# SOME NEWLY OBSERVED LINKS IN THE NITROGEN CYCLE.

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It is now nearly seventeen years since the present writer had the honour of delivering a second Presidential Address to the Indian Chemical Society and chose as his subject the field of work with which he was most familiar, viz. 'Recent Researches on the Biochemistry of the Nitrogen Cycle'. Following that address he was called upon some years afterwards to deliver a course of lectures as Sakraj Ray Reader in Natural Science in the Patna University and chose the same subject. Finally in 1934 he collected such knowledge as he possessed in the book entitled: 'An Introduction to the Biochemistry of Nitrogen Conservation'. This volume was based on material concerned with the same subject extracted from a former publication, by then out of print, bearing the more extended title of 'An Introduction to Bacteriological and Enzyme Chemistry'.

The decade following the publication of the later volume in 1934 was mainly preoccupied with the second world war and in consequence there was during most of the time an almost complete cessation of new public works construction.

Nevertheless, perhaps indeed on account of this, it has been possible to devote closer attention to the actual operations for the disposal or purification of waste organic matter whether by means of sewage 'works' or sewage 'farm'. As a result new phenomena have been studied and in some cases quite new view-points have developed, from which older knowledge can be usefully criticised.

In the absence of such research work those who were closely occupied with the daily duties of works or farm were content to base their operations on the simple sequence with which most would be familiar, viz. the production of ammonia or amino compounds in the sewage tank by the bacterial decomposition of protein, the oxidation of ammonia to nitrate, and the recovery or fixation of nitrogen from the air by the activity on the farm of leguminous plants. By the application of such knowledge it was possible to run a works or a farm with reasonable success. There still remained the question of *sludge*, the 'slimy deposit' left in the settling tanks. This was 'disposed of' in various ways or 'digested' with production of methane to be used for power, leaving behind the same residue as would be found at the bottom of a bog and with the same lack of agricultural usefulness.

Besides liquid nitrogenous waste or sewage, i.e. 'water after it has been used', is the dry or semi-dry refuse arising from the 'conservancy' methods of towns, this material being either burnt in destructors, with resultant piles of useless dust, or disposed of in 'controlled heaps' not infrequently becoming a paradise for rats.

It has been well said that if the same attention were to be given to sludge and other kindred waste material as has been given to coal, which, after all, is only an advanced stage of sludge, results of even greater value might be expected. The subject, as a valued correspondent put it after a talk with Sir Robert Robertson, affords not merely a field but a 'perfect prairie' of research.

Clearly, to vary the old proverb, if a chain is to be strong every link must be able to stand the strain. Thus for the economic conversion of waste organic matter into food for man and animal there must not be preventable loss at any point.

Pondering upon the many possibilities of new knowledge the writer was led to reconsider some important observations of his earlier years connected with the function of nitrate in the sequence of changes involved in the complete conversion of putrescible organic waste matter into inoffensive plant food. This and no less, it may be said, is the true objective of the sanitary chemist and engineer.

Consequently, the writer put together his thoughts on the subject in a tentative paper entitled: 'The Function of Nitrate in Sewage Purification' and sent a few copies to his fellow-workers in the sewage field for their criticism. A considerable file of interesting and important comments resulted and the present paper is based on the original draft amplified by still more recent observations which have been made in this and other allied aspects of the cycle.

The paper may thus be conveniently divided into two parts, viz.—

I. The Function of Nitrate in Sewage Purification.
II. Other Links in the Life Cycle.

## Part I.

### *The Function of Nitrate in Sewage Purification.*

Early in the writer's experience in the field of Sewage Purification, viz. as far back as 1901, he published a paper before the Royal Institute of Public Health entitled: 'Some Points in the Management of Septic Tanks and Bacterial Contact-beds.'

The paper was accorded a good deal of attention at the time and the main conclusions were embodied in the Annual Report of the Manchester Corporation for the year ending March, 1901. The following extracts have a special bearing on the subject now under consideration:—

> The thorough drainage of a bacteria bed is of the first importance in securing a good effluent. If the water cannot get out, the air cannot get in, and the lower parts of the bed rapidly become putrid and the nitrates decrease, perhaps are quite absent. Here it must be emphasised that when the nitrates decrease and simultaneously there will always, as a rule, be an increase of nitrites, the bed must be rested.
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> On examining the material of a contact bed in active condition, every piece of it will be found coated over with a slimy growth. If this is removed it is found to be a stiff jelly which after a little drying can be cut with a knife.
>
> If placed in a tube containing air, and connected with a manometer, the jelly will rapidly absorb all the oxygen with production of carbon dioxide. This action will sometimes produce a vacuum equal to several inches of mercury. This experiment shows that there is little need to force air into a bed.
>
> For the successful working of bacteria beds, the following methods of procedure will be calculated to give the best results: The bed must be worked very slowly at first in order to allow it to settle down and the bacterial growths to form.
>
> The burden should not be increased till analysis reveals the presence of surplus oxygen, either dissolved or in the form of nitrates, in the effluent.

As a result of the careful adherence to this procedure in the days of early enthusiasm effluents of exceptionally high nitrate content were obtained so that it was possible to adopt a policy of mixing highly nitrified effluents with those less efficiently purified giving a mixture which was non-putrefactive and which was accepted by the statutory authorities.

There can be no doubt that when a contact-bed is worked strictly according to the rules laid down in the early days of intensive study, high nitrification results, and in the case of secondary bed 10*a* (at the Davyhulme Works) an almost indefinite prolongation of the life of the bed was achieved. This bed was stopped on the 22nd February, 1932, after having been in operation for 27 years. During the last two years in which the bed was in operation the nitric nitrogen content in the effluent was maintained steadily at an average of 0·82 parts per 100,000. From the corresponding figures for ammoniacal nitrogen in the influent and the effluent, viz. 1·81 and 1·09 respectively, there would not appear to be much loss of nitrogen during the passage of the influent through the contact bed. In this somewhat remarkable case it is evident that the bacterial jelly with its adsorbed organic colloids was continually converted under steadily maintained aerobic conditions into easily drainable *humus*. Some of this no doubt escaped as fine particles in the effluent and the remainder formed part of the active body of the contact bed.

It is at any rate clear that the presence of well oxidised humus is favourable to active nitrification.

The relation between the presence of humus and the nitrification of organic matter would seem to have been first emphasised by Adeney in his classical researches on the conditions of oxidation of sewage matter.

Adeney concluded that oxidation of organic matter proceeds in two well-defined stages which may be briefly described as the *carbon* oxidation stage and the *nitrogen* oxidation stage.

While confirming the conclusions of earlier workers that the nitrous organism cannot carry oxidation beyond the stage of nitrite whereas the nitric organism only oxidises nitrites to nitrates, he added the further important conclusion that the presence of peaty *humus* matter appears to preserve the vitality of the nitric organisms during the earlier stages of the fermentation process and to establish the conditions whereby it is possible for the nitric organisms to thrive simultaneously with the nitrous.

The somewhat curious fact of the apparent necessity of the presence of humus, if sewage matter is to be fully nitrified, is left unexplained and undiscussed. The importance in publications of scientific research of accurately recording facts, even if not completely understood, is once more emphasised in these observations of Adeney.

A very important paper has since been published entitled: 'Some Further Considerations on the Oxidation of Sewage' by F. R. O'Shaughnessy and S. J. Roberts (*J. Soc. Chem. Ind.*, Vol. 57, p. 281, 1938) in which the somewhat indefinite reference of Adeney's is shown to be of great significance.

O'Shaughnessy and Roberts state that under conditions such as may obtain in practice carbon and nitrogen oxidation may proceed simultaneously but the oxidation of ammonia is dependent not upon the absence of carbon but upon the presence of *humus* solids.

It is evident from this observation that the sludge produced after nitrification has set in, i.e. what may be termed a 'nitrifying sludge', is of a quite different character from that obtained when aeration is continued merely to the 'clarification' or even 'carbon oxidation' stage. The earlier partially oxidised product may be described as *floc*, the other as *humus*.

There is, however, a still further stage to be considered. In describing the biochemical changes taking place in the contact bed it was pointed out that there is an alternation of active nitrification while the bed is emptying and draining and of *denitrification* when it is standing full. The denitrification process is at the same time one of carbon oxidation, and cellulose and hemi-cellulose derivatives are then converted into *humus* much as they are in the gardener's leaf mould pit.

To limit the aeration in an activated sludge tank merely to what may be termed the flocculation stage is to fail to utilise the reserve of oxygen in the nitrate producible from the ammonia still present and to leave a sludge disposal problem of increased difficulty, *floc* being much less easily drainable than *humus*, and since there is no reserve of nitrate in the interstitial water the sludge is liable to offensive decomposition if allowed to accumulate under anaerobic conditions.

Moreover, this denitrification process can be utilised for the final purification of semi-purified effluent by a mere mixing together either in a final denitrification tank or actually in the stream receiving the effluent.

As already noted, this method of final purification was actually advocated by the Manchester chemists in the days of the contact bed and was accepted as adequate by the supervising authorities. The writer would urge that close study be given to this cycle of nitrification and denitrification changes in the light of all the experience which has accumulated since those early days.

In the old and often quoted experiments of Scott-Moncrieff a high degree of nitrification was obtained by trickling an effluent with high ammoniacal nitrogen content over a series of superimposed trays containing filter media. The course of nitrification was considerably interfered with if the sequence of the trays was altered. The assumption was that the activity of the nitrifying organisms was inhibited.

Recent experience would point to a different explanation. Researches published by the Water Pollution Research Board in their Annual Report for the year ended June 30th, 1938, show that when two percolating filters are operated in series great efficiency results if the action is from time to time reversed and No. 2 filter becomes No. 1 filter and *vice versa*.

It would thus appear more likely that denitrification takes place resulting in more complete oxidation of the organic matter as a whole but less *apparent* yield of nitrate.

That such alternate nitrate formation and reduction takes place even in a compost heap is clear from the early experiments of Rege (*J. Ind. Inst. Sc.*, Vol. 8A, Part XIII, 1925) which laid the foundation of the technique followed in the production of what is known as 'activated compost'.

Rege found that by aerating a mixture of sulphate of ammonia, calcium carbonate and grass powder with activated sludge the ammonia at first was rapidly converted intro nitrate, while on further aeration the nitrate disappeared with, however, no loss of total nitrogen. From this point repeated additions of ammonium sulphate and grass powder were made, when it was found that disappearance of nitrate from the solution took place more and more rapidly with successive additions. Finally, the additions of ammonium sulphate and grass powder were made simultaneously, and at this stage the whole process of nitrate formation and disappearance took place within 24 hours.

Experiments (unpublished) carried out a few years ago at the Indian Institute of Science showed that if finely powdered cellulose matter was added to the aeration tanks in an activated sludge plant (i.e. in presence of ample aeration by means of diffused air) all the soluble nitrogen was removed from solution and was built into the resulting sludge which was very easily drainable and was of the nature of *humus*.

A study of recently published discussions on closed percolating filters leads to the conclusion that improved conditions for nitrification due to rise of temperature is the most important factor in the apparent increased output of a closed filter.

The old observations mentioned earlier of the measurable vacuum caused by the 'breathing' of the bacterial jelly on the medium of the contact bed would seem to render unnecessary the forced aeration of the closed filter. Provided the natural air supply is not impeded it is doubtful whether any artificial increase over the natural draught will seriously accelerate the rate of oxidation of the black film frequently present on the under-surface of the slimy coating on the filter medium. On the other hand, the infiltration of a solution of nitrate would facilitate the oxidation of the black film with simultaneous reduction of nitrate.

No doubt in absence of adequate aeration *nitrites* may be formed with consequent loss of nitrogen either in the free state or as nitrous oxide which gas was actually found by Letts to be present in a contact bed under certain conditions. It must be remembered that every *percolating* filter is really intermittent in its action. Unless the influent is actually run through in a single mass as in a mechanical water works filter there is an interval between the arms of a rotary distributor or the return journey of the travelling distributor on a rectangular filter which must produce conditions alternating between nitrification and denitrification.

Careful study of the conditions of compost making shows the necessity for adequate aeration if loss of nitrogen is not to take place. Howard, indeed, advocates that compost heaps should be provided with aerating chimneys and ample under-drainage.

The periodic 'turnings' of a compost heap again are likely to produce conditions of alternate nitrification and denitrification, although simultaneously carbon oxidation will go on. If there is no actual loss of nitrogen in this case, and careful frequent analyses seem to negative this possibility, then the ammoniacal nitrogen must eventually be 'demineralised' and be 'built in' to the *humus* which finally results, passing no doubt during the process through the bodies of many living organisms characteristic of the special conditions obtaining in any given case.

Attempts have been made by various workers in the past to purify sewage merely by the addition of nitrate of soda in quantity sufficient to supply adequate oxygen through denitrification. It is doubtful whether the conditions thus arranged are really the most suitable for efficient purification. The following passage from the Annual Report of the Rivers Department of the Manchester Corporation for the year ending March 27th, 1901, p. 73, is not without significance:—

> 'It was found that when a primary filtrate was allowed to stand overnight in contact with air, or when it was aerated by shaking every quarter of an hour during two hours, a distinct improvement resulted, though almost invariably at the expense of the nitrate present.
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> 'The economical bacterial purification of sewage on a large scale depends essentially upon the addition of oxygen *in presence of the requisite bacteria* [1]; consequently, although aeration alone is incapable of appreciably purifying sewage (see City Surveyor's Report, 1897, page 29), yet when aerobic bacteria are introduced by addition of a volume of well-nitrified filtrate, it is probable that aeration may play a much more important part.'

Here possibly may be seen an adumbration of the Activated Sludge process.

Finally, reference may be made to the remarkable results obtained by Lockett at Mogden by what he terms the 'complete process'. In this technique Lockett relies upon the frequent complete conversion of ammoniacal into nitrate nitrogen. This is effected by recirculating a large proportion of the highly nitrified effluent together with a high proportion of nitrifying sludge. Lockett describes the sludge so obtained as settling very rapidly and being easily filtered and has stated in a letter that it contains more than 8% of nitrogen. It might be assumed that this high percentage of nitrogen is primarily due to what may be termed the 'fractionating' of the sludge by the removal in presettlement tanks of a large proportion of the mineral matter which would otherwise form part of the final product. In view of other observations, however, it is possible that this high percentage of nitrogen content is due to the 'demineralisation' of ammoniacal nitrogen already referred to in connection with Rege's researches on compost. In any event Lockett's results entirely confirm the conclusions of workers in other spheres, notably Howard who writes from the point of view of an agriculturalist, that aerobic conditions are necessary if true *humus* is to be obtained and, it might be added, if the maximum conservation of nitrogen is also to be effected in the various techniques employed in the utilisation of habitation waste.

The present writer's conclusion is that the true conditions for the efficient and economical purification of sewage consist in the building up of an initial mass of highly active nitrifying sludge and mixing this with the sewage under conditions of *adequate aeration.* A state of things is thus set up in which the nitrate present is alternately reduced and re-formed from fresh additions of ammoniacal nitrogen, the humus which is simultaneously produced showing a high percentage of nitrogen

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[1] Italics absent in original reference.

due to the building of ammoniacal nitrogen into the bodies of living organisms and possibly also to the formation of complex organic molecules by the combination of the nitrogen with the carbohydrate residue present.

In this way the nitric nitrogen present virtually acts as a catalyst and once the correct conditions are established a considerable reduction in the power consumption required for aeration should be achieved. A hint of such a result is given in his Report for 1937 by the Chief Sewage Chemist of Shanghai. He speaks of having more or less by chance obtained a sludge in all respects satisfactory with a *low power consumption*. This means that it is the *quality* of the sludge, quite as much as the quantity of air employed, which has to be studied and properly understood.

The present writer would urge the importance of renewed careful and exact research with the object of obtaining further knowledge on these lines. Such researches may result in very valuable additions to the underlying theory of the activated sludge process and make ultimately for greater economy and efficiency without loss of the agricultural value of the end products.

## Part II.

### *Other Links in the Life Cycle.*

The foregoing pages are thus chiefly concerned with the two end products of sewage purification, viz. *nitrate* and *humus*, and their relation to the actual technical processes employed in the 'sewage *works*'.

Essentially, the same changes go on in the 'sewage *farm*' when nitrogenous waste matter, whether liquid in the form of sullage or sewage, or semi-solid in the shape of various forms of organic manure, is brought into contact with the soil, there to provide food for the growing plant.

Here, however, other factors enter into the cycle. Through the interaction of oxidisable matter with the soil particles, purely physico-chemical factors have greater scope. Forms of life other than specific and well-known types of bacteria play their part, and finally the conditions of life of the growing plant affect the changes occurring in the soil complex in which it grows.

Just as knowledge gained on the sewage works may be applied in the running of the sewage farm, so observations made on the 'sewage farm' may find useful application in the operation of the 'sewage works'.

The 'Newly Observed Links' may, therefore, be considered under the following categories. The active agencies comprised in these categories contribute, in collaboration with the two end products (i.e. nitrates and humus) so far considered, to the life of the growing plant and so to the life of men and animals:—

1. Physico-chemical factors.
2. Bacteria concerned with proteolysis, nitrification and denitrification changes, and immobilization or translocation of nitrogen.
3. Biological factors concerned with nitrogen fixation. Bacteria, free living and symbiotic. Other living agencies.
4. The Mycorrhizal association.
5. Protozoa.
6. Animal and man.

### *1. Physico-Chemical Factors.*

It is to Dr. N. R. Dhar and his co-workers that we owe the most recent and continuous research on this subject. While it is commonly believed that the processes of ammonification, nitrification and nitrogen fixation are mainly the result of bacterial activity, Dhar has emphasised the importance of purely physico-chemical factors, sunlight being the source of energy. The researches of Dr. Dhar and his colleagues up to 1937 are collected and summarised in a valuable paper in the *Proceedings of the National Institute of Sciences of India* (Vol. III, No. 2, pp. 75–131) under the title of 'Nitrogen Transformations in the Soil'.

Thus to take the simpler stage of *nitrification* of ammonium salts, experiments in vitro when ammonium salts were mixed with sterilised and unsterilised soil, exposed to sunlight and kept in the dark, a much greater percentage of ammonia was oxidised to nitrite and nitrate in the case of the mixtures exposed to sunlight whether the soil was sterilised or unsterilised. Similarly dilute sodium or potassium *nitrite* solutions are readily oxidised to nitrate when exposed to light.

Dr. Dhar, therefore, considers that the bacterial factor in nitrification has been somewhat over-emphasised especially in tropical countries.

This point of view finds confirmation in the old experiments of Cavendish who brought about oxidation of ammonia on surfaces in 1777. There is also the well-known technical method of producing nitric acid by the passage of ammonia gas over heated platinum gauze, as well as the experiments by Warburg confirmed by Norris and Ranganathan showing the effect of animal charcoal in accelerating the ordinary bacterial nitrification process.

While these purely physico-chemical factors, especially the effect of tropical sunlight, may be freely admitted and deserve further study, especially in connection with production of nitre in so-called nitre beds, yet it is difficult to believe that sunlight has much influence in the nitrification process as effected in the various filtration processes in the sewage works where it is often highly efficient, and where it must occur in the inner depths of the filter bed entirely cut off from sunlight. The same conditions are present in the activated sludge tank and also in the compost heap, although to a less extent, since there is in both cases a periodical change of surface at longer or shorter intervals. It would be of interest to find out by experiment whether any different results were obtained when these processes were operated under conditions differing only in the presence and absence of sunlight.

*Ammonification.*

Dhar and his colleagues found that substances like egg white, gelatine and blood serum, yielded ammonia when exposed to air in presence of sunlight the amount being greatly increased in presence of solid surfaces like $TiO_2$, $ZnO$, $SiO_2$, etc., the best results being obtained with $TiO_2$.

It was found that these reactions were autocatalytic inasmuch as when the $p$H increased through the increase of ammonia the rate of oxidation increased proportionately. Thus it would appear that the formation of ammonia in the soil is a surface reaction and chiefly an oxidative process, taking place on the soil surface with liberation of energy.

Similarly experiment indicated that nitrite could be converted to nitrate by direct aeration especially in presence of inductors like ferrous hydroxide, sodium sulphite, etc., where another physico-chemical action is involved, viz. *induced* oxidation.

Dhar, therefore, concludes that the processes of ammonification and nitrification can be photochemical rather than bacterial especially in tropical countries where the number of bacteria is small being mostly killed by the high temperature of the soil during the summer months.

That oxidation changes in the soil, resulting in the quicker availability of organic manures, can be accelerated by purely *chemical* agencies has been shown by the work of C. R. Harihara Iyer and V. Subrahmanyan who have studied the effect of small quantities of manganese and iron salts on the fertilising activity of the soil during the life-time of the crop, thus compensating in some measure for the losses of plant food ingredients during periods of fallow, which in tropical countries are greater than in temperate zone climates. Their main findings have been confirmed by others. Thus Vyas has reported that manganese and iron oxides could be used to counteract the toxic principles left in the soil by 'jowar' and thus increase the yields of the succeeding crop.

These inorganic chemicals could be applied directly to the soil or be incorporated with the manure prior to application to the soil.

The researches of the aforesaid workers agree in showing that the purely oxidative changes concerned with the conversion of organic matter into end products of humus and nitrate are at any rate capable of being considerably affected by non-living agencies both chemical and physical.

For the practical application of these results experiments are called for which would determine the economic factors involved. Of these one of the most obvious is the cost in labour or mechanism incurred by adequate exposure to sunlight of the surface both of soil and of compost heap.

It would be of interest to compare the results from two sections of an activated sludge plant comparable in all respects save that one was exposed to sunlight and the other 'blacked out'. In both sections there would be an equivalent exposure of fresh surfaces only one of which would be exposed to sunlight.

The same principle might be applied in the case of compost heaps though here the change of surface could hardly be complete.

In view of recent unpublished experiments by Pillai and Subrahmanyan on the effect of dilution of sewage in presence of oxygen and the observed streaming effect of dissolved oxygen, e.g. in the rusting of strips of suspended iron, it would be useful to compare the changes taking place in sterilised and unsterilised material not only in presence and absence of sunlight but also in presence or absence of air. Does sunlight influence anaerobic changes?

*Nitrogen Fixation.*

Purely physico-chemical factors play a considerable rôle in the more complicated changes concerned with the important 'link' of nitrogen fixation, important since without it there would be permanent loss of nitrogen from the cycle of life.

Dhar brings evidence to show that the energy available by direct oxidation of organic matter in the soil as described in the foregoing paragraphs can be utilised to bring about the oxidation of other organic matter by induction or by catalysis, as well as by bacterial action. Thus the addition of molasses to soil in presence of sunlight brings about an appreciable increase in the nitrogen content of the soil under conditions of adequate exposure to air and sun.

The same arguments therefore in regard to the economics involved, i.e. the labour or mechanism needed for continual exposure of fresh surfaces to sunlight holds also in the derived reactions concerned with nitrogen fixation.

It is of interest in this connection to note that the activity of worms in chewing up raw organic debris such as leaves, etc. must result in making a product which is more readily oxidisable and so any system of manuring which affects the worm population such, e.g. as an excess of 'mineral' manures, will tend to retard nitrogen fixation.

*Trace Elements* particularly boron and zinc may play some part in these physico-chemical effects, as well as in the ammonification effects already referred to. Although the quantities of these may be infinitesimally small, a few parts per million in the soil and less in the plant, they are found to be essential although larger amounts may be toxic. In a recent address Dr. W. G. Ogg, the new Director of Rothamsted, divides trace elements in Agriculture into four groups:—

(i) Those necessary for normal healthy plant growth. Among these are comprised Boron, Manganese, Copper and Zinc.
(ii) Those which are toxic to plants, e.g. excess of Manganese.
(iii) Those necessary for animals if not for plants, viz. Cobalt and Iodine.

(iv) Those poisonous to animals but not to plants. Among these may be classed Molybdenum and Selenium.

For research on such traces quantitative spectrography has been found valuable. The necessary apparatus is hardly likely to be found in the normal sewage works laboratory.

Besides the necessary presence of these minute quantities of trace elements, the absence from the soil, or it may be from the fertiliser, of certain minerals may have serious effects. Thus a deficiency of calcium may be detrimental to the rape plant or may cause manganese toxicity to develop in cauliflowers. Potash deficiency may adversely affect barley and mangold plant. Magnesium in defect is shown by leaf disease in tomatoes and pears.

Clearly in a prepared sludge for the application to a given crop all these deficiencies can be suitably adjusted.

### 2. *Bacteria concerned with proteolysis, nitrification and denitrification changes, and immobilisation or translocation of nitrogen.*

Although it may be admitted that the three first of the above-mentioned natural processes occurring in the normal life-cycle can be brought about by physical or physico-chemical agencies, yet there is abundant evidence that under normal circumstances of the sewage works or sewage farm the chief agencies are specific bacteria. Accounts of their nature and mode of action will be found in text-books dealing with the bacteriology of agriculture and sewage purification.

Reference has been made in Part I to the immobilisation of soluble nitrogen in the compost heap and in the activated sludge tank by aeration of soluble ammoniacal salts in presence of hemi-cellulose material. This reaction is of considerable practical importance in connection with the utilisation of the effluent from the activated sludge tank for the irrigation of crops since it is frequently the case that an excess of nitrogen is prejudicial to the crop at certain stages of its growth. Scientific control of the amount and character of the nitrogenous content both of effluent and sludge is therefore needed if the nutritional requirements of the crop are to be properly met.

### *Humus and Plant Nutrition.*

The extensive and world-wide observations which are being made by Sir Albert Howard and his numerous fellow-workers and which have been published in sundry volumes and in the issues of the *Compost News Letter*, now appearing as *Soil and Health*, make the old time crude efforts to dispose of the sludge on the land for crop production seem rather elementary. In those days N.P.K. percentages were almost the sole criterion of value and short-time results and ease of application made 'artificials' popular and the 'law of return' was ignored. Many years ago I remember while we were washing up the old contact beds at Davyhulme there was a demand for almost any kind of dried sludge, or humus forming material, to bring back fertility to the eroded and wind-swept soils of Canada.

In the last issue of *Soil and Health* there is the first of a series of papers by J. E. R. McDonagh, F.R.C.S., on the 'Nature of Health and Disease in Plants' in which particular attention is drawn to the *Rôle of the Sap Protein in Health or Life*.

In a recent address on *Food and Phylogeny* Dr. C. S. Haynes, F.R.S., classifies nutrients as (*a*) sources of cellular energy, and (*b*) sources of specific chemical molecules required for growth which a particular organism is unable to synthesise for itself.

Nutritional requirements are the reflection of basic biological problems of biosynthetic capacities of different organisms.

It can hardly be expected that such complex requirements can be met by mere addition of elementary chemicals like sulphate of ammonia any more than any kind of protein molecule will serve as human food. More than a decade ago it was shown by Rose that only certain amino acids were of nutritional value. Recently these observations have been extended and comparatively small differences in molecular structure of various isomeric amino acids have been shown greatly to affect their susceptibility to enzyme action and consequently their nutritional value. Similarly the detailed structure of complicated anti-malarial drugs affects their toxic properties.

It is evident that careful preliminary preparation of putrescible sludge: (i) by final oxidation by direct aeration or by composting in presence of air, (ii) by elutriation, (iii) or by other means which may be revealed by research, will convert the one-time 'slimy deposit' into valuable plant or even animal food. It has been already hinted that there may be a future before sludge chemistry comparable to the coal-tar industry. Unlike coal which has to be taken as nature left it, sludge can be modified in composition and properties while it is in the course of production. Already in the U.S.A. household implements have been devised for eliminating household kitchen waste by disintegration and discharge into the sewer to be treated along with other sewage solids and recovered with the remaining sludge. If there is a large proportion of hemi-cellulose residues present it may have the effect of immobilising a certain amount of soluble nitrogen and withdrawing it from the effluent into the sludge. Such a process is important when the effluent has to be used for irrigation of crops as these may find too much nitrogen detrimental at certain stages of growth. In the absence of refuse cellulose material it may indeed pay to add some suitable source of hemi-cellulose such as chopped grass for the specific purpose of immobilising ammoniacal nitrogen. Preliminary experiment at Bangalore has shown this to be possible.

Briefly it may be stated that anaerobic action transfers nitrogen from the sludge to the effluent, aerobic action in presence of cellulosic material transfers nitrogen from the effluent to the sludge.

### 3. *Biological factors concerned with Nitrogen fixation. Bacteria, free living and symbiotic. Other living agencies.*

An excellent compendium of information on the 'Fixation of Atmospheric Nitrogen in Living Forms' has been published by T. R. Bhaskaran and S. C. Pillai in *The Indian Journal of Agricultural Science*, Vol. XII, Part I, February 1942.

They confirm the general thesis that the amount of nitrogen fixed is proportional to the energy developed by carbon oxidation in a given time.

In their summary they set out no less than 50 items. Only the more striking and recently observed of these can be here mentioned.

*Azotobacter* and *Clostridia* are the important non-symbiotic organisms which fix nitrogen in the soil. The azotobacter is typical of the aerobes and the clostridia of anaerobes. Azotobacter uses carbohydrates, salts of organic acids and alcohols as energy sources. Soil humus has been found to exert a stimulatory influence on the organism for nitrogen fixation. Vitamin $B_1$ and phytonucleic acid stimulate growth and nitrogen fixation. Certain minerals in optimum concentration are necessary for the growth of azotobacter and among these calcium (replaceable by strontium) and molybdenum (replaceable by vanadium) are specific for nitrogen fixation; manganese and uranium accelerate nitrogen fixation. Iron plays no specific rôle in the process.

Light is not without effect on the activity of this organism; thus, in a measure, confirming the conclusions of Dhar, but yellow light is better than blue.

*Hydroxylamine* would appear to be the first intermediate product formed during nitrogen fixation.

Bhaskaran and Subrahmanyan have reported that the fixation of nitrogen by the mixed flora of the soil follows a different course from that of azotobacter alone in artificial media. In the latter case fixation only proceeds so long as the sugar lasts in the medium. With a mixed flora only a small quantity is fixed in presence of sugar while the major part, amounting to over two-thirds of the total quantity fixed, is fixed in the later stages. They have further shown that the products of decomposition of sugar are utilized in this subsequent fixation.

These products of decomposition of sugar consist of simple organic acids and alcohols. It is likely that the energy resulting from their oxidation is utilised for nitrogen fixation by purely physico-chemical reactions in accordance with Dhar's observations.

*Other agencies.*

Long ago Jamieson of Aberdeen contended that nitrogen fixation took place primarily through the agency of the leaf hairs which produce albumin from the nitrogen of the air. According to Bhaskaran and Pillai several workers have reported from time to time that different parts of higher plants exhibit the power of fixing atmospheric nitrogen either by themselves or by their association with the bacteria present in them but evidence so far obtained is still inadequate to draw any definite conclusion regarding the relative importance of these as nitrogen fixers.

It may well be that the extent of nitrogen fixation in any given case depends on the conditions obtaining in each case, viz. the presence of symbiotic agencies whether plant or micro-organism or on the availability or otherwise of nitrogen from other sources.

The activities of protozoa in the field of nitrogen fixation will be referred to when considering their specific functions in other portions of the cycle.

## 4. *The Mycorrhizal Association.*

The mycorrhizal association may be defined as the mechanism by which living fungous threads (mycelium) invade the cells of the young roots and are gradually digested by these.

This important link in the nitrogen cycle has received detailed attention in the writings of Sir Albert Howard and his school. I well remember the visit that Sir Martin Forster (Dr. Forster as he then was) and I paid to Pusa in the early nineteen twenties and the fascinating examples then shown us by Howard illustrating the importance of root development and of how a well-developed root system was virtually a mirror image of the plant above ground. This lesson I have since striven to impress upon sundry mahlis who prefer to souse a plant with water rather than to do a little careful digging in order to maintain a reasonable amount of root aeration. Fully to expose the root system involves, as Howard showed us, very careful washing away of the surrounding soil.

At that time no mention was made of the mycorrhizal association the significance of which was not fully understood, although the association of fungus mycelium with root cells had been observed, and the term mycorrhiza given to the mycelium, as early as 1829.

The careful researches of Dr. Rayner on the mycorrhizal association in relation to conifers at Wareham in Dorsetshire where small additions of properly made compost had produced spectacular results, led Howard in 1937 to consider the possibility of the phenomenon being general and of its having some special function in connection with the nutrition of the plant on the roots of which it was observed.

Careful observations were then made by Dr. Rayner and Dr. Ida Levisohn and others of the root systems of many plants for evidences of the mycorrhizal association. It was found that plants manured with artificials or grown on derelict land showed poor development. Dr. Rogers of East Malling in Kent devised an observation chamber for root studies. He arranged a vertical darkened glass window on the side of a deep pit in an orchard. In this it was possible with the assistance of a conveniently arranged low-power microscope to observe some of the soil fungi actually at work. (A photo of one such observation is given in Howard's recent book, p. 35.)

The universally beneficial effect of organic manure whether in the form of compost or other commonly employed humus-forming material is thus seen to be due to the support given to the necessary fungous mycelium.

These careful microscopic observations show that in the invaded cell the mycelium exhibits a regular sequence of changes from invasion to the clumping of the hyphae around the cell nuclei, digestion and disintegration of their granular contents and the final disappearance of the products from the cells. In this way the digestion products of the proteins of the fungus pass into the cell sap and thence into the green leaves.

The mycorrhizal association has been found to be a very widespread phenomenon. The following important crops are all mycorrhiza formers : wheat, rice, tea, coffee, cacoa, sugarcane, cotton, sisal, maize, cocoanuts, bananas, citrus fruits, grapes, apples, pears and peaches.

Some singular exceptions occur, viz. tomato and cabbage. The beneficial effect of organic manure is nevertheless clearly observable in these cases and it appears likely that the protein requirement supplied by the mycorrhizal association is in these cases derived from the dead bodies of the bacteria present in the organic debris.

While it might be supposed that legumes would be sufficiently supplied with nitrogenous nutriment through the intervention of their root nodules it appears that they also need the assistance of the mycorrhizal association if they are to retain the power to produce seed.

The relation between the intake of protein and the observed power of disease resistance is explained by J. E. R. McDonagh by the character of the protein digestion products supplied to the cell sap as already referred to in section 2.

The ultimate consequences of these new observations and conclusions are very far-reaching. In order to maintain the necessary supply of organic manure, 'mixed farming' is essential, i.e. proper rotation of crops and the intervention of livestock. There must indeed be a definite ratio between the number of livestock and the crop acreage.

## 5. *Protozoa.*

It has been contended that the ordinary British mind prefers action to meditation with the result that experience is gained through the encountering of practical difficulties which might have been avoided by the expenditure of more time and thought on preliminary investigation. A further consequence of this method of procedure is that important work of an apparently recondite character is overlooked and its true significance is only appreciated when its bearing is seen on more practical issues. In few instances is this feature more strikingly exhibited than in the history of the *activated sludge process* of sewage purification.

I must admit my full share in this apparent blindness to what are now fairly obvious clues to a true scientific theory of the process. In preparing my earlier book on 'Bacteriological and Enzyme Chemistry' I must have become aware of Munro's experiments on the acceleration of the nitrification process by a technique of *Activation* identical with that used in the building up of activated sludge. Yet, unless subconsciously, it had no direct influence on the course of the early research work on which the process was based, although it was fully recognised later. Of possibly even more importance has been the failure to recognise until quite recently the fundamental significance not only in the activated sludge process but in the nitrogen cycle generally of the activity of *protozoa.*

That the presence of protozoa in sewage and effluents was long ago recognised is clear from a discussion held at a meeting of the Royal Sanitary Institute in 1909 on the effect of biological conditions on the quality of effluents. Here the discussion centred round the question of how far merely chemical figures of analysis really were sufficient to define the effect of an effluent on the stream into which it flowed. It was contended that an effluent adequately purified so far as the chemical figures indicated might yet start up various living growths—fungoid, algal or protozoan—which in turn might adversely affect the amenities of the stream. Among the protozoa then under observation was the vorticellid known as *Carchesium Lachmannii* and Mr. Glover, chemist in charge, under my direction, at the Withington works of the Manchester Corporation, made some excellent *camera lucida* drawings of the development of this organism and established the fact that fission of one head and formation of two complete organisms took place within three quarters of an hour. These drawings were reproduced in the Annual Report of the Rivers Committee and were copied by my former good friend the late Dr. Calmette in one of the admirable reports he was then compiling for the advancement of sewage purification technique in France. This remarkable rapidity of reproduction was of great significance in relation to the possible function of this or kindred organisms in nature. It was to be emphasised that the growth did not occur in effluents which would be classified by chemical standards as unpurified but in discharges which were actually in process of nitrification. Thus an abundant growth of *carchesium* was afterwards noticed in the effluent channel from a final or 'secondary' contact bed at the Davyhulme works, this bed by the way being operated on the continuous flow system. All these observations can now be seen to have a close bearing on the function of protozoa both in the operation of continuous filters and of the activated sludge process. Unfortunately again the findings of distinguished workers in other fields tended unduly to influence the conclusions of many of the earlier workers on the activated sludge process, and so it is only in quite recent years that the true function of protozoa has been recognised and fully investigated. The early history of the Activated Sludge process is admirably set out in the remarkable book by A. J. Martin (*The Activated Sludge Process* by Arthur J. Martin—London, Macdonald and Evans, 8 John St., Bedford Row, W.C. 1, 1927) which becomes of greater historical value as the years go on.

Protozoa of differing species were described by various observers but were thought to be incidental to the process rather than essential. The study of the experimental activated sludge plant at the Indian Institute of Science by Swaminathan, done largely under my supervision, was influenced by the results of Russell and Hutchinson on the apparently favourable effect on plant growth, notably of tomatoes, by the elimination of the protozoal population by the old gardeners' recipe of heating the soil. This was confirmed by Fairbrother and Renshaw who used methylene blue as a partial sterilisation agent. Swaminathan's observations did not indicate any marked effect favourable or otherwise on the process from the application of this treatment to the activated sludge as distinct from soil, although it was confirmed that the effect of partial sterilisation was to increase the number of bacteria in the sludge as moderate heating had increased the number of bacteria in the soil.

Apart from protozoa it was suggested by Dr. Bartow, one of the most distinguished of the early scientific investigators of the activated sludge process (Dr. Bartow later was elected President of the American Chemical Society), that the red worms *Aelosoma Lemprichii*, often found in decaying organic matter, played some useful part in the purification process. This idea was however soon abandoned. The ordinary 'blood worm' the larvae of *chironomus* is unfortunately well known as a parasite on the useful forms of life in the activated sludge tank.

As a consequence of all these observations, incomplete as we now know them to be, most workers including myself were of opinion that the activated sludge process was mainly dependent on bacterial activity, associated as in the case of the well-known $M_7$ with a certain proportion of organic iron compounds as a precipitating agent.

I looked upon the process as one of intensive bacterial oxidation, acitification being included under this general category. Unfortunately among bacteria could be classed those higher thread like species such as *sphaerotilus* and *leptomitus* characteristic of polluted but partially aerated streams. Such growths have been classed as a form of activated sludge, although they have nothing in common with the true product and have little or no clarifying still less purifying effect.

Apart from bacterial activity it was also recognised that physical factors, notably the mechanical flocculation of colloidal particles, played their part.

Martin's book was published in 1927. At that time the remarkable researches of Cramer of Milwaukee had not been published (1931) and the important detailed working out of the subject by Pillai and Subrahmanyan, and their collaborators had not been undertaken and the detailed work of Gurbaxani only recently successfully submitted as a Ph.D. thesis is still awaiting publication. Dr. Gurbaxani is now pursuing his studies under the leading sewage purification specialists in the U.S.A.

Although Cramer's work finds mention in my book published in 1935 it even then appeared more as supporting an interesting theory than as of basic and fundamental importance not only to the theory of the activated sludge process but to the operation of the nitrogen cycle in general. Consequently, although a brief mention of it appears in my book, the importance of the remarkable details described in the original paper was not fully realised partly perhaps because the methods of investigation were somewhat unusual. In the light of the tabulated experimental results given in Gurbaxani's thesis their importance becomes obvious. Cramer employed an original method for preserving an aerobic atmosphere. Instead of bubbling air through the liquid under investigation he used a small quantity of sodium chlorate in order to operate under more easily controlled conditions.

Cramer draws attention to the fact that while the activated sludge process may give fairly satisfactory results without the formation of nitrates yet adequate oxygen is essential showing the process to depend on living agents. Purely mechanical flocculation of colloidal matter either by mechanical stirring or by injection of nitrogen or $CO_2$ does not deal with impurities in solution.

Free access of air was assured by employing dishes of only 4·3 cm. in depth with a surface area of 23 sq.cm. Under these conditions heat sterilised sewage if left alone became septic. The addition of sodium chlorate to the extent of 0·3% prevented the sewage from becoming septic but did not produce clarification. Further addition of 1 c.c. of raw sewage produced clarification. Sewage bacteria in separate culture did not clarify nor did yeasts or the enzymic solution obtained by crushing activated sludge with sand and filtering.

It was found that if activated sludge was heated to 60°C. for 30 minutes all protozoa were killed but many bacteria remained alive. Inoculation with this sludge did not produce clarification in sterile sewage which contained 0·3% of sodium chlorate. Further addition of a drop of water containing a *single protozoan* produced clarification in a week.

If a small amount of sludge from this clarified sample which contained many individuals of one type of protozoan only was added to sewage that had been first sterilised and then inoculated with bacteria and yeasts only and to which 0·3% of sodium chlorate had been added, clarification resulted in 48 hours or less.

In all the experiments in which air was allowed access to the surface of the liquid the neck of the bottle was closed with a sterile cotton wool plug to prevent contamination. When this plug was removed from a bottle containing sterile sewage and 0·3% of sodium chlorate the sewage did not clarify. Microscopic examination showed that it contained bacteria and yeasts but no protozoa.

Further experiments by Cramer himself, for details of which the original paper may be consulted, showed that if the sludge is heated to 50°C. for 5 minutes all protozoa are killed except vorticella—an observation of great interest in view of the obviously resistant character of this organism as shown by the Bangalore researches and also in view of its mode of sustenance which can be actually seen to consist in the ingestion of bacteria and of foecal organic matter. It was observed that when the protozoa die they rapidly disintegrate and become sludge particles. It was believed, although the observation needs confirmation, that during the process of disintegration bacteria could actually be seen emerging from within the protozoa. If true this phenomenon partly explains the increase of bacterial population following partial sterilisation.

Cramer draws the practical conclusion that clarification depends on (1) Aerobic bacterial life; (2) Live protozoa; (3) Oxygen in solution, and consequently that the activated sludge process can best be controlled microscopically and that a stock of normally active sludge should always be kept on hand for inoculation in case of deterioration from any incidental cause of the main bulk in circulation. Cases of temporary stoppage of efficiency through a flush of trade waste, or interruption of aeration, might be met in this way.

Cramer expresses the opinion that the protozoa arrive in the sewage through storm water, infiltration water, or kitchen waste.

The work was performed in the Research Laboratory of the Sewerage Commission of the City of Milwaukee under the immediate direction of Dr. J. A. Wilson. Some portion was done in R. Cramer's own laboratory. Dr. Wilson considers that clarification is proportional to the relation between organic dispersed matter and the amount and vigour of the protozoa.

*Bangalore Researches.*

It was in 1938 that I was concerned with the putting into operation of an up-to-date diffused air activated sludge plant for the Military authorities at Cossipore, Calcutta. It was designed to deal with the sewage from a population of from 8,000 to 10,000. Having seen that the installation was functioning in mechanical order I placed Mr. S. C. Pillai of the Department of Biochemistry at the Indian Institute of Science in immediate supervision until the plant could be handed over to the authorities. In the absence of local facilities for detailed chemical analysis it was fortunate that through the courtesy of the resident medical officer Pillai was able to follow the building up of the sludge by regular microscopical observations. It was then that he confirmed the statement of Cramer and his colleagues that efficient clarification was coincident with the appearance of protozoal life particularly *vorticellids*. Following these observations he undertook, in collaboration with the Professor of Biochemistry, Dr. V. Subrahmanyan, and with the detailed assistance particularly of Dr. M. Gurbaxani, a detailed study of the function of protozoa, with reference not only to the Activated Sludge process but also to the nitrogen cycle throughout nature. He was indebted to the late Prof. B. L. Bhatia of Lahore for specific identifications and to Dr. B. R. Seshachar of Central College, Bangalore, for assistance in the photo-micrographic work. The general results of these researches are available in recent literature although many valuable details still await publication. The following conclusions may be held as established.

*Flocculation of Colloids.*

The special function of protozoa in flocculating sewage colloids is clearly evident. Amongst the active species *vorticellids* are predominant and among these *epistylis* is specially efficient being twice as effective as the ordinary type of *vorticellids*. Observations of the sludge from the installation at the Madura Mills, Tuticorin, where the tanks are operated with sea water show that protozoa are active even under these conditions; indeed a species has been isolated and identified as *Zoothamnium* for the first time reported in India perhaps on account of its habitat in sea water.

*Oxidation effects.*

Of even greater interest than the flocculating effect of protozoal activity is the demonstrated conversion of crude foecal emulsion to the stage certainly of the formation of *nitrite* if not completely to *nitrate*. There would seem to be a process of digestion of protein matter akin to the cellular activity of growing plants in digesting the threads of mycorrhizal mycelium and in the case of protozoa of the further oxidation by means of an *oxidase* of the amino compounds so produced. Here then it would appear that we have a direct conversion of human waste matter into plant and even animal food since the masses of epistylis have been found to be readily consumed by rats and poultry in much the same manner as yeast. Indeed many years ago Buswell and Lang considered that the purification of sewage by microscopic communities is entirely similar to the disposal of garbage by feeding it to hogs.

In connection with the remarkable oxidation activity of protozoa and their need for abundant air supply interesting reference is made to an observation of H. M. Vernon in 1897 that protozoa have the largest respiratory coefficient of all invertebrates, one case being cited where this function was forty times that of a frog.

*Elimination of Pathogenic Bacteria.*

A further advantage of this function of the protozoa is that in the course of it, pathogenic bacteria have been found to be completely eliminated. This was shown in early days by the empirical observations of Col. Stewart, then Director of Public Health in Bengal, and is now confirmed in detail by Gurbaxani with specific cultures both of protozoa, viz. *epistylis*, and of *B. Typhosus, B. dysenteriae* and *V. cholera.* The sanitary importance of these findings is obvious.

*Sensitiveness to pH conditions.*

On the other hand, experiment showed that the protozoa were sensitive to changes in *p*H, sudden flushes of acidity proving to be destructive. The bearing of this result on the regulation of trade effluents is important.

*Sources of Protozoa.*

The Bangalore workers agree with Cramer that the protozoa do not derive directly from human excreta but from soil. In dry soil the *vorticellids* exist in the form of cysts, becoming active under conditions of waterlogging. They are naturally to be found in all kinds of stagnant and polluted waters. The interesting fact is recalled that the very first protozoan described by Leeuwenhoek, the inventor of the microscope, was a species of *vorticella* which he had seen in standing rainwater in 1675.

From these observations together with the laboratory experiments it would appear likely that nitrification changes under natural conditions are assisted by the specific activity of certain forms of protozoa.

*Confirmation of Bangalore Researches.*

The findings of the Bangâlore workers have been confirmed by investigators in widely separated centres. Thus Reynoldson has noted the activity of *vorticellids* in the percolating filters at Huddersfield. Hardin of Stanford University, California, records a clear cut case of the flocculation of bacteria through a protozoan *Oikomonas termo*. Watson of the Wellcome Bureau of Scientific Research reports a similar activity of a soil ciliate *Balantiophorus minutus*.

It is of particular interest that in an important report from Manchester by Messrs. Wishart, Jepson and Klein on the Dewatering of Sludge, the abundance or otherwise of *vorticella* revealed by microscopic observation, is taken as an indication of the 'condition' of the sludge. Since the sewage reaching the Davyhulme works contains almost every type of trade waste it may be concluded that provided these do not unduly affect the *p*H of their environment the protozoa continue to thrive and perform their function in producing flocculation. Careful study is required in order to ascertain the economic limit of air supply, as between the production of fully active protozoa resulting in a sludge of high purifying power, and excessive aeration resulting in the 'burning' out of the protozoa.

*Protozoa and Nitrogen Fixation.*

Besides the flocculation of organic colloidal matter and its further oxidation to harmless end products there is evidence, e.g. through the work of Cutler and Bal that *nitrogen fixation* is facilitated by the symbiosis of certain protozoa with the normal nitrogen fixing organisms of the type of *Azoto bacter chroococcum.*

### 6. *Animals and Man.*

Apart from their laboratory studies in connection with the experimental activated sludge plant at the Indian Institute of Science the Bangalore workers have been deputed by the Imperial Council of Agricultural Research to undertake a continuous investigation of *Sewage Farming* under a Scheme of Research approved by the Council. Valuable reports have been published by Messrs. S. C. Pillai, R. Rajagopalan and V. Subrahmanyan. The researches thus reported have been mainly concerned with the local sewage farms at Bangalore, with the municipal sewage farm at Madura, and with the treatment of mixtures of sewage and trade waste as instanced by the municipal sewage farm at Ahmedabad.

In the course of this work many opportunities naturally were afforded to recognise the numerous links which hold together the chain of living activity from man through animal and plant back to man again. These observations have been admirably summarised in a paper by Pillai and Subrahmanyan in the issue of *Science and Culture* for May 1946. Thus they point out that at Madura where some two million gallons of sewage is treated daily on 100 acres of underdrained land, the sewage as it filters through the land and travels down to the effluent channels undergoes rapid oxidation and emerges as a fairly clear effluent supporting micro-organisms including numerous types of protozoa as well as higher forms of life such as worms including earthworms, a variety of insect larvae including those of *chironomus*, gastropods, crabs, frogs, fish (some eight varieties), tortoises and water snakes. Some of the visible forms attract birds of prey and many of the fish are consumed for human consumption. Since the sewage irrigated areas are intensely farmed for grass, vegetables and fruit trees there is thus at the Madura Sewage Farm an unbroken life cycle.

The function of the protozoan link in transforming the organic matter of sewage has been described in the last section. It would be of interest if the roots of the various crops were examined for the mycorrhizal association.

The various constituents of *plankton*, including protozoa, as food for fish need careful investigation from the important point of view of increased production of fish for human consumption. The authors suggest that a stage intermediate between protozoa and fish might be cultivated, e.g. in special fish tanks from which the fully purified effluent might be used for the irrigation of crops.

All these researches have their final bearing on the fundamental issue of the production of adequate and health giving food for man. Increasing evidence is forthcoming to show that of even more importance than quantity is the quality of food supplied. The researches of McCarrison and his fellow-workers now many years ago showed, e.g. that although by plant breeding it might be possible to produce double the yield, e.g. of the rice plant, yet unless the crop was grown under suitable conditions a double ration was required in order to provide an equally sustaining meal. The researches on the mycorrhizal association, and on the effects on crop production of the use of organic manure in the form of compost, have indicated that the critical issue is the synthesis of suitable proteins. If these are not supplied the nutritive and disease-resisting properties of the crops are depreciated, as well as the health and sustenance of the men and animals consuming them.

### *Summary and Conclusions.*

In the foregoing review an attempt has been made to collect together the outstanding contributions which have been made during the past decade to our knowledge of what is now more than ever seen to be the most vital subject of scientific enquiry, viz. the basic economics of food production. By this is meant the full utilisation of all available material for the maintenance of the cycle, plant—animal—man.

It has been found during this decade of intensive fundamental research, i.e. research concerned with what is often termed 'academic' as contrasted with 'technological' objectives that, as so often occurs in the history of scientific research, what had seemed simple sequences or reactions really involved many until then unknown or unobserved 'links' the provision or understanding of which was necessary for the full control of the system under investigation. The work of Dixon, Brereton Baker, Bone and others on the effect of traces of moisture on combustion phenomena may be cited in illustration. In the present review the end product of the 'Nitrogen cycle', viz. nitrate, has been seen to function as a *catalyst* through alternate reduction in presence of carbonaceous waste material and reoxidation in presence of adequate oxygen, in addition to its direct utilisation as plant food. In addition to the commonly accepted agents in the breaking down and mineralisation of nitrogenous waste material, viz. various specific bacteria, sundry purely *chemical* or *physico-chemical agencies* have been shown to play an important part. Reference in particular has been made to the researches of Dhar and his co-workers on the effect of sunlight on the sequence of fundamental changes resulting in the conversion of nitrogenous organic materials into nitrate. The interesting influence of *trace* elements has been noted.

Among living agents other than bacterial, special reference has been made to the *mycorrhizal association* the far reaching importance of which has been dealt with in detail in the writings of Sir Albert Howard, Lady Eve Balfour and their worldwide associates. The nature and importance of this 'link' between plant and soil has only thus recently been recognised.

Another living 'link', the study of which has been intensively pursued of recent years, with very important results, is that of the activity of *Protozoa*. Not only has their useful activity been observed throughout the natural operations of agriculture but they have been shown to be the essential agent in the economic functioning of the activated sludge process of sewage purification. These organisms, particularly certain species of *vorticellids*, have been shown to be capable of causing flocculation of colloids, and consequent *clarification* of the sewage, but also of digesting protein matter, including, as a most important corollary, *pathogenic bacteria*, and finally of developing an *oxidase* activity manifested in nitrification. Eventually these protozoa may themselves become food for fish which in turn increase the food supply of man.

The researches discussed in the foregoing review have introduced a new viewpoint into the control of crop production and of the operations of sewage purification, biological factors attaining much greater prominence.

Once more it has been shown that Nature's storehouse of wonders is inexhaustible and that if it is carefully and honestly investigated, and not greedily rifled, we may look forward to an increasing supply of food for the starving millions of the world in place of the wind swept areas of erosion which in our ignorance we have so far created.

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* Denotes papers under the single authorship of S. C. Pillai.