes of carbohydrate fermentation would result in an increased retention of nitrogen.

The exact mechanism of the fixation process is not yet completely understood. The bacteria apparently receive carbohydrate material from the plant and return a complex nitrogenous substance to the plant. This actually appears as a sort of slime when nitrogen fixation takes place in presence of sugar apart from the plant.

Thomas Jamieson, Director of the Agricultural Station at Aberdeen, has consistently held a theory that nitrogen fixation takes place primarily through the agency of the leaf hairs which produce albumin from the nitrogen of the air and that the nodules have no real connection with the process. The fact that bacteria can be shown to fix nitrogen by flask experiments easily repeated in the laboratory has naturally tended to confine the attention of workers to this side of the subject and whether Jamieson's views ultimately find acceptance or not, the role of bacteria will always be of fundamental importance in this connection.

Many attempts have been made artificially to induce nitrogen fixation by inoculation of the soil or of the plant seeds.

Thus Nobbe grew nitrogen fixing bacteria on gelatine and sold the cultures as "nitragin". It was found however that the gelatine contained too much nitrogen for efficient growth of these bacteria, which are naturally most active in fixing nitrogen from the atmosphere when deprived of any other source of nitrogen.

To avoid this difficulty American bacteriologists endeavoured to transport active cultures on cotton wool. Small scale experiments on these lines had some success, but it was really only when the actual earth containing the necessary bacteria was transported to soils poor in nitrogen that any marked effect on a large scale was produced.

Bacterised peat or Humogen.

These various attempts led up to the work of Bottomley on "bacterised peat". He found that the bacteria kept best when raised with earth, and a product was put on the market called "nitro-bacterine" which was the forerunner of "bacterised peat". The experiments with soil showed the importance of "humus" as a medium for growth and it was found that peat if kept moistened for about a week, decreased in acidity and increased in humus and was hence termed "humogen". Peat treated in this way was steamed to destroy competing organisms and was then inoculated with *Azotobacter* and *B. radicola*, dried at a low temperature and mixed with the soil. Pot experiments and small scale trials were most successful, but owing apparently to difficulties in the preparation of the material on the large scale, field trials were less satisfactory.

Apart from the actual value of the material as a manure the scientific studies in connection with bacterised peat are of great interest and open up many attractive lines of inquiry.

In the first place the two chief species of bacteria concerned in nitrogen fixation viz *Azotobacter* and *B. radicola*, have been carefully studied and it has been found that they work much better in symbiosis than in pure culture.

It has been further found that certain special kinds of nutrient material tend to increase their activity when acting symbiotically. Thus while mannite appears to be the best food for *Azotobacter* and maltose for *B. radicola*, a mixed culture thrives best on dextrin rather than on a mixture of the above substances. Moreover basic slag has been found to have special advantages as a neutralising agent. These facts are of considerable practical importance. The disposal of effluents from breweries and distilleries is a difficult and troublesome problem, but it would seem likely that under suitable conditions such effluents, containing as they generally do unfermented dextrinous matter, could be disposed of in such a way as to stimulate nitrogen fixation, especially if basic slag is used to prevent the development of acidity.

The very striking effects obtained by the use of 'bacterised peat' as a manure led however to the conclusion that something more was involved than could be explained by the mere increase in the amount of nitrogen or in the improved physical conditions of cultivation.

The work of Hopkins and others in accessory food bodies known as "vitamines" suggested that substances of the same character might be present in bacterised peat.

Careful investigation showed this to be the case.

An aqueous extract of bacterised peat was found to have a stimulating effect on plant growth comparable to the original material.

Absolute alcohol also extracted a substance capable of greatly stimulating nitrogen fixation.

Such a substance could not be obtained from raw peat by extraction either with water, absolute alcohol or carbonate of soda.

Further chemical investigation of this extract by fractional treatment with phospho-tungstic acid and silver nitrate served to isolate nitrogenous compounds which were found to be derivatives of so called nucleic acid, such as adenine, guanine, cytosin and uracil.

Schreiner and others have shown that plants can absorb such nitrogenous bases direct. That the growth promoting bodies extracted from bacterised peat are definite chemical substances is rendered likely by the fact that they are unaltered in their stimulating properties after heating to 134°C for half an hour.

These growth promoting substances have been termed by Bottomley *auximones* (Greek *auximos*, promoting growth).

He has devised an ingenious bacteriological test for their presence in solution. About 10 grams of ordinary fertile soil is incubated for two days in contact with a solution containing nitrifying organisms. The solution to be tested for *auximones* is added and the mixture further incubated for 24 hours. If *auximones* are present a scum is formed proportional in amount to the quantity of *auximones* in solution.

Florence A. Mockridge working under Prof. Bottomley's direction has found that while *auximones* tend to increase nitrogen fixation and also nitrification they depress the rate of denitrification and do not appreciably affect the rate of ammonification or the breaking down of complex nitrogenous matter. It would appear therefore that *auximones* play some definite part in the building up of the complex nitrogenous molecule.

Bottomley indeed has found that even when nitrogen fixing bacteria are grown in a pure synthetic medium their products can function as growth promoting substances for higher plants.

The general conclusion is warranted that bacteria produce auximones through the decomposition of organic matter of various kinds, or even by synthetic processes of their own, these auximones are taken up by the plant and assist in the production of vitamins which are of importance for animal metabolism.

The experiments of Mockeridge have shown that auximones are present in well-rotted stable manure to a greater extent than in fresh manure. They are also present in leaf mould and in good well-manured garden soil.

All Bottomley's experiments on the growth stimulating properties of auximones were done with full mineral plant food as control, which was found insufficient to maintain vigorous growth.

It will thus be seen that the character of the organic matter and of the bacteria present in a manure are of first importance.

From this point of view the possibilities of activated sludge are of especial interest.

The increase in yield which it shows over other nitrogenous manures may well be due to the presence of the necessary bacteria in a nidus of organic matter suitable for the production of auximones.

VI. The Nitrogen in Activated Sludge,

It is important therefore to discover the source of the nitrogen in activated sludge and whether by any method it can be increased in amount.

A number of workers have studied this problem the majority under the direction of the writer, others independently. A brief account of this work may now be given.

Experiments at the Frankland Laboratory, University of Manchester.

Experiments on the fixation of nitrogen by activated sludge have been made in the Frankland Laboratory of the Chemical Department of the University of Manchester by

Mrs. E. M. Mumford and Mr. Ernest C. Gaul in 1915 and 1916, respectively, and the results were included in the reports to the Board of Agriculture already referred to.

Experiments by Mrs. Mumford.

These experiments were carried out under the author's direction in the following way.

Two lots of 50 cc. of sludge were taken in each case, one to act as a check, while to the other was added 1% of glucose.

The sludges were placed in similar wash bottles and to each wash bottle was fixed another containing 50 cc. standard acid to absorb any ammonia which might be given off.

These bottles were attached to each other in series, and a current of air previously freed from ammonia by strong acid and then saturated with water to prevent evaporation, was drawn through the bottles. The current was kept constant by a mercury valve inserted in the series.

After a fortnight, the experiment was stopped and the acid titrated with alkali. No trace of ammonia was found. The nitrogen content of the sludge was then estimated as free and saline ammonia, as albuminoid ammonia and as Kjeldahl nitrogen. Portions of the sludge were taken and each estimation was repeated. The results obtained are given in the following table:—

Nitrogen as	Sample A.	Sample A. + glucose.	Sample B.	Sample B. + glucose.
Kjeldahl nitrogen	... 2.4%	6.8%	2.02%	6.1%
Free and saline ammonia03%	.12%	.08%	.06%
Albuminoid ammonia81%	.44%	1.11%	.94%
Nitrite and nitrate059%	.15%	.23%	0.45%

These results show that an increase in nitrogen content has certainly taken place simultaneously with the carbonaceous fermentation.

In another experiment it was decided to allow freshly activated sludge to remain in contact with air without pore aeration than an occasional shaking and by analysis of the air to see if any nitrogen had been absorbed.

The sludge was contained in a large separating funnel which was inverted and connected by means of a glass tube and india rubber stopper with a flask containing water, so that if any absorption of gas took place in the funnel water would be sucked

into the funnel in equal amount. Similarly the pressure could be adjusted from a reservoir connected with the flask.

After the sludge had been allowed to stand with occasional shaking for 14 days the pressure was adjusted to that of the atmosphere and the volume of the gas present in the funnel marked. Samples of gas were removed for analysis by opening the tap of the funnel and raising the reservoir. Two samples of gas were analysed with the following results :—

Sample 1.	Carbon dioxide	...	
	Oxygen	...	2·82%
Sample 2.	Carbon dioxide	...	
	Oxygen	...	

The volume of air in the funnel before fermentation measured	925 cc.
do do after	do. 790 cc.

From the above analysis it can be calculated that the residual gas contains only 707·5 cc of nitrogen as against the 740 cc originally present. An absorption of 32·5 cc of nitrogen has therefore taken place.

The actual weight of dry sludge used in the experiment was 8·41 grams. Under the circumstances of the experiment it was therefore calculated that 1 gram of sludge would fix 0·5 mgms of nitrogen.

The experiment while so far confirmatory of the more striking gravimetric results requires to be repeated with perhaps a rather better devised form of apparatus before useful conclusions can be drawn.

Further experiments were made later by Mrs. Mumford in 1916 to determine whether there was any increase of nitrogen during the ordinary process of purification as the experiments of Ardern (J. S. C. I. Vol. XXXIV No. 16, 30th September 1915) led him to conclude that this was the case.

The activated sludge and raw sewage were obtained from the Withington Sewage Works of the Manchester Corporation by the courtesy of Mr. (now Dr.) Ardern, then Chief Chemist to the Rivers Committee of the Corporation.

Analyses of the original activated sludge and of the raw sewage added were made and aeration started in a liquid which contained 25 % activated sludge by volume and 75 % raw sewage.

Tyro fillings of raw sewage were made per day. In drawing off the effluent the sludge was allowed to settle for two hours by shutting off the air supply and the clear liquor at the top siphoned off and the volume carefully measured.

The nitrogen figures and the total and suspended solids were determined in the raw sewage added and in the effluents drawn off.

Thus the total weight of original sludge plus solid matter added could be compared with the total weight of the final sludge plus solids which passed away in the effluent.

An analysis of the original sludge was also made together with that of the final sludge and the total of nitrogen in each compared. The following figures were obtained:—

	Before Experiment.	After Experiment.
Volume of sludge ...	550 cc.	566.4
Weight of sludge dry ...	18.865 gms.	23.625 gms.
Analysis of sludge : mineral matter ...	30.57%	33.31%
" " organic matter ...	69.43%	66.69%
Nitrogen ...	5.20%	4.92%
Weight of original sludge + solids added =	29.442 grams	
Weight of final sludge + solids in effluents =	34.199 "	
Production of sludge (34.199—18.864) =	15.334 "	
Gain in sludge	— 4.76 "	
Total volume of sewage added	= 15.050 litres	<i>i. e.</i> , 3.31 gal-
		lons sewage produce 0.54 oz. sludge
Weight of nitrogen in original sludge + nitrogen added	= 1.481 grams	
Weight of nitrogen in final sludge + nitrogen in effluent	= 1.964 "	
Total gain in nitrogen	= 0.483 "	= 32.6%

From these figures it appears that a million gallons of Withington sewage as represented by these samples produce 4.5 tons of sludge. Analyses showed that this gain is in excess of that due to the addition of ordinary suspended solids in the raw sewage and is evidently in part at least produced by coagulation of colloids. From the nitrogen figures however, it would appear that a proportion of this gain is due to the absorption of nitrogen from the air.

It should be mentioned that the Kjeldahl nitrogen was only determined in the initial and final sludge. Only the ammoniacal and the albuminoid nitrogen were determined in the sewage added and in the effluent. It is possible therefore that the value given for the gain in nitrogen may in consequence be somewhat too high, a greater proportion of Kjeldahl nitrogen being present in the sewage added than in the effluents passing away.

Later experiments by Sarangdhar and Nayak confirm this supposition.

During 1917 further experiments were made by Mr. Ernest Gaul in the Frankland Laboratory on the lines of drawing ammonia—free air through activated sludge suspended in water together with a little calcium carbonate and with precautions to arrest any ammonia passing away.

Two experiments, both in duplicate, were done, the air being drawn through flasks 1 and 2 for four weeks and through flasks 3 and 4 for ten weeks. At the end of these periods, the contents of the flasks were submitted to the Kjeldahl process.

The following results were obtained.

	Flask 1.	Flask 2.	Flask 3.	Flask 4.
Gain in nitrogen actual per cent	Nil	·007	·02	·016
Percentage gain on original nitrogen.	Nil	6·8	19·6	15·5

The figures for flasks 3 and 4 are quite beyond experimental error and point unmistakably to fixation of nitrogen g taken place,

Rothamsted

Gaul's experiments were largely due to a suggestion of Dr. Russell's and the method employed was similar to that used by Mr. E. H. Richards in his experiments demonstrating the presence of nitrogen fixing organisms in the fœces of animals *viz.*, in horse and cowdung (Journal of Agricultural Science, Vol. viii, pt. 3, 1917, p. 298-311).

Among Richards' conclusions are the following:—

Under the most favourable conditions 1 gram of dry matter in the fœces will fix: four mgms of nitrogen.

Evidence is adduced to show that fixation is brought about by a mixed culture of *Azotobacter* and *B. lactis a*

Of these the latter is normally present in fœces, *Azotobacter* is not but readily infects fœces. Both organisms are present in the soil used and will fix nitrogen in raw fœces but not in sterile fœces.

In a discussion of a paper by himself and Dr. Winifred E. Brenchley on "The Fertilising Value of Sewage Sludges" (*loc. cit.* p.—240) Richards spoke of experiments to be carried out at Rothamsted on the nitrogen fixing power of activated sludge.

Experiments at the Indian Institute of Science.

These experiments were carried out in the Department of Applied Chemistry under the writer's direction:—

Experiments by Mes-rs. Sarangdhar and Nayak.

500 cc of activated sludge and 1500 cc of raw sewage were introduced into a glazed porcelain diffuser of about 2500 cc capacity. Samples of both the sludge and raw sewage were analysed for total nitrogen which gave the amount of nitrogen put into the diffuser at the beginning of the experiment. The contents of the diffuser were aerated for six hours and then allowed to settle for two hours. A known volume of the clear effluent was siphoned off and a known volume of fresh sewage put in. Samples of the effluent taken out and of the raw sewage put in were analysed for total nitrogen. This was repeated a number of times. Finally the resulting sludge was analysed for total nitrogen. The following results were obtained:—

Nitrogen added to the diffuser in the form of sludge and raw sewage.		Nitrogen removed from the diffuser in the form of effluent and nitrogen in the final sludge.	
TOTAL NITROGEN.		TOTAL NITROGEN.	
1st day	... 500 cc. sludge 1500 cc. raw sewage 3.000 cc. " J = .125	1000 cc. effluent = .100 gr. 700 cc. " = .063 "	
2nd day	... 1000 cc. " J = .250 1000 cc. " J = .250	1500 cc. = .080 1000 cc. = .080 1000 cc. = .080 1000 cc. = .050	
3rd day	... 1000 cc. = .100 1000 cc. = .100	1000 cc. = .060 100 cc. = .060	
4th day	... 1000 cc. = .250 1000 cc. = .250	1000 cc. = .060 100 cc. = .060	
5th day	... 1000 cc. = .060	Total nitrogen in the remaining sludge 1.152	
Total nitrogen added 1.793		Total nitrogen obtained ... 1.865	

It will be seen from the left hand column that the total nitrogen added amounts to 1.793 grams and from the right hand column that the total nitrogen obtained is equal to 1.865 gms. The total percentage increase, due presumably to fixation, is about 4 per cent, but the total increase in the sludge nitrogen is nearly 15 per cent, doubtless owing to colloidal nitrogenous matter having been precipitated.

An interesting experiment was made with some activated sludge which had been used to purify the effluent from the fermentation process of acetone production. As this effluent contained residual carbohydrate material it was thought that it would possibly be a suitable medium for the development of nitrogen fixing bacteria.

An extract from this sludge was therefore incubated at 39°C with a medium containing glucose and potassium phosphate.

For comparison an extract from sweet pea nodules was similarly incubated.

In both cases blanks were also incubated. The nitrogen content of the flask was determined at the end of one and two weeks with the following results.

		Milligrams of nitrogen.		
		Blank.	One week.	Two weeks.
Acetone sludge extract	...	21.68	25.34	23.8
Percentage increase of nitrogen	16.7%	
Nodule extract	...	9.48	13.4	12.8
Percentage of increase of nitrogen	41.3%	

Probably the drop of nitrogen in the second week is due to some of the nitrogen of the sludge being oxidised to nitric nitrogen which was not determined.

Experiments by R. R. Deo.

During the past year further experiments have been carried out by Mr. R. R. Deo.

He made use of the following medium similar to that used by Walton* in his work at Pusa except that di-potassium

* (J. Walton—Azotobacter and Nitrogen Fixation on Indian Soils—Memoirs of the Department of Agriculture of India Bacteriological Series I, No. 4, August 1915).

hydrogen phosphate was substituted for the mono potassium salt.

Mannite	20	grams
Dipotassium phosphate	0.2	„
Magnesium sulphate	0.2	„
Sodium chloride	0*	
Calcium sulphate	... 0.1	
Water	... 1000	„

A number of small flasks containing this medium were inoculated with activated sludge which had been prepared at Jamshedpur and stored in a tin for upwards of a year.

Some of these were placed in the incubator at 35°0 and incubated without artificial aeration. Air was bubbled through others at laboratory temperature for about 10 hours daily with precautions against gain or loss of ammonia. The results are summarised in the following table :—

Summary of Results.

Incubated at 30°C.

Time in weeks.	Weight of activated sludge. at the start.		N at the end.	N fixed.	fixed.	Mean.
	f	0.6713	0.02942	0.03888	•00446	15.16
	t	0.9158	0.04014	0.04627	•00613	15.28
	f	0.5147	0.02279	0.02730	•00451	19.79
	t	0.7970	0.03493	0.04193	•00700	20.04
4	f	0.5367	0.02353	0.02863	•005100	21.67
	t	0.5443	0.02385	0.02912	•005270	22.10
5	f	0.5123	0.02246	0.02807	•00561	24.98
	t	0.4421	0.01937	0.02422	-0.0485	25.03
6	f	0.5493	0.02407	0.03038	•00631	26.22
	t	0.4401	0.01929	0.02429	•00500	25.92

Aerated with ammonia free air for about 10 hours daily.

Room temperature.

2	S	0.6662	0.02919	0.03507	•00538	20.15
	t	0.7333	0.03236	0.03864	•00628	19.41
3	S	0.9321	0.04084	0.05026	•00942	23.07
	t	0.6711	0.02941	0.03712	•00671	22.82
4	f	0.5941	0.02604	0.03255	•00651	25.00
	t	0.5890	0.02581	0.03234	•00653	25.30
5	f	0.6224	0.02746	0.03444	•00698	25.42
	t	0.6267	0.02747	0.03437	•00690	25.11

In every case it will be seen that there was a gain of nitrogen.

In the incubator without any artificial aeration the gain in nitrogen was about 15 % in 2 weeks rising to 26 % in six weeks.

With aeration at laboratory temperature there was a gain of 25 % in 4 weeks.

Conclusions.

All the various investigations described in this section though they are of a preliminary character and do not carry the inquiry very far, yet unite in showing that an increase of nitrogen greater or less under different circumstances unquestionably takes place when activated sludge is kept in contact with air and especially if the necessary carbohydrate medium is present. They have the advantage that they have been carried out by different workers using different methods and working quite independently.

There is little doubt therefore that not only does the activated sludge process recover the nitrogen present in the faecal matter of sewage but through fixation from the air an actual increase takes place over what can be recovered from the sewage.

The extent to which this fixation can be carried and the conditions of its maximum efficiency have yet to be determined.

Certain observers *e. g.* Clark, (Fourth Annual Report of the State Department of Health of Massachusetts p. 115) and Russell (private communication) speak of the loss of nitrogen in connection with the activated sludge process. In the experiments they refer to, however, aeration was not continuous. Under such circumstances, denitrification changes may easily set in with resultant loss of nitrogen.

VII Green manuring.

In the foregoing pages some indication has been given of the bio-chemical changes which result in the assimilation of nitrogen by the plant particularly the leguminosae which work in association with nitrogen fixing organisms in the soil.

Such plants when ploughed into the soil are broken down by bacterial action yielding simpler nitrogenous bodies which are again utilised by the growing plant.

The utilisation of such nitrogen containing plants in this way is known as "green manuring and the proper use of green manures is generally decided by the practical experience of the agriculturist.

The bio-chemist however can by a careful study of the changes taking place when plants are buried in the soil assist the agriculturist to avoid as far as possible the loss of nitrogen by such reactions as are indicated on p.

Thus Hutchinson and Milligan (Pusa Bulletin No. 40 1914) have studied the effect of the age of the plant at the time of burial and the subsequent treatment of the soil. They advise a partial preliminary decomposition under controlled conditions before actual use. This is in accordance with what is known of the fundamental changes involved. Thus it is probably better for the cellulose matter to be broken down under anaerobic conditions, not only because this is as a rule more rapid, but its aerobic decomposition generally involves the destruction of nitrates as has been recently confirmed by Joshi's experiments on the biochemical decomposition of cowdung and urine in the soil. On the other hand actual water-logging resulting in prolonged anaerobic decomposition has been shown to be deleterious to the plant, probably through production of toxic products, as well as defective root aeration.

Experience of the anaerobic decomposition of nitrogenous matter, along with cellulose fermentation in the septic tank would indicate that nitrogen is rarely lost in the gaseous state under these conditions. (*cf.* Chap. vii Sewage Disposal in the Tropics—W. W. Clemesha).

There are however exceptions, the most important of which is the case of swamp rice soils which have been the subject of very interesting researches by Harrison & Subramania Aiyar. They have shown that there is a free gaseous interchange between the roots of the plant and an active bacterial film which forms on the surface of the soil. Where green manuring is resorted to marsh gas and hydrogen are evolved doubtless through anaerobic decomposition of cellulose, but the film bacteria have the power to oxidise marsh gas and hydrogen to carbon dioxide and this is assimilated by the green algae also present in the film with production of oxygen, which in turn is dissolved in the water, bringing about greater root aeration and therefore greater root development and cropping power. Under these circumstances it may be assumed that any toxic products reaching the roots are oxidised.

The root system under these circumstances remains near the surface and the nitrogenous food consists of ammonia and probably various amino-compounds derived from the decomposition of the proteids of the green manure. Nitrates do not appear to be formed and in fact may be deleterious owing to rapid denitrification with production of toxic nitrates.

There has been found to be an optimum rate of drainage at which the plant thrives best, viz., a rate insufficient to disturb the surface film which is the source of root aeration and at the same time enough to enable the roots to penetrate to a greater depth and obtain in consequence a better food supply.

In this case the green manure besides furnishing by its decomposition, nitrogen derivatives suitable for plant food induces through the gases evolved a greater activity on the part of the surface film which leads to better aeration of the roots.

In their last paper (Mem. Dept. of Agric. India. Vol. V, No. 1, Chem. Series) proof is given of considerable loss of nitrogen from cropped paddy soils in presence of green manures. This is ascribed to the anaerobic decomposition both of the green manure and also of the roots of the growing plant, as the action of the surface film and consequent aeration of the soil was eliminated by addition of copper sulphate in small quantities to the water covering the soil.

In view of the results from the decomposition of sewage in septic tanks already referred to and the fact that loss of nitrogen from manure heaps is minimised by strict exclusion of air, it is not clear what the exact re-actions are which give rise to the evolution of gaseous nitrogen if the conditions are strictly anaerobic. The authors admit, on the evidence of Leather who discovered argon in paddy soil gases, that some of the nitrogen may be of atmospheric origin perhaps from air occluded in the roots of the plant which is liberated on decomposition.

If this be so, it might be suggested that small quantities of oxidised nitrogen compounds are formed and immediately reduced giving rise *e. g.* to nitrous oxide which has been shown by Letts to be present in solution in the liquid contents of a sewage "contact bed." The presence of hydroxylamine also is possible under partially aerobic conditions in accordance with the experiments quoted on p. 247. Such compounds might well act as catalysts resulting in large losses of nitrogen through interaction with complex ammo compounds.

Although the experiments of Harrison and Subramania Aiyer indicate that in the case of paddy the effect of green manures is mainly indirect yet they rightly urge the importance of further study in particular of the effect of mixtures of green manures with direct manures such as bone meal, superphosphate, ammonia and cyanamide.

The presence or absence of auximones among the products of the decomposition of green manures is a factor which has so far received little attention, but which may account for sundry anomalies in the behaviour of these manures.

VIII. General conclusions and problems for research.

In the foregoing pages evidence has been given to show that by the scientific and economic use of waste nitrogenous material and also of green manures the necessary nitrogen for the supply of the world's agriculture can readily be obtained.

(2) That very large populations are adequately supported in many parts of the world particularly in China without the need for the nitrogen of artificial fertilisers.

(3) That inorganic nitrogen in the form of nitrate, sulphate of ammonia or calcium cyanamide even when combined with other mineral nutrients such as potash and phosphoric acid, is not alone adequate for healthy plant development.

(4) That minute quantities of accessory food materials, elaborated by bacterial action, and which have been termed *auximones* are necessary if vigorous plant life is to be sustained

(5) That these products are only to be found in organic manures.

(6) That among organic manures *activated sludge* has been found to give the most striking results.

(7) That provided the nitrogen in the sludge and effluent can be economically utilised the activated sludge process of sewage purification offers the possibility of the recovery for agricultural purposes of all the nitrogen in town sewage, as not only is there no loss of nitrogen in the process but an actual gain owing to the occurrence of nitrogen fixation.

In order that the best results may be obtained from organic nitrogenous manures further research is required in numerous directions which are briefly indicated in the following paragraphs.

The various lines of investigation may be classified broadly under :—

- (a) Laboratory experiments, including pot or water cultures.
- (b) Field experiments.
- (c) Technical experiments.

A. LABORATORY EXPERIMENTS.

(i) *The acceleration of nitrogen fixation**

Experiments on the purification of special trade effluents by activated sludge as well as general experience would indicate the possibility of building up activated sludges capable of bringing about specific bio-chemical changes at a greater velocity than is possible by the usual less intensive methods of Mo-chemical activity. The acceleration of nitrogen fixation in this way is a peculiarly attractive problem, it being now unquestionable that fixation does take place.

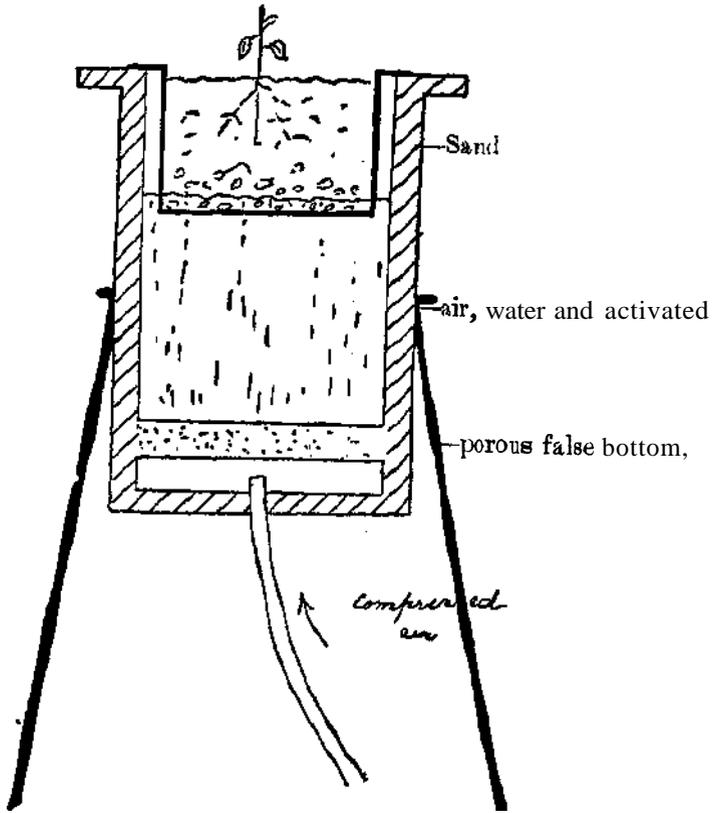
It will be necessary in the first place to determine what proportion of this nitrogen is fixed in the soluble or insoluble condition, whether *e. g.* complex nitrogenous slimes are formed directly or whether simpler and soluble nitrogen compounds are first formed and are afterwards built up into more complex bodies. It may well be that the advantages of symbiosis of different organisms pointed out by Bottomley, Richards and other workers are due to a difference of function in this respect.

It will then be to determine whether by suitable choice of carbohydrate food, of the necessary symbiotic organisms and by the maintenance of proper temperature conditions, it may be possible to build up a special activated sludge which will fix nitrogen in quantity and at a rate sufficient to make the process one which might compete with electro chemical or thermo chemical plants.

(ii) *The relation of the plant to the nitrogenous material.*

It is evident that the bio-chemical changes brought about in organic nitrogenous matter such as activated sludge by the agency of the bacteria present in it will be greatly modified by the activity of the roots of the growing plant.

This is clearly brought out in Harrison & Subramania Aiyer's work referred to in the section on green manures. It would seem likely that the withdrawal by the growing plant of the products of bacterial activity in the nitrogenous matter would stimulate further production of these, so that the maximum rate



of change takes place under conditions when both plant and bacteria exert their maximum efficiency.

Some preliminary work in this direction has been done by the writer and Mr. R. K. Deo at the Indian Institute of Science.

For the purpose of studying the effect of activated sludge and the products of its decomposition on plant growth a small circular metal container with brass wire bottom was suspended on the rim of a diffuser and filled with clean white sand. The sand was immersed about half an inch below the surface of the water leaving some two inches of moist sand above.

A seedling plant (castor oil) was placed in the sand so that air, activated sludge and the products of its decomposition came up against the roots which thus were fully aerated and provided with a continuous supply of food. (See diagram opposite).

Two control diffusers were set up one containing activated sludge and a plant but without air and another with activated sludge and air but with no plant. 5 grams of activated sludge were added to two litres of water in each case.

A few cc. of the liquid from each diffuser were examined day by day for ammonia and nitrous acid (as an index to nitrification) and observations were taken of the growth of the plant.

The plant growing over the aerated activated sludge throve much better than the one without aeration, in fact the latter died at the end of a month while the former was flourishing.

The necessity for good aeration of the soil when activated sludge is used as a manure is thus emphasised, and the wisdom of Bartow's suggestion of manuring some months before seeding is confirmed as a means for eliminating toxic substances which may be present in the raw sludge or sludge stored under anaerobic conditions.

In the case of the two aerated liquids the ammonia present in the liquid was oxidised quite appreciably faster in the case of the diffuser with the plant. In the case of the plant without aeration comparatively large quantities of ammonia appeared in the liquid but oxidation to nitrites was practically negligible, such small amount as took place being presumably due to aeration through diffusion from the atmosphere.

Thus after three weeks observations the figures were as follows in parts per 100,000 :—

	Ammonia	Nitrous nitrogen.
Plant and sludge and air	0·03	0·2
Sludge and air only	0·50	0·05
Plant and sludge only	2·80	nil

Judging from the composition of the liquid in the diffuser the actual quantity of nitrogen taken up by the plant was very small, but as analyses of the total nitrogen left in the plant, the sludge and the liquid at the end of the experiment gave conflicting results, no final conclusion can be drawn and many more such experiments will have to be carried out, possibly with some improvements in the details of the method before the exact sequence of changes which take place, can be properly defined, and the precise factors determined which produce increase in growth.

In this connection it will be necessary to examine samples of activated sludge of different origin and after different periods of oxidation, for the presence of *auximones*.

The same line of inquiry may well be carried out with different kinds of green manure at different stages of decomposition.

The effect of the presence of the plant on the rate of nitrogen fixation by means of activated sludge will also have to be studied.

(iii) *Loss of Nitrogen.*

The possibilities of loss of nitrogen by purely chemical action or by a combination of bacteriological activity and chemical change referred to on p. 246 afford an extensive field for inquiry. The interaction of complex amino compounds and nitrites in presence of nascent hydrogen or carbon dioxide can be studied in almost unlimited permutations and combinations of interest.

A further problem in this connection will be to find how far such loss is diminished or inhibited in the presence of the growing plant or of nitrogen-fixing organisms. The fact of there being no loss of nitrogen in the activated sludge process would indicate that the activity of the nitrogen fixing organisms may

mask a certain amount of denitrification change, as in the activated sludge tank there is considerable evolution of carbon dioxide in presence of the products of the breaking down of proteid matter, together with nitrites and nitrates, a condition of things under which loss of nitrogen might be expected.

The conditions of loss of nitrogen from green manures and from ordinary manure heaps are still somewhat obscure in spite of the considerable amount of study which they have received. The experiments with amino derivatives of known composition just referred to should throw some light on the decomposition of the complex proteids of green and other manure.

(iv) *Intensive nitrification.*

The possibility of the economic conversion of ammonium salts into nitrates through the agency of activated sludge has been referred to (p. 245) and experiments are already in hand by Mr. M. B. Eoy under the writer's direction at the Indian Institute of Science.

(v) *Bacterial hydrolysis of calcium cyanamide*
(*Nitrolim*)

The precise conditions for the most advantageous use of nitrolim are not fully understood, (see p. 251) and it will be of use to make a careful study of the possible transformations which may be effected in this substance by bacteriological agency. A knowledge of the changes which may take place in a mixture of this substance and activated sludge may lead to a useful combination of the artificial nitrolim and the different varieties of organic manures

B. FIELD EXPERIMENTS,

i. *Selection of plants responsive to activated sludge.*

The trials already made with activated sludge and ordinary crops should be extended and in particular selection made of such plants as respond most readily to the stimulus of this manure with a view to discover the possible reasons for this response. The effect of mixtures of activated sludge with other manures such as superphosphate and basic phosphate should be studied.

ii. *Effect of activated sludge on special Indian crops.*

Important Indian crops which respond to nitrogenous manure, particularly indigo, sugar-cane and paddy should be manured with activated sludge and the effect noted.

iii. *Conditions of availability of nitrogen in activated sludge.*

The 'availability' of the nitrogen in the sludge when the latter is put on the ground in the wet state as compared with the results when the sludge is dried for transport should be determined both by laboratory and by field experiments. Experiments by Mrs Mumford and by Mr. Ernest Gaul have shown that the lower the temperature of drying, the more immediately 'available' is the nitrogen while the nitrogen in the wet sludge is the most quickly 'available' of all.

iv. *Intensive cultivation by means of activated sludge.*

Accounts occasionally appear in the press of extraordinary profits up to *e. g.*, £ 1,000 per annum per acre made by intensive culture of market produce, &c. Experiments on these lines might well be made with activated sludge. Possibly a kind of hanging garden could be constructed over an activated sludge tank on the principle of the laboratory experiments described on p. 269 to produce quick growing and succulent crops for the market such as lettuce, onions and tomatoes, peas and beans, as well perhaps as special flowering plants which have shown themselves responsive such as lilies and hydrangeas.

The Chinese are accustomed to produce succulent crops by intensive cultivation as these show the quickest response to manure, are easily digestible, and may have a relatively higher nitrogen content than fully matured crops. Moreover the same plot will produce more crops in a given time (King, *loc cit*).

v. *Fishponds.*

The scientific study of fishponds fed with the effluent from activated sludge installations should reveal the life cycle best suited for the growth of fish and the recovery of nitrogen thereby. Thus it may be anticipated that aquatic plants will thrive on the nitrates and other salts present, infusoria of various kinds will feed on the bacteria and will afford food for fish, and both fish and plants will be a source of nitrogen for subsequent return to the soil.

The writer in the past once made some attempts with an effluent of a moderate standard of purity to establish a small fish pond but fungoid growths made their appearance and the conditions of a healthy life cycle were evidently not present.

The absence of colloids in an effluent properly purified by activated sludge, as well as the high content of dissolved oxygen either present or readily obtained, should greatly facilitate the possibility of maintaining suitable conditions for fish culture.

C. TECHNICAL EXPERIMENTS.

(i) *The drying of activated sludge for transport.*

The most important technical problem to be solved in connection with activated sludge, as distinguished from those directly connected with bio-chemistry or agriculture is undoubtedly the drying of activated sludge for transport. Activated sludge fresh and in good condition, is a highly flocculent precipitate which readily settles and is easily drained from superfluous water but the resulting mass is very gelatinous and contains over 90% of water. The problem is therefore the economic 'dewatering' of this jelly. In view of the results published and the numerous patents taken out in connection with the dewatering of peat and of kaolin &c., electric endosmose seems a very attractive line of attack. Recent laboratory trials by the writer and Mr. Malandkar with gelatinous precipitates of alumina would tend to confirm the conclusion arrived at by workers in Manchester that with such material any effect apparently due to electric endosmose is, in large measure at any rate, produced by a rise of temperature. Even if this be so, it would indicate that a very moderate rise of temperature will tend to separate water from the jelly.

Moderate heating therefore, combined with centrifugal action in one of the several continuous centrifuges described in various recent papers and patent abstracts, *e. g.* in the Journal of the Society of Chemical Industry, is likely to reduce the water content of the gelatinous activated sludge to such a point that it can be economically dried by direct methods.

The final process of drying will need to be scientifically conducted, as not only will excessive or too rapid heating tend to harden the albuminoid matter present and render the nitrogen less readily "available" but the bacterial life present in the sludge and necessary for its maximum efficiency will also be destroyed.

It is probable that warm air in large volumes will be the best drying agent. Many tons of humus tank sludge have been dried in this way at the Davyhulme works of the Manchester Corporation and the method is being used at a recently established factory in Madras for the purpose of drying glue.

During the hot weather in India and other tropical countries no difficulty should arise in drying the material in ordinary well constructed sludge drying beds in the open air as the sludge is inoffensive in character.

If the bacterial factor in the sludge is neglected and its value simply calculated on the units of nitrogen present more drastic procedure is possible. In this connection the conclusions so far reached by the city of Milwaukee are of interest*. After several years of large scale experimental work, in the earlier stages of which the present writer was professionally consulted, a complete scheme has been devised to treat the whole of the sewage by the activated sludge process at an ultimate cost of £ 1,000,000. The methods decided upon for conversion of the riudge into fertiJiser are briefly as follows. The sludge is somewhat concentrated in the treatment tanks by subsidence, and removal by means of special sludge ploughs known as "thickeners". As it leaves the tanks it contains 97"5 per cent moisture, 4.5 cc. of sulphuric acid are added per gallon of sludge which is then pressed in filter presses of specially selected design to a moisture content of 80%. This pressed sludge is then dried in mechanical driers of normal type. It is estimated that the dried sludge containing 10% of moisture will be worth at least £ 3—10—0 per ton.

If this sludge is to be used in soil already containing a full supply of the necessary bacteria, its complete or partial sterilisation may be of small moment. Where it is to be applied to poor soil the admixture of a certain proportion of sludge dried at a temperature which will not destroy the necessary bacteria will probably greatly enhance the value of the manure.

ii. The treatment of concentrated Latrine Sewage by activated sludge.

In many cities in the East and generally in towns where the rateable value is low compared to the population a large consumption of water per head for the purpose of transporting sewage is not available and so the liquid to be dealt with is likely to be very concentrated. In many cases it is necessary to "dump" excreta &c., removed from the houses in pails by hand direct to the sewers. The conditions for successful treatment of such material by the activated sludge process have not yet been fully investigated.

* Surveyor Vol. LVIII, pp. 21 and 48.

the many lines of work shortly to be undertaken in Shanghai this question will receive attention and an installation is to be fitted up in connection with a latrine which can be kept under exact control. It may well be found that the sewage will have to be diluted in the initial stages with extra water and afterwards with its own effluent on the principle which was adopted by the writer several years ago in dealing with highly concentrated and germicidal trade effluents*.

It is evident that much work still remains to be done in the large field of inquiry which forms the subject matter of the present paper.

Success can only be achieved by correlating the results of the activity of many workers, investigating different aspects of the subject in different parts of the world.

The labours of the bio-chemist, the bacteriologist, the botanist, the agriculturist and the engineer will all be needed.

Such work however will be well worth while if it results, as there is every reason to hope it will, in an increase in the productivity of the soil, and a consequent great addition to the real wealth of mankind.

IX, Summary of Literature.

In addition to the individual papers incidentally mentioned in the text the following list of books, monographs and other literature will be of use to the student of the problem of nitrogen conservation.

(a) *The bio-chemical aspects of sewage purification.*

An Introduction to Bacteriological and Enzyme Chemistry—Gilbert J. Fowler—Thacker Spink and Co. Es. 7. Published by Edward Arnold, London.

Principles of Sewage Treatment—Dunbar and Calvert.

Sewage Disposal—Kinnicutt, Winslow and Pratt.

American Sewerage Practice—Metcalf and Eddy. Vol. III, Disposal of Sewage. McGraw Hill Book Co. New York and London, Chapter V.

Sewage Disposal in the Tropics—W. W. Clemesha, M. D. Thacker Spink and Co. Rs. 10.

Colloid Chemistry and its General and Industrial Applications, Second Report of the Committee. Section 4, Sewage Purification, Edward Arden, D. Sc., F. I. C.

* Fowler and Holden J. S. O. I. Vol. 30 1911 p. 180.

Fowler and Shepherd do p. 181.

(b) Nitrogen Fixation by Physico-Chemical Methods.

Fixation of Atmospheric Nitrogen—Joseph Knox. Gurney and Jackson, 33, Paternoster Row, London, E. C. 2 s net.

Utilization of Atmospheric Nitrogen—Thomas H. Norton—Department of Commerce and Labor Bureau of Manufactures, special Agents series No. 52—Government Printing Office, Washington.

The Present Status of Nitrogen Fixation. Alfred H. White, Journ. Industrial and Engineering Chemistry, March 1919. p 231.

Nitrogen Production Committee Final Report Published by H. M. Stationery Office London, 1920. Price 4/-.

(c) The activated Sludge Process of Sewage Purification.

The Activated Sludge Process of Sewage Treatment—A Bibliography of the Subject by J. Edward Porter, Chemical Engineer, General Filtration Company, Inc. Rochester, N. Y. Price 25 cents.

This bibliography contains brief abstracts of all papers, articles, patent specifications and other references to the subject which have appeared since the "Preliminary note on the bacterial clarification of sewage" by G. J. Fowler and E. M. Mumford, (Jour. Royal San. Inst. 34, 1913. No. 10.)

In the original edition published early in 1917, 221 references are given. A new edition is in course of preparation bringing the subject up to the end of 1920.

The greater number of the publications occur in the Journal of the Society of Chemical Industry, the Surveyor and the Engineering News and Record of New York.

Special references may be made to :—

A Resume of the present position of the Activated Sludge Process of Sewage Purification by Edward Arden, M. Sc. Reprinted from the Journal of the Society of Chemical Industry, July 31st 1917, No. 14, Vol. XXXVI.

The Activated Sludge Process of Sewage Purification. G. J. Fowler—Jour. Inst. San. Engineers. March and April 1916. (cf. Surveyor 49, 148—51.)

The Manchester experiments are all to be found in the Annual Reports of the RIVERS Committee of the Manchester Corporation published by P. S. King and Son, Orchard House, Victoria Street, Westminster, S. W. price 2/6 beginning with 1914.

(d) *The Bio-chemistry of Bacterised Peat.*

- The Spirit of the Soil—G. D. Knox. This is a brightly written popular monograph on the subject published in 1916 by Constable and Co. Ltd., London.
- The following is a list of the chief scientific papers by Prof. Bottomley and Miss Mockeridge as well as certain other researches specially referred to by them :—
- The Assimilation of Nitrogen by certain nitrogen fixing bacteria in the soil. (Proc. Roy. Soc. B. Vol. lxxxii, 1910.)
- Home effects of Bacterio-toxins on the Germination and Growth of Plants. (Report Brit. Association, 1911.)
- The Fixation of Nitrogen by free-living soil bacteria. (Report Brit. Association, 1911.)
- Some conditions influencing nitrogen fixation by aerobic organisms. (Proc. Roy. Soc. B. Vol. lxxxvi, 1912.)
- Some effects of Humates on Plant Growth. (Report Brit. Association, 1912.)
- Ammonium Humate as a source of nitrogen for plants. (Report Brit. Association, 1913.)
- The effect of soluble Humates on nitrogen fixation and plant growth. (Report Brit. Association, 1913.)
- The Bacterial treatment of Peat. (Jour. Roy. Soc. Arts. Vol. lxii, 1914.)
- Some Accessory Factors in plant growth and Nutrition. (Proc. Roy. Soc. B. Vol. lxxxviii, 1914.)
- The Significance of certain Food Substances for Plant Growth, (Annals of Botany, Vol. xxviii, 1914.)
- The Formation of Humic Bodies from Organic Substances. (Bio-chemical Jour. Vol. ix, 1915.)
- A Bacterial Test for Plant Food Accessories (Auximones) (Proc. Roy. Soc. B. Vol. lxxxix, 1915).
- Bachmann (1919) Journ. Biol. Chem., 39, 235.
- Bottomley (1914) Ann. Bot., 28, 581.
- (1911) 1. Proc. Roy. Soc. B. 89, 481.
- (1917) 2. " " " B. 90, 39.
- (1919) " " " B. 91, 83.
- (1920) 1. Ann. Bot., 34.
- (1920) 2. " " 34.
- Clarke and Schryver (1917). Biochem. J., 11, 319.
- Jones (1914). Nucleic Acids. (Longmans).
- Livingston (1907) U. S. Dept. Agric. Bureau of Soils, Bull., 86.
- Mockeridge (1917) Proc. Roy. Soc. B., 89, 503.

- Schreiner and Lathrop (1912) U. S. Dept. Agric. Bureau of Soils, Bull., 89.
- Eeed and Skinner (1907) U. S. Dept. Agric. Bureau of Soils, Bull. 47 and Shorey (1910; U. S. Dept. Agric. Bureau of Soils, Bull. 74 and Skinner (1912) U. S. Dept. Agric. Bureau of Soils, Bull. 87.
- Shorey (1913; U. S. Dept. Agric. Bureau of Soils, Bull. 88.
- Williams (1919) Journ. Biol. Chem. 38,465.
- The Occurrence and Nature of the Plant Growth-Promoting substances in various organic Manurial Composts—F. A. Mockeridge—Bio-chemical Journal, July 1920. Vol. XIV p. 432

(e) Agriculture and Green Manuring.

- Report on the Improvement of Indian Agriculture. J. A. Voelcker, printed by Eyre and Spottiswoode, London.
- Green Manures and Manuring in the Tropics. P. de Sornay. John Bale, Sons and Danielsson, Ltd, Oxford House, 83—91, Great Titchfield Street, Oxford Street, London, W.
- Farmers of Forty Centuries F. EL King, D. sc. Published by Mrs. F* H. King. Madison Wis, 1911. A very complete account of agricultural methods in China and the Far East.
- Soil Conditions and Plant Growth. Edward J. Russell. Longmans Green and Co., London.
- Plant Products and Chemical Fertilisers. S. Hoare Collins. Rideal's Industrial Chemistry. Bailliere Tindall and, Cox, 8, Henrietta Street, Oovent Garden, London.

Agricultural Research Institute, Pusa Bulletins.

- Green Manuring in India by A. C. Dobbs, Bull. No. 56.
- The Indian Saltpetre Industry by J. Walter Leather and Jatin-dranath Mukerjee, ptice 9 d. Bull. No. 24.
- Green Manuring Experiment, 1912—13 by C. M. Hutchinson and S. Milligan, price 5 d. Bull. No. 40.
- Saltpetre—Its origin and extraction in India by C. M. Hutchinson. Bull. No. 68.
- A modified Method of Green Manuring by Hutchinson. Bull. No 63, 1916. Development of No. 40.

Memoirs of the Department of Agriculture in India. Chemical Series.

- The Gases of Swamp Eice Soils, Part I by Harrison and Subra-
raania Aiyer, Vol. III, No. III. Price Rs. 1—8—0.
- do part II. Vol. IV. No. I. Price Re. 1.
- do part III. Vol. IV, No. IV. Price As. 12.
- do part IV. Vol. V. No. I. Price Rs. 2.

Bacteriological Series.

- Studies in Bacteriological Analysis of Indian Soils, No. 1, 1910-11 by Hutchinson, price Es. 2—8—0. Vol. 1.
- A New Nitrite-forming organism by N. V. Joshi. price Re. 1. Vol. 1, No. III.
- Azotobacter and Nitrogen Fixation in Indian Soils, by J. H. Walton, price Re. 1 Vol. 1, No. IV.
- Studies in the Rootnodule Organism of the Leguminous Plants, N. V. Joshi, Vol. 1, No 9, price Rs. 1—4—0.

Agricultural Journal of India.

- Effect of Drainage on Rice Soils by C. M. Hutchinson, Agric. Journ. of India. Vol. VIII, 1913, p. 35.
- Nitrogen Fixation—Hutchinson, Indian Journ. of Agric. Vol. 14, pp. 205—214.
- Studies in Bio-chemical Decomposition of cowdung and urine in soil by N. V. Joshi, Agric. Journ. of India, Vol. XV, Part IV, July 1920, p. 398.
- Note on Exhaustion of Indian Soils—R. V. Norris. Agric. Journ. of India, Vol. XV, Part IV, July 1920, p. 433.

DEPARTMENT OF APPLIED CHEMISTRY.
INDIAN INSTITUTE OF SCIENCE,
BANGALORE.