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## Report for 1934

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## Microbiology

### Rothamsted Research

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structure stability. The circumstance that water within the compact masses will not drain away and can only be removed by suction of plants on neighbouring drier soil, or by direct evaporation, has its reflection in traditional agricultural practices.

xxxv. G. W. SCOTT BLAIR. "*Definition and Translation of Rheological Terms used in Soil Physics.*" Transactions of the First Commission of the International Society of Soil Science, 1934, pp. 159-167.

Rheology is defined as the science of the flow of matter, and a great many terms used in soil physics therefore may be classed as "rheological terms."

The use of such terms in the international literature is subject to two types of uncertainty: (a) uncertainty as to definition of terms; (b) uncertainty as to translation of terms. With regard to (a), it is desirable that definitions should conform as far as possible to established usage not only in Soil Science, but also in other fields, and should not conflict with the traditional meaning of words. The term "plasticity" is discussed in this connection. Although (b) is a separate problem, it is a closely allied one. As an example, the translation of the English term "stickiness" is discussed.

It is recommended that a committee should investigate these problems, and standardise usage as far as is possible.

#### MICROBIOLOGY.

(Departments of Bacteriology, and Fermentation.)

##### (a) BACTERIA.

xxxvi. H. G. THORNTON and P. H. H. GRAY. "*The Numbers of Bacterial Cells in Field Soils, as Estimated by the Ratio Method.*" With appendix by R. A. FISHER, F.R.S. Proceedings of the Royal Society of London, Series B, 1934, Vol. CXV, pp. 522-543.

There are two serious difficulties in estimating the bacterial numbers in a soil sample from the microscopic examination of stained films of that soil. The first is that of determining with sufficient accuracy the mass of soil in the film and the second that of estimating from random microscope fields the numbers of organisms in the film when these organisms are not distributed through it at random.

A technique is here described in which these difficulties are avoided, by determining, in random microscope fields from a parallel series of stained films, the ratio between the number of bacteria and the number of indigo particles, of which a counted suspension has previously been added to a given mass of soil. The bacterial numbers calculated from such ratios are, of course, independent of the mass of soil in the film.

It is found that the ratios obtained from parallel microscope fields are distributed at random, although counts of bacteria taken by themselves from the same fields are much less uniform.

The accuracy of the method has been tested in the following experiments:

(a) The bacterial numbers in four portions of a single soil sample agreed within a standard error of 3.3 per cent.

(b) The numbers found in films prepared by three workers from the same soil sample showed no significant differences.

(c) The numbers found by two workers independently counting different microscope fields from the same films agreed as random samples.

(d) Counted suspensions of bacteria added to sterilized soil were estimated with a standard error of 3.5 per cent.

The bacterial numbers found in Rothamsted field soils by this method range from 1,000 to 4,000 million per gram of soil.

Samples taken from some of the Hoos field plots showed a relationship between the total bacterial numbers and the average yield of straw taken over a number of years.

Caution is at present necessary in discussing results obtained from samples taken on a single occasion, since there is evidence of rapid changes in numbers of bacteria with time. Successive samples taken from garden soil showed significant changes in total bacterial numbers during the course of a day.

#### (b) BIOLOGICAL ACTIVITIES.

XXXVII. C. N. ACHARYA. "*Studies on the Anaerobic Decomposition of Plant Materials. I. The Anaerobic Decomposition of Rice Straw (Oryza sativa).*" *Biochemical Journal*, 1935, Vol. XXIX, pp. 528-541.

Many of the changes taking place in the soil during the cultivation of rice are brought about by micro-organisms acting in the presence of small amounts of air. The anaerobic decomposition of organic manures is therefore of particular interest in the growing of this crop.

The course of anaerobic decomposition of rice straw and its major constituents has been followed. Decomposition appears to proceed in two distinct stages, *viz.* (1) the formation of organic acids, and (2) the conversion of these into gaseous products: acetic and butyric acids are the main products of the first stage, and carbon dioxide and methane the main products of the second. From 100 gm. of straw either about 20 gm. of organic acids, or over 20 litres of gas containing 50 per cent. methane, are obtainable. This yield is equivalent to about 7,200 cu. ft. of gas per ton of straw. The nitrogen requirement for anaerobic digestion is low, 100 gm. of straw requiring about 0.1 gm., or less, of nitrogen, while for aerobic decomposition 0.6-0.7 gm. of nitrogen is necessary.

XXXVIII. C. N. ACHARYA. "*Studies on the Anaerobic Decomposition of Plant Materials. II. Some Factors Influencing the Anaerobic Decomposition of Rice Straw (Oryza sativa).*" *Biochemical Journal*, 1935, Vol. XXIX, pp. 953-960.

A temperature range of 30-35°C, pH 7.5-8.0 and straw: water ratio of about 1 to 10 are found optimum for the anaerobic digestion of rice straw by mesophilic organisms. The organisms required to decompose the straw are contained by the straw itself and further inoculation is unnecessary. Additions of phosphate have no stimu-

lating effect. Finely ground straw does not ferment quicker than chaffed straw.  $\text{NH}_4\text{HCO}_3$  and  $\text{KHCO}_3$ , either singly or better still in admixture, are found most suitable for neutralising the organic acids formed during anaerobic digestion. The reaction of the medium can also be controlled by periodic additions of alkali. Urea or cyanamide may replace ammonium bicarbonate, but not proteins. The amount of  $\text{CO}_2$  and  $\text{CH}_4$  produced is approximately the same irrespective of the volume of free space above the culture medium, provided accumulated gases are removed so as to prevent high concentrations of  $\text{CO}_2$ , or the reduction of the pH value of the medium.

- XXXIX. C. N. ACHARYA. "*Studies on the Anaerobic Decomposition of Plant Materials. III. Comparison of the Course of Decomposition of Rice Straw under Anaerobic, Aerobic and Partially Aerobic Conditions.*" *Biochemical Journal*, 1935, Vol. XXIX, pp. 1116-1120.

The greatest loss of dry matter is recorded when rice straw is fermented under aerobic conditions. The loss is less under partially aerobic conditions, such as those existing in swamp soils, and least of all under strictly anaerobic circumstances. Cellulose and lignin are more easily attacked under water-logged than under anaerobic conditions. Partially aerobic conditions resemble anaerobic conditions in regard to the nature of the products obtained. Under the former set of conditions smaller amounts of organic acids and methane, and larger amounts of  $\text{CO}_2$ , are formed. The nitrogen factor and nitrogen equivalent decrease in the following order: aerobic (0.536; 1.11 respectively), water-logged (0.395; 0.961), and anaerobic (0.069; 0.169), which also shows a progressive decrease in the amount of protein accumulated. The protein formed under aerobic conditions is mostly insoluble in water, while under water-logged conditions it remains in solution.

- XL. E. H. RICHARDS and J. G. SHRIKHANDE. "*The Preferential Utilization of Different Forms of Inorganic Nitrogen in the Decomposition of Plant Materials.*" *Soil Science*, 1935, Vol. XXXIX, pp. 1-8.

Experiments were carried out to discover whether the organisms concerned in the decomposition of cellulosic matter exercise any preference for ammoniacal or nitric nitrogen, when both forms are available to them in equal concentrations. The results showed that in the early stages of breakdown there is a definite preference for ammonia rather than for nitrate. This selection appears to be shown only in the early stages; after straw had been fermented under optimum conditions for 14 days, about equal amounts of ammonia and nitrate remained unassimilated.

The nitrogen factor is always lower when nitrate is the source of nitrogen than when ammonia is used. The loss of nitrogen is greatest when nitrate is present. As a result of this loss of, presumably,

elementary nitrogen from nitrate, the relative assimilation of ammonia may be greater than the figures indicate, since the drop in nitrate includes nitrogen lost besides that assimilated.

XLI. S. H. JENKINS. "*The Biological Oxidation of Carbohydrates. IV. The Phosphorus Requirements of Percolating Filters.*" *Biochemical Journal*, 1935, Vol. XXIX, pp. 116-132.

Previous work has shown that the oxidation of carbohydrates in biological filters is influenced by the supply of available compounds of nitrogen. In the work described in this paper, experiments were made to find the requirements of phosphorus compounds by a filter supplied with sucrose and an ample amount of nitrogen. The results showed that the biological film of organisms responsible for sugar oxidation reached maximum activity in a very short period when excess of available phosphorus compounds was supplied; once this efficient state had been reached, the film was able to exist without any diminution in activity on a much smaller ration of phosphorus.

Inorganic compounds of phosphorus were found to be more readily available to micro-organisms than the organic phosphorus compounds present in beet sugar factory effluent. The former were used preferentially when both sources were present simultaneously.

Although sugar is believed to be oxidised in the living cell *via* the stage of hexose-phosphate, all the evidence obtained indicated that intermediate compounds of sugar and phosphorus were absent from the solutions undergoing oxidation: the hexose-phosphates exist within the cell itself, and under natural conditions are not ordinarily found outside it.

XLII. A. G. NORMAN. "*The Biological Decomposition of Plant Materials. Part IX. The Aerobic Decomposition of Hemicelluloses.*" *Annals of Applied Biology*, 1934, Vol. XXI, pp. 454-475.

The decomposition of the hemicelluloses of plant materials occurs rapidly under normal aerobic conditions. Over seventy common fungi were tested as to their ability to utilise on agar plates the crude hemicellulose from straw. All of the fungi grew well, the majority at least as well as on glucose, and a number better than on that sugar. It seems likely that the ability to ferment hemicelluloses is a general property of common fungi.

From soil and manure were isolated twenty aerobic bacteria capable of utilising hemicelluloses. Their morphological characters and biochemical reactions were determined. All the organisms utilised an exceptionally wide range of sugars and polysaccharides, so that differentiation by the usual means was difficult. Certain of the forms agreed with the description of *Achromobacter ubiquitum*. None of these organisms in pure culture could be described as very active in decomposing isolated hemicellulose, or that *in situ* in straw.

This survey led to the view that fungi are more active and important in the natural decomposition of hemicelluloses than bacteria.