I.—BIOLOGICAL OXIDATION OF SULPHUR. PART II[1]. Effect on the Microflora of Activated Sludge.

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The value of microbiological analysis as a guide to the changes occurring in the soil has been much debated with a growing tendency to depreciate the reliability of inferences drawn from such work. The reason for this attitude lies in the scarcity of standard methods and the disregard of many factors limiting their applicability. Waksman (*Soil Sci.*, 1922, **14**, 343) has, however, shown that the plate method, when carried out under well defined conditions, gives much reliable information in regard to such changes, the number of the organisms thus revealed serving to indicate the soil conditions. Since the inferences drawn from such investigations have agreed with field results obtained with various crops over a number of years, it is clearly helpful to obtain information regarding the manner in which the soil population is affected by external conditions.

As in our previous investigation on the biological oxidation of sulphur (*J. Indian Inst. Sci.*, 1928, **11A**, 85), forming Part I[1] of the present series, we have used activated sludge, which is to be regarded as an intensified form of soil; the results may therefore be taken to indicate the general type of changes occurring in a soil population resulting from development of acidity. The work was also expected to indicate improved means for the isolation of sulphur-oxidising bacteria from this source.

EXPERIMENTAL.

Suspensions of activated sludge were aerated under different conditions for several weeks. Samples from each series were taken every 24 hours for enumeration and examination of microflora. Three sets of trials were conducted :—(1) Activated sludge alone, serving as control, (2) activated sludge with sulphur and (3) activated sludge with sulphur and mineral phosphate. The samples taken after suitable dilution (usually 1 in 40) were plated out on (1) nutrient agar, (2) wort-agar and (3) a special sulphur medium (*J. Bact.*, 1922, **7**, 606). The plates were incubated for 24 hours at 37° in the case of (1) and for 7 days at 30°, in those of (2) and (3). Comparative studies of the number and type of colonies developing on the plates were made. The reactions of the samples were also determined. The results are presented in Figures I, II and III.

Activated sludge alone.—There was very little change in reaction, the P_H remaining about 5·9 throughout. No marked decrease in bacterial numbers was observed as aeration proceeded. Considerable

changes, however, took place in the nature of the organisms found. At first spreading colonies predominated but soon died out, as did also putrefactive and chromogenic bacteria. It was however observed that the filamentous organisms believed to be responsible for the bulking of sewage (*J. Indian Inst. Sci.*, 1929, **12A**, 133) persisted in spite of continued aeration although the phenomenon has generally been regarded as a symptom of inadequate air-supply. The number of organisms developing on wort-agar or on the sulphur medium was small, the reaction not being favourable to the development of fungi or yeasts, and distinctly unfavourable to the growth of sulphur-oxidising organisms.

Activated sludge with sulphur.—In this series the results obtained were quite different. As aeration continued, owing to biological oxidation of sulphur, the acidity increased steadily, the reaction at the end of ten days being P_H 5·4. The conditions then seemed favourable to acid-producing organisms as there was an abrupt rise in acidity, the P_H after another week reaching 2·8. On continuing aeration, the rate of change slowed down considerably, but eventually a P_H of about 2·1 was attained after another 14 days' aeration.

This change in reaction had a marked influence on the flora. In the beginning, when the development of acidity was slow, the bacterial numbers as shown by agar counts tended to increase, this increase being particularly marked when the P_H reached 5·4. The agar counts in consequence reached a maximum between the 10th and 15th days. The development of further acidity, however, checked the increase of bacteria, and encouraged that of fungi and sulphur-oxidising organisms. The intermediate stage was characterised by a rapid increase of *Actinomyces* and moulds which became the predominating types, a result which is in close agreement with Waksman's observations in the case of soil (*Soil Sci.*, 1922, **14**, 74 and 151). At first the *Actinomyces* predominated, but as the acidity rose these decreased more rapidly than the moulds; among the latter, varieties of *aspergillus*, *fusarium*, *penicillium* and *trichoderma* were the most common. By the time, however, that the acidity reached a P_H of 2·4 most of the moulds also had died out with the exception of *fusarium* which seemed able to withstand a highly acid reaction, but did not survive when the P_H was about 2·2. No large increase in the number of sulphur-oxidising organisms took place until the P_H fell to 3·0. Counts on the sulphur medium then increased rapidly while those on nutrient agar and wort-agar became very low, the sulphur organisms not growing on those media. Practically all other types were now extinct. It was then easy to isolate sulphur-oxidising types even in liquid cultures, they being almost the sole survivors when oxidation was allowed to continue until a P_H of 2·2 was reached.

Fig. I

P_H Changes during the Oxidation of Sulphur

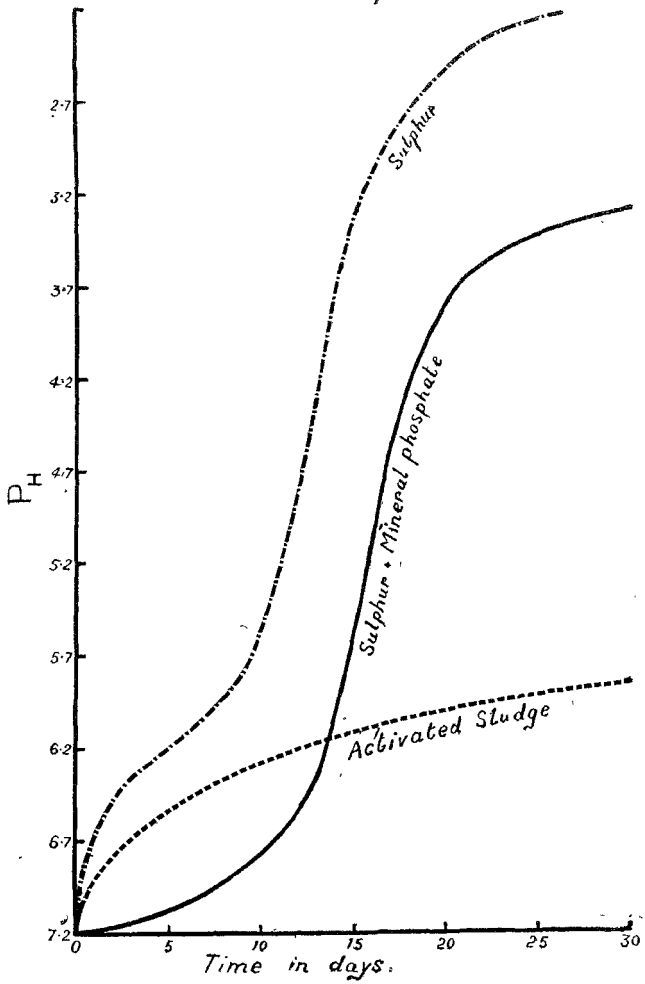


Fig. II

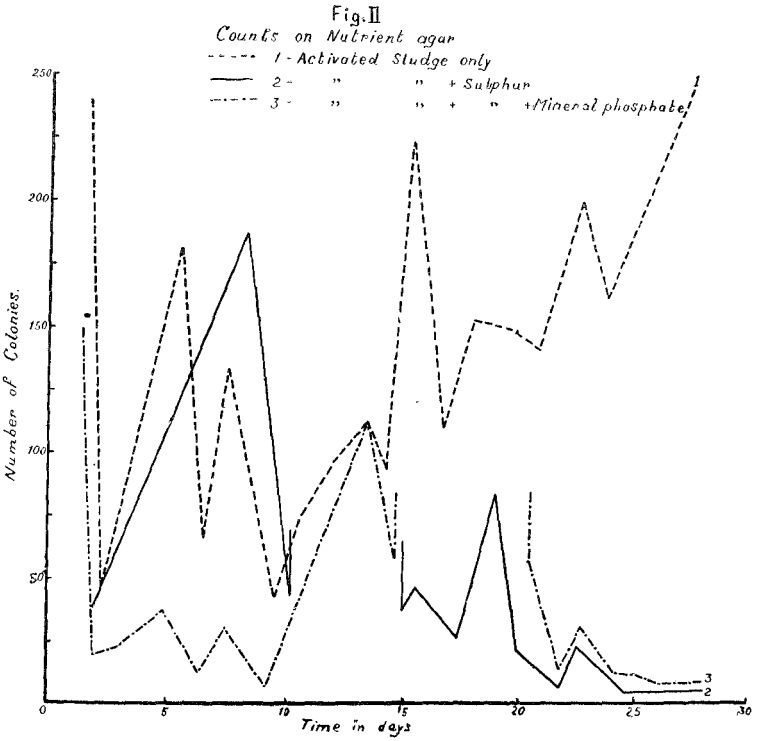
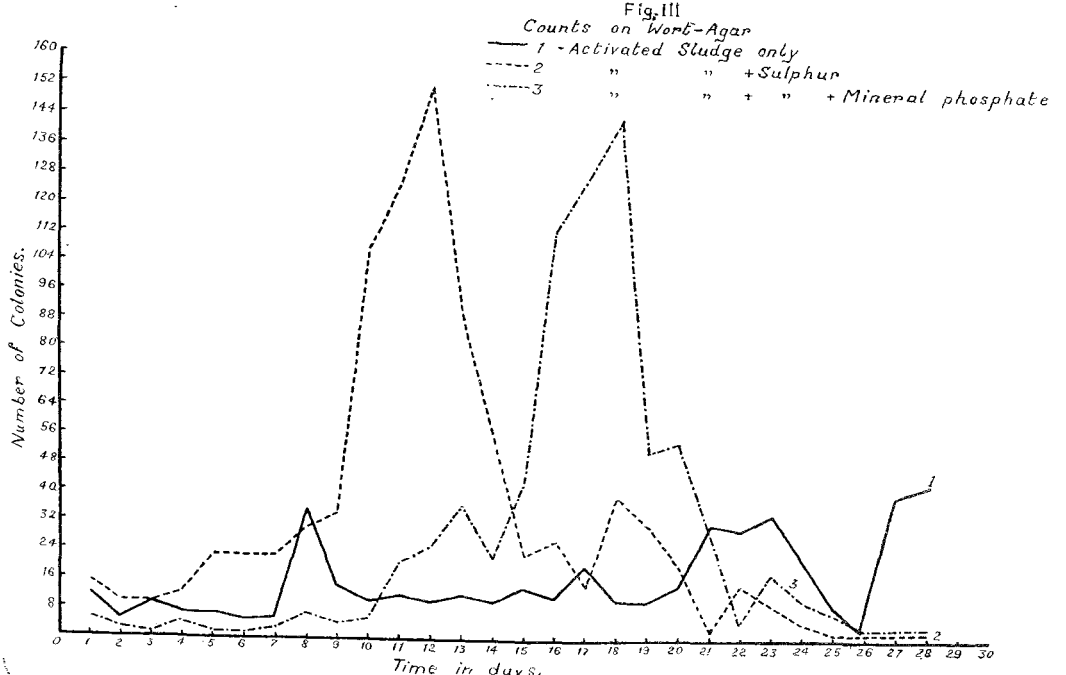

Counts on Nutrient agar
1 - Activated Sludge only
2 - " " + Sulphur
3 - " " + " +Mineral phosphate
Number of Colonies.
250
200
150
100
50
0
5
10
15
20
25
30
Time in days
1
2
3

Fig. III


Counts on Wort-Agar
1 - Activated Sludge only
2 " " + Sulphur
3 " " + " + Mineral phosphate
Number of Colonies.
Time in days.
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
8 16 24 32 40 48 56 64 72 80 88 96 104 112 120 128 136 144 152 160

Activated sludge, sulphur and phosphate.—The results were intermediate in character between those found in series I and II. This was due chiefly to the buffer action of the phosphate added, the P_H in consequence undergoing less rapid variation. The phosphate added was a mineral phosphate from the Trichinopoly deposits and contained about 17 per cent. of calcium carbonate together with impurities such as iron and alumina. The initial P_H was slightly alkaline being about 7·2. Due to the buffer action already referred to (*J. Indian Inst. Sci.*, 1928, I IA, 87) the P_H changed but slowly in the initial stages and not till 10 days had elapsed did the figure fall to 6·6. The acidity then increased rapidly, the reaction changing from 6·6 to 4·0 in ten days, after which P_H 3·2 was reached.

In the beginning the bacterial counts were lower than in series I and II, but later increased, changes similar to those described under Series I taking place. Owing to the slower development of acidity, these were later in developing, increase in *Actinomyces* occurring between the 16th and 20th days. The counts on wort-agar and sulphur media varied somewhat from those previously described. The presence of lime from the mineral phosphate appeared to check the development of moulds and consequently the wort-agar counts did not show marked increases until the P_H had fallen to about 4·0. On the other hand, the multiplication of the sulphur-oxidising types took place at a rather earlier stage, the numbers beginning to rise when the P_H reached 3·2. It is possible the phosphate had a stimulating influence on those organisms.

SUMMARY.

During the progress of aeration and oxidation of sulphur there was decrease in the number of faecal and putrefactive bacteria and of chromogenic organisms. At a latter stage, further specialisation took place and only a few well defined soil types persisted. When the P_H reached about 5·2 *Actinomyces* were the predominating organisms, dying out at a reaction between 3 and 4, when moulds appeared in large numbers. The latter survived until the P_H was about 2·1, *fusarium* being usually the most resistant type. At this last stage, the sulphur-oxidising organisms began to multiply vigorously; they were almost the sole survivors and continued to increase and function actively. Owing to the absence of nearly all other organisms, it was then easy to isolate the sulphur-oxidising organisms in pure culture.

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