Oxidation of Thiosulphate by Certain Bacteria in Pure Culture.

By William T. Lockett.

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In the course of investigations on the oxidation of thiosulphate on bacterial sewage filters,* it was found that partially oxidised filtrates, still containing appreciable quantities of thiosulphate, were slowly but completely oxidised by simple aëration in the presence of living organisms, practically no oxidation taking place in the control experiments with corresponding solutions rendered sterile by steaming.

Further investigations were undertaken with a view to the isolation of the organism or organisms capable of bringing about this oxidation.

Accordingly, gelatine and agar plates were made from active filtrates from time to time and in general about 100 organisms per cubic centimetre were noted, which were mainly of the non-liquefying and chromogenic types. Subcultures in peptone water and peptone broth of several of the predominating types were made and after a few days' incubation added to solutions of thiosulphate, which were then aërated under sterile conditions. Many experiments were carried out in this manner without success. Variations were introduced with regard to the age of the cultures and the nature of the culture media, without effect, practically no oxidation of the thiosulphate solutions taking place after several weeks' aëration.

Subsequently it was observed that a bacteriological slide made of a loopful of an active filtrate showed proportionately a greater number of organisms per cubic centimetre than was indicated by the gelatine and agar plates of the same solutions. Further, the microscopic appearance of these—consisting mainly of one particular type—was very different from that of the organisms previously subcultured.

All attempts to grow the particular and characteristic organism on the usual media, *e.g.* nutrient gelatine and nutrient agar, failed. Minor investigations indicated that the organism was most active in neutral solutions containing only small quantities of organic matter, whilst ammonium sulphate was a decided stimulant.

Ultimately it was found on plating out very small quantities (*e.g.* 0·001 c.c.) of an active filtrate on a solid gelatine medium made without bouillon, but

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containing ammonium sulphate (0·1 per cent.) and sodium thiosulphate (0·4 per cent.), that a great number of slow growing, circular, non-liquefying, bluish-white colonies were obtained.

Plates made with such a medium showed that active filtrates contained 100 to 1000 times more organisms per cubic centimetre than was shown on gelatine and agar. In addition the microscopic appearance of the organism was apparently identical with those previously noted in the slides of the filtrates.

Streak cultures of the organism made on media of the same composition as the above produced clearly defined, thin, bluish-white growths after 10 to 15 days' incubation at 20° C.

Experiments were then made to ascertain how far this particular organism was able to bring about the oxidation of thiosulphate. At first difficulties arose with regard to the finding of a suitable liquid medium for the growth of the organism; eventually good growths were obtained by the use of a medium of the following composition :—

1·0 grm. sodium thiosulphate, 0·5 grm. ammonium sulphate, 0·5 grm. potassium biphosphate, 0·025 grm. sodium chloride, 0·01 grm. magnesium sulphate, 2·0 grm. Rochelle salt, dissolved in 1000 c.c. distilled water.

To this solution it was found necessary to add sufficient acid (N H_2SO_4) to reduce the alkalinity to methyl orange by approximately one-half, thereby presumably liberating free tartaric acid. Before and after sterilisation clear solutions were obtained of this mixture, which were alkaline to methyl orange.

A suitable solid medium for the growth of the organism is also obtained by the addition of gelatine (10 per cent.) to this solution.

In testing the oxidising power of the organism the procedure generally adopted was as follows :—A pure streak culture was taken, and a small quantity of the growth, attached to the end of a sterile platinum needle, was introduced into 10–12 c.c. of the above sterile solution contained in a test-tube, the usual bacteriological precautions being observed.

After a few days' incubation at 20° C. a slight white, stringy growth was observed in the inoculated solutions. Later, after 14–21 days a distinct turbidity was apparent, and the solutions on examination at this period were found to be free from thiosulphate. Complete oxidation had taken place with the formation of acid sulphate, the final solution being slightly acid to methyl orange.

Uninoculated solutions showed no change after several weeks' incubation.

A large number of experiments have been made on these lines with complete success. Solutions inoculated directly from colonies found on

ammonium sulphate gelatine plates were similarly oxidised, and other experiments have been conducted which confirm the above results.

The following is a typical example of the chemical results obtained :—

Results in parts per 100,000.

	Oxygen absorbed in three minutes from acid permanganate.	Reaction with mercurous nitrate.
Inoculated solution after 21 days' incubation at 20° C.	1·00	White ppt.
Solution of control experiment after 21 days' incubation at 20° C.	28·80*	Black ppt.

* Equivalent to 83·8 parts $Na_2S_2O_3$ per 100,000.

That the thiosulphate is bacterially oxidised to sulphate and that the change is not a simple decomposition due to the formation of acid by the organism seems evident from the fact that (1) there is no deposition of free sulphur, (2) the final solutions do not absorb appreciable amounts of oxygen from acid permanganate, this excludes the presence of thionic acids.

The following are comparative results obtained with three solutions, to one of which had been added before incubation 1 c.c. of normal sulphuric acid, thereby making the solution decidedly acid to methyl orange :—

Results in parts per 100,000.

	Oxygen absorbed in three minutes from acid permanganate.	Reaction with mercurous nitrate.	Remarks.
1. Inoculated solution after 21 days' incubation at 20° C.	1·80	White ppt.	Slight turbidity.
2. Solution of control after 21 days' incubation at 20° C.	21·00*	Black ppt.	Clear solution.
3. Solution made decidedly acid to methyl orange prior to 21 days' incubation at 20° C.	14·60	Yellow ppt. (thionic acids).	Deposit of sulphur.

* Equivalent to 61 parts $Na_2S_2O_3$ per 100,000.

The organism is apparently able to live in slightly acid solutions, although prolonged contact with free acid appears seriously to impair its activity and growth.

Further experiments are in progress relating to the morphology and classification of the organism, which appears to be one hitherto unknown, and to its effect on other sulphur compounds, *e.g.* tetrathionate.

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The Production of Anthocyanins and Anthocyanidins.

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The idea that the anthocyan pigments are closely related to the flavone and flavonol glucosides is by no means new. Attempts to solve the problem of their relationship have come chiefly from botanists, and, as a result of their researches, a number of hypotheses have sprung up around which quite considerable controversy has been centred.

Miss Wheldale* puts forward the suggestion that anthocyan pigments are the oxidation products of colourless or faintly coloured chromogens; and that these chromogens are products of hydrolysis of glucosides present in the tissues of the plant (probably glucosides of flavone or flavonol derivatives). The hydrolysis of the glucoside she considers as essential to the production of the anthocyan pigment. She represents the changes taking place by means of the following equations:—

$$\text{Glucoside} + \text{water} \lesseqgtr \text{chromogen} + \text{sugar}.$$

Then— $\text{Oxidation of chromogen} \rightarrow \text{anthocyan pigment}.$

If this hypothesis be accepted, then either the anthocyan so produced will remain a non-glucoside, *i.e.*, it will be an anthocyanidin, or in the presence of sugars the anthocyanidin first formed must unite with sugar to form an anthocyanin (glucoside). Her more recent suggestion that in flavone glucosides all the hydroxyl groups are substituted by sugar molecules, hence partial hydrolysis could produce glucoside anthocyans,† has apparently no foundation upon experimental evidence, most of the flavone and flavonol glucosides containing one or two sugar residues only.

Now, in view of the fact that it has recently been shown that in no case

* 'Camb. Phil. Soc. Proc.,' vol. 15, p. 137 (1909); 'Journ. Genetics,' vol. 1, p. 133 (1911).

† 'Biochem. Journ.,' vol. 7, p. 87 (1913).