PART IV.—BIOLOGICAL OXIDATION OF SULPHUR.

Influence on Ammonification and Nitrification in Activated Sludge.

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With the recent advances in improved systems of sewage disposal, the importance of nitrogen transformations in sewage has been recognised (*J. Indian Inst. Sci.*, 1927, **10A**, 99), but the influence of other processes such as sulphur-oxidation on the nitrogen cycle in activated sludge has not been so well understood as in the case of soils. A study of biological sulphur-oxidation affecting the nitrogen transformations in suspensions of activated sludge was therefore undertaken.

Boullanger and Dugardin (*Compt. rend.*, 1912, **155**, 327) attributed the favourable action of sulphur on soil to stimulation of the ammonifying bacteria and, to a small extent, of the nitrifying organisms. They also observed that there was no influence on nitrogen-fixing or denitrifying bacteria. Organisms decomposing complex nitrogenous substances into ammonia appeared, however, to be greatly favoured. Pitz (*J. Agric. Res.*, 1916, **5**, 771) studied the influence of sulphur on higher and lower forms of plant life and assigned the beneficial effects of sulphur to increase in the production of ammonia and decrease in the total number of organisms. Ames and Richmond (*Soil Sci.*, 1918, **5**, 320) in recording the role of sulphur-oxidation in relation to nitrogen transformations in acid and basic soils explained the depression that they observed in the activities of nitrifying organisms as being due to absence of bases like lime to neutralise the acid produced. Ammonium sulphate was formed by reaction with the ammonia of the soil and not by stimulation of ammonifiers. Shedd (*J. Agric. Res.*, 1919, **18**, 329) who studied the effect of sulphur-oxidation on the solubility of rock phosphate in soils observed, on the other hand, that nitrification proceeded even in presence of free sulphuric acid that tended to accumulate, the amount nitrified being 20 per cent. of the total nitrogen present. It may be gathered from the above that (*a*) there is no general agreement with regard to the mode of production and accumulation of ammonia, and (*b*) nitrification is completely inhibited in soils devoid of bases while in those containing some, the inhibition is noticeable in the later stages.

To study the effect of sulphur-oxidation in suspensions of activated sludge, with and without phosphate, on the various forms of nitrogen, experiments were designed and the products closely studied during a period of six weeks, while keeping the suspensions under constant aeration by compressed air. Samples were drawn daily from each series for the different determinations. In addition to study of changes in the different forms of nitrogen, observations on changes in (*a*) reaction, (*b*) counts on albumin agar (Waksman, *Soil Sci.*, 1916, **2**, 113) and (*c*) nature of colonies developing on raisin-agar were also made.

METHODS.

The details of experiment were the same as those described in an earlier communication (*J. Indian Inst. Sci.*, 1929, **12A**, 275) except that ammonia-free sterile air was used for aeration. Before drawing samples for analysis, aeration was stopped, the sludge allowed to settle, and the supernatant liquid drawn under aseptic conditions was used for plating and nitrogen estimations. Ammonia was estimated by the method of Melling (Chemists' Year Book, 1927, 639), 5 c.c. being used for each determination, and nitrates by the phenoldisulphonic acid method. Reaction was determined colorimetrically (Medalia, *J. Bact.*, 1920, **5**, 441 ; **7**, 589).

Before making the plates, trials were conducted on albumin agar to determine the dilution favourable to development of 150–200 colonies per plate after incubation for 7 days at 28° to 30°. A dilution of 1 in 100 was thus found to be the most efficient. Samples drawn under sterile conditions were accordingly diluted and plated in triplicate.

The total period of observation was divided into halves, of three weeks each, based on the nature of the different changes that were observed. The quantities of ammonia and nitrate, counts on albumin agar, and reaction at different stages have been represented in figures I, II, III and IV respectively.

THE FIRST PERIOD.

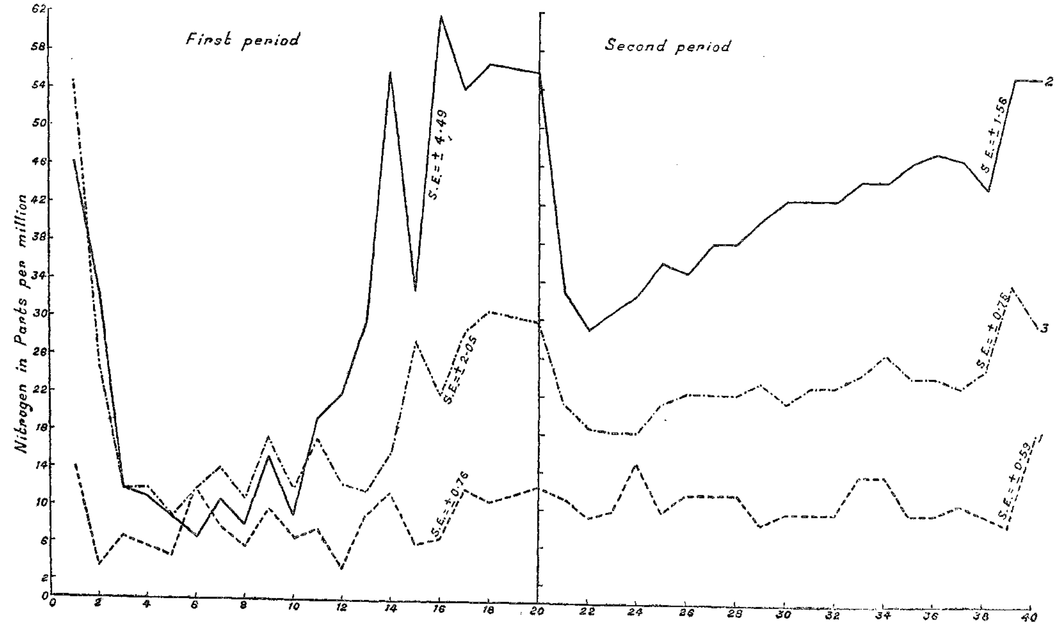
Activated Sludge series.—The reduction in ammonia and nitrate observed initially might have been due to either (*a*) loss of the former during vigorous aeration and the latter through denitrification or (*b*) assimilation of both by micro-organisms and consequent transformation into complex forms.

It was observed that the air leaving the jars did not contain even traces of ammonia. On inoculating the effluent into a nutrient medium containing nitrate and incubating for 14 days at 30°, no gas-production was observed. A study of the bacterial counts for the same

Fig. 1
Ammoniacal Nitrogen

----- Series 1 Activated Sludge only
——— " 2 " " + Sulphur
-·-·- " 3 " " + " + Mineral Phosphate

S.E. = Standard Error



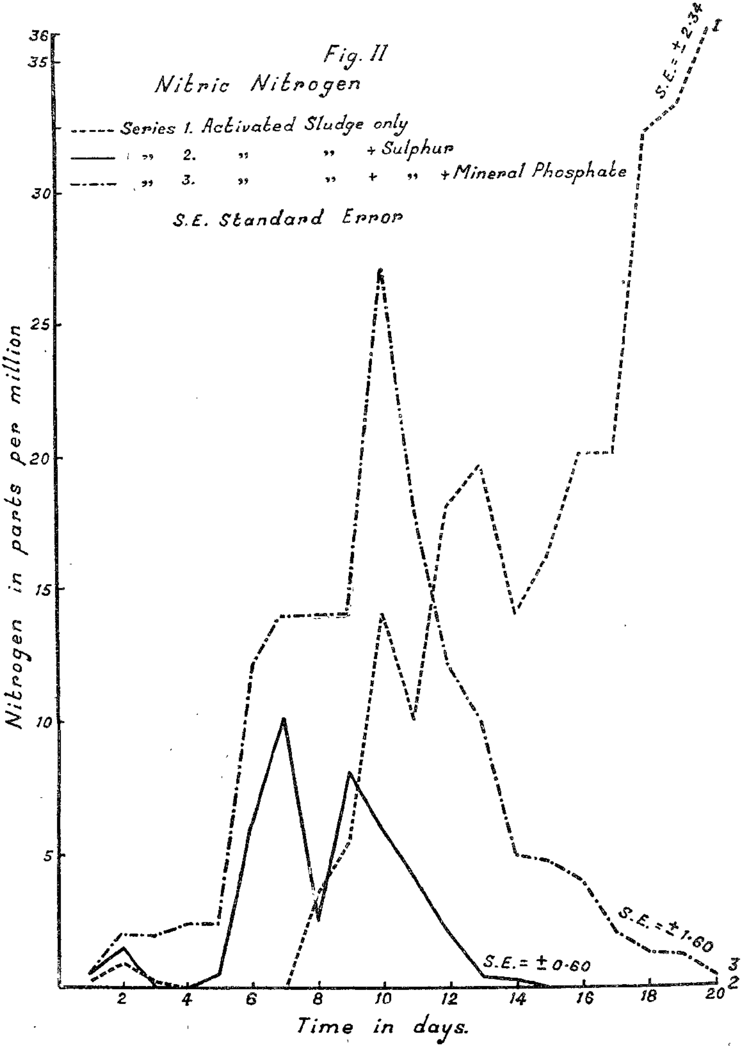


Fig. II
Nitric Nitrogen
Series 1. Activated Sludge only
" 2. " " + Sulphur
" 3. " " + " + Mineral Phosphate
S.E. Standard Error
S.E. = ± 2·34
S.E. = ± 1·60
S.E. = ± 0·60
Nitrogen in parts per million
Time in days.
36
35
30
25
20
15
10
5
2
4
6
8
10
12
14
16
18
20
1
2
3

Fig. III
Colonies on Egg albumin agar

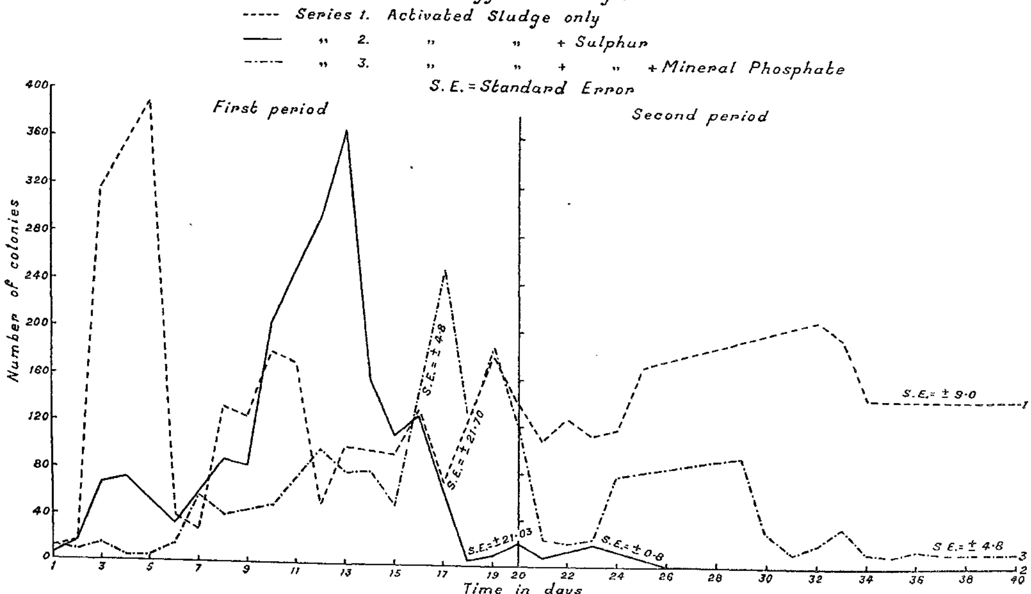
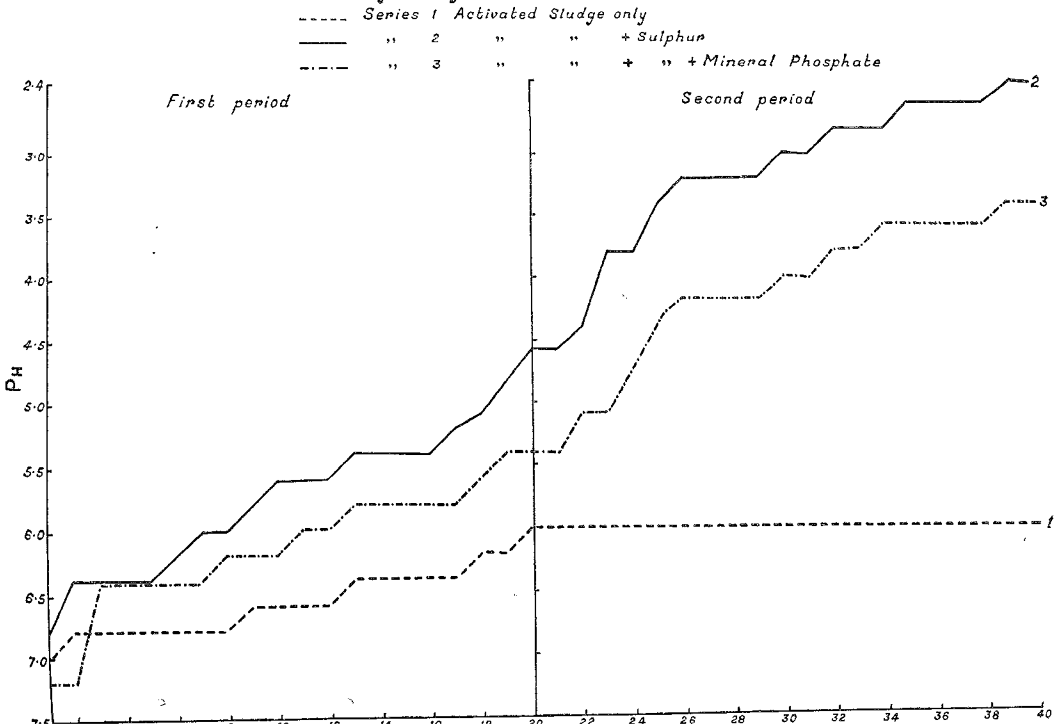


Fig. IV
Changes in Reaction



period, together with the observation that fungal colonies were then appearing in increasing numbers suggested, however, that ammonia and nitrate were being used up by the growing microflora.

After the tenth day, a steady production of ammonia was maintained. The increase in bacterial numbers at the same time suggested that (*a*) there was marked increase in the number of ammonifiers and (*b*) ammonia was being produced at a rate much too fast for its assimilation. The steady formation and accumulation of nitrates indicated that the conditions were favourable to the growth and functioning of nitrifying organisms.

Sulphur-activated sludge series.—There was rapid fall in the ammonia content during the first three days, and the corresponding diminution in bacterial numbers suggests that those organisms were not responsible for such a change. There was rapid production of ammonia from the eleventh to the eighteenth day, when the rate slackened. The development of intense acidity during the second three weeks was evidently responsible for the weakening in production of ammonia during that period.

As the reaction became more acid, nitrification appeared to have been hindered; the bacteria which increased in numbers up to the thirteenth day were probably responsible for the complete utilisation of even the small quantities of nitrates produced.

Simultaneously with the rapid production of ammonia, large numbers of fungi were observed on the raisin-agar medium, suggesting that those organisms were in some way associated with the production of ammonia from the albuminoid matter present in the sludge. It has been recorded by many workers, and recently by Thakur and Norris (*J. Indian Inst. Sci.*, 1928, **11A**, 149) that fungi are to a large extent responsible for the production of ammonia in the soil. Since it is also generally recognised that the microflora of activated sludge are essentially those of the soil, it appears probable that the organisms chiefly responsible for the production of ammonia in the present series were also fungi. The cultural characteristics of the fungi developing on raisin-agar were similar to those previously observed on wort-agar (*J. Indian Inst. Sci.*, 1929, **12A**, 275).

The foregoing observations are in conformity with those of Pitz (*loc. cit.*) who observed that sulphur-oxidation favoured the development of ammonifiers, but retarded, with the development of acidity, the growth of commoner bacterial types. They also suggest that the enhanced ammonification noticed by Boullanger and Dugardin (*loc. cit.*) during the oxidation of sulphur in the soil was due to increased activity of fungi that decomposed the complex organic matter of the soil.

With prolonged aeration and development of acid, the colour of the sludge changed to brown, as is generally observed with activated sludge under similar conditions.

Phosphate-sulphur series.—Nitrogen transformations in this series may be described as being intermediary to those observed in the two previous ones. Owing to the calcium carbonate in the rock phosphate partially neutralising the acid produced, and the dissolved phosphate acting as buffer, active nitrification in this series proceeded for nearly a week longer than in the sulphur series. With further development of acid, however, nitrification was hindered and ammonification favoured, as observed already in the sulphur series.

The limiting reaction for nitrification in the acid range lay between P_H 6·0 and 5·8 as observed by Ranganathan and Norris (*J. Indian Inst. Sci.*, 1927, **10A**, 114) in their studies on intensive nitrification in activated sludge. After that stage no further quantities of nitrate were produced. At P_H 5·4 they disappeared almost entirely having probably been utilised by the fungi which then developed in large numbers.

THE SECOND PERIOD.

In the activated sludge series the reaction, bacterial numbers and nitrate content remained almost unaltered. There was a slight but significant increase in ammonia.

In the sulphur series the reaction became steadily more acid, finally attaining P_H 2·4. Bacterial counts diminished steadily. Plates made after the twenty-sixth day did not carry even a single colony, thereby confirming the previous observation of Ramaswami Ayyar and Norris (*J. Indian Inst. Sci.*, 1929, **12A**, 277) that the commoner sewage organisms could not withstand the acidity. Nitrates were absent, having been entirely used at the end of the first fortnight, the reaction subsequently developed being unfavourable to nitrification. The ammonia content showed marked decrease in the initial stages, thereafter increasing steadily to the end.

The last observation is one of considerable interest. The sudden decrease in ammonia noticed between the twentieth and the twenty-second days could not have been due to loss in the gaseous form because none was detected in the air leaving the jars in which aeration was being conducted. As the counts made during the same period showed a marked decline in the number of bacteria, the latter could not have been responsible for the rapid decrease. Only the fungi were much in evidence at that stage and it is probable that they were responsible for the initial transformation of free and saline ammonia into less readily available forms.

The steady increase in ammonia after the twenty-second day could not have been due to the activity of the commoner ammonifying bacteria because the reaction was distinctly unfavourable to them. As the sulphur-oxidisers, then increasing, were found incapable of bringing about nitrogen transformations, it may be inferred that there were other agencies responsible for this appreciable increase in free and saline ammonia in the medium. Two possible causes were suggested; (*a*) sulphuric acid then developing in the medium flocculated the colloids and leached out the adsorbed ammonia, or (*b*) activity of the abundant fungi in the sludge.

To investigate the direct chemical action of sulphuric acid alone, experiments were designed wherein the action of fungi was suppressed by toluene. Ammonia was extracted after adding dilute sulphuric acid to give different H-ion concentrations and estimated. The results did not show any significant increases over controls without acid, indicating the absence of any direct action of sulphuric acid. It therefore appeared probable that the increase in free and saline ammonia observed in the later stages was also due to the activity of fungi.

In the phosphate-sulphur series, the changes taking place during the second period were of the same type as those of the sulphur series, though generally less intense. Probably owing to sulphur, the reaction became steadily acid in spite of the buffering action of the phosphate, the final reaction being P_H 3·4. The quantities of ammonia passing into the supernatant liquid were less than in the sulphur series. Most likely owing to the same causes as in the sulphur series the nitrates disappeared and the bacterial numbers decreased steadily, with development of acidity.

The conclusions reached in the foregoing paragraphs were confirmed by a statistic study of the different sets of data, those for reaction, ammonia and nitrates being recognised as characteristic of the respective treatments. The bacterial counts during the first period were not, however, significantly different from each other, though they accounted for the corresponding changes in ammonia and nitrate.

DISCUSSION.

Even though the nature and function of activated sludge are still imperfectly understood, yet it is now generally recognised that it (*a*) contains an intense mass of living organisms, which are essentially aerobic and which can bring about a variety of oxidation changes in air ; and (*b*) conserves, in its mass, the major part of the nitrogen and the phosphate of the sewage in a readily available form (Richards, E. H., and Sawyer, G. C., *J. Soc. Chem. Ind.*, 1922, **41**, 62). Activated sludge cannot however be regarded as a form of purified sewage and its utility as an all round manure, except when fully dried, is marred by its tendency to putrefy, causing offensive odours and probably endangering public health (Whitehead, cited from Martin, The Activated Sludge Process, 1927, 180). Collection and marketing are rendered difficult because of its occurrence as a jelly, often containing over 97 per cent. of moisture which is not easily removed.

Seven years of vegetation experiments carried out at the Indian Institute of Science have shown that when the effluent from an activated sludge tank is utilised for irrigation undiluted, many of the plants show the effects of excessive supply of nitrogen. For this reason and perhaps others, some of the crops have been found susceptible to certain bacterial and fungal diseases. In view of the fact that such occurrences will discourage the extensive use of undiluted effluent in farm practice, it will be desirable to have a modification that will yield a weak effluent conserving all the plant nutrients in the sludge itself.

The investigations so far carried out on the biological oxidation of sulphur in suspensions of activated sludge (*J. Indian Inst. Sci.*, 1928, **11A**, 85; 1929, **12A**, 275; 1929, **12A**, 278) have shown that (*a*) with the progress in sulphur oxidation, there occurs a selective functioning of oxidising organisms, (*b*) the pathogenic and putrefactive organisms commonly found in the sewage sludge become extinct, (*c*) the oxidation of sulphur leads to gradual development of acidity and to the formation of a floc which settles readily on standing, (*d*) the effluent obtained after moderate aeration in presence of sulphur contains less nitrogen than the one from the activated sludge alone and (*e*) the sludge formed during sulphur oxidation conserves plant nutrients more effectively than activated sludge alone.

In addition, sulphur-oxidation has a bearing on dewatering activated sludge, since it has been recognised that sludge which has been treated with sulphuric acid parts readily with its water. Thus the Maclachlan process of concentrating sewage sludge is based on passing sulphur dioxide fumes mixed with steam into the sludge followed by pressing (*Eng. News Rec.*, 1923, **87**, 947). At Milwaukee, preliminary treatment with sulphuric acid prior to pressing is practised (Martin, Activated Sludge Process, 1927, 345).

Further study of the phenomenon is required before the foregoing and the allied observations can find practical application. The quantities of sulphur and inorganic catalysts needed to provide active flocs under a variety of conditions and for different types of sewage have to be determined. The mode of application and different stages of treatment required to give a flocculum that will (*a*) have a uniform composition, (*b*) not settle heavily in the tanks and clog the pores of the diffusers and (*c*) be readily reactivated, have to be determined. Liming and other treatments necessary to neutralise the effects of the acid in the effluent on soil have also to be standardised. It is hoped that the foregoing and the allied problems will form subjects of subsequent communications.

SUMMARY.

The effect of sulphur-oxidation on ammonification and nitrification in suspensions of activated sludge is discussed.

In the presence of sulphur, nitrification was found to be completely inhibited, but ammonification, brought about largely by fungi, proceeded vigorously almost to the end.

Changes in the type and number of microflora, and in reaction were studied and correlated with other observations.

The possible bearing of the different observations on their application to the purification of sewage, with special reference to the conservation of plant nutrients in the sludge, is indicated.

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