MICROBIOLOGY : COPENHAGEN 1947
Report of Proceedings
Rosenkilde and Bagger . Copenhagen 1949

The microbiological estimation of available copper and molybdenum in soil.

DR. E. G. MULDER (*Holland*).

COPPER

For the determination of the available copper Aspergillus niger may be used. Cultivated in a copper-free nutrient solution the fungus develops a white mycelium, without spores. With 0.2 γ of Cu in 40 ml. of nutrient solution spores are formed, the colour of which is yellow, with 0.4 γ of copper added the colour of the spores is yellowish-brown, with 1.0 γ gray-brown, with 1.5 γ gray-black and with 2.5 γ and more black. So the colour of the Aspergillus spores is a measure of the quantity of available copper in the nutrient medium.

For the estimation of the available copper in soils, one gram of airdried soil is added to 40 ml. of a copper-free nutrient solution in 1 l. Erlenmeyer flasks. This medium is inoculated with a suspension of Aspergillus spores and after four days of incubation at 30° C the colour of the spores is compared with the colour scale of a series of standard cultures to which copper in increasing quantities from 0—2.5 γ a culture is added.

By comparing the results of pot- and field experiments with cereals with those of the Aspergillus test we found that soils containing 0.6 γ of copper or less per 1 g of airdried soil produce cereals with heavy symptoms of Cu-deficiency. From 0.6—1.5 γ Cu per 1 gram of soil light deficiency symptoms in the crops may be expected whereas with 2 γ and higher in general healthy crops can be obtained.

By adding 60 mg of nitrogen as NH_4NO_3 beyond the normal nitrogen concentration of the 40 ml nutrient solution of Aspergillus niger considerably more copper is required to induce black spores. The same effect as NH_4NO_3 gives the addition of small quantities of cadmium to the nutrient solution.

The addition of ammonium nitrate or cadmium sulfate may be used to extend the copper scale of Aspergillus niger so that greater amounts than 2.5 γ per gram of soil can be estimated.

MOLYBDENUM

For the normal development of Aspergillus niger small quantities of molybdenum are indispensable. An insufficient supply of this element gives a poor, mucous mycelium without spores. Increasing amounts of molybdenum added to the nutrient solution result in an increase in weight of the mycelia and in a more abundant spore formation. The optimum yield already is reached with 0.02 γ of Mo per 40 ml of nutrient solution.

The molybdenum requirement of Aspergillus niger is connected with the compound in which the nitrogen is added to the nutrient medium, nitrates requiring a considerably higher concentration of molybdenum than ammonium compounds. Much evidence was obtained that molybdenum catalyses the nitrate reduction in Aspergillus niger.

The importance of Mo in the reduction of nitrate was clearly demonstrated in experiments with denitrifying bacteria and higher plants. The bacteria were

cultivated in 100 ml. stoppered bottles. Two of the strains did not show any grow in the molybdenum-free medium, the other two brought about an insignificant denitrification. With 5 γ of Na_2MoO_4 added per bottle, however, all strains showed an intensive denitrification.

In experiments with barley and tomatos the significance of molybdenum for the reduction of nitrate in the green plants was demonstrated.

From the above-mentioned results it can be concluded that for the determination of small quantities of Mo with Aspergillus niger the nutrient solution has to contain nitrogen as a nitrate. In this case it is possible to estimate quantities of molybdenum as small as a hundred thousandth of a milligram.

This method can be used to estimate the Mo-content of soils and of plant material. 100 mg of an airdried soil are added to 50 ml. of a Mo-free solution in 1 l. Erlenmeyer flasks. After 4—5 days incubation at 30° C the mycelia of Aspergillus are harvested, washed, dried and weighed. Comparing of these weights with those of a standard series with increasing amounts of molybdenum enables the estimation of available molybdenum. For the estimation of Mo in plant tissues this material has to be incinerated and the ash added to the nutrient solution of Aspergillus. It was found that nodules from pea plants are very rich in molybdenum. This is in good agreement with the results of our investigations with peas in nutrient solution in which it was found that without molybdenum the pea plants develop normal nodules but these are unable to fix gaseous nitrogen.