Short Communication

THE EFFECT OF PIPETTING ON THE CONCENTRATION OF HOMOGENEOUS SPORE SUSPENSIONS

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Viable counts of micro-organisms are commonly used in the assessment of antibacterial substances. These usually involve microscopic counts of a suspension of test organisms followed by dilution before enumeration by roll tube methods.

Dilutions of about 10^{-3} are made by pipetting and it is assumed that these are accurate. We have found that certain unsuspected errors can occur in the preparation of diluted suspensions of micro-organisms and the factors involved have been investigated.

Spore suspensions of *Penicillium spinulosum* of various concentrations were prepared and the optical densities were found to be related to haemocytometer counts. Aqueous dilutions of spore suspensions, ranging from 1 in 4 to 1 in 10, were prepared using a calibrated 1 ml. pipette and a graduated 10 ml. burette. The optical densities of the volumetric dilutions were higher than the expected values, calculated from the dilution ratios. When the suspensions were diluted by weighing, these discrepancies did not occur. It was concluded that pipetting had a concentrating effect on the diluted suspension.

A volume of a spore suspension was withdrawn with a pasteur pipette from a test tube and ejected back. The optical density of the suspension before and after 24 such operations was found to be unchanged using the same pipette. When a fresh dry pipette was used for each operation the optical density of the suspension was found to increase by approximately 4 per cent.

Twenty-four serial transferences of suspension were made from test tube to test tube using a fresh pasteur pipette for each tube. Every six transferences increased the optical density by approximately 8 per cent.

A spore suspension drawn up a vertical capillary tube was allowed to descend and viewed through a microscope. Streamline flow of spores was observed. Spores at the periphery moved at a slower rate than those in the centre. The downward movement of peripheral spores changed as the meniscus "overtook" them, and they passed rapidly across under the meniscus and then joined the fast moving central stream. Spores were not left in the residual film on the capillary wall.

A glass tube (length 80 cm. bore 5 mm.), provided with a tap at the lower end, was filled with water. A concentrated spore suspension was layered both on the top and half way down the column. The liquid was allowed to run down the tube and the behaviour of the spore zones closely observed. The suspension layers assumed the form of progressively elongating parabolas and a clear layer appeared over the top of the upper

spore zone. The velocity at the tips of the parabolas was double that of the meniscus while the spores at the periphery of the lower suspension did not move. These results showed the radial velocity distribution of the spores to be in accordance with the Poiseuille equation, and indicated the return of slower peripheral spores to the faster moving central stream on approach of the meniscus, which travelled at half the speed of the fastest spore.

2 ml. of a spore suspension was drawn into a pipette and 1·0 ml. ejected into each of two test tubes. The optical densities of the original suspension and of the deliveries were 0·537, 0·538, and 0·548 respectively. Similarly deliveries of 1·5 and 0·5 ml. were made giving readings of 0·535, 0·542 and 0·554 (means each of eight experiments).

The spore concentration was higher in the last portion delivered, and was greater in the last quarter than in the last half. Since the fastest spores moved at twice the speed of the meniscus the first ml. was the same as the original. When deliveries of 1·5 and 0·5 ml. were made the 1·5 ml. delivery was composed of 1 ml. original strength and 0·5 ml. of concentrated suspension. The theoretical value would be

$$\frac{(2 \times 0{\cdot}535) + 0{\cdot}554}{3} = 0{\cdot}541$$

and the experimental value was 0·542.

Deliveries made between two graduation marks on a pipette, where the distance between the marks was less than that from the tip of the pipette to the lower mark, showed no increase in optical density.

Dilutions of 10^{-5} were made by (i) using two 1 ml. pipettes and dilutions of 100 and 1,000 times and (ii) by using five 1 ml. pipettes and five 10 times dilutions. Colony counts were made on roll tubes inoculated with one ml. samples of the last dilution. The colony count/total count ratio by the first method was 80 per cent, and 90 per cent by the second method.

It is usually assumed when spore suspensions are pipetted that the residual liquid remaining on the wall of the pipette has the same composition as the original suspension. This has not been found to be true; this can be a source of error in making serial dilutions. With *Penicillium spinulosum* spores the residual film contains few spores and pipetting has a concentrating effect. Similar effects have been observed with red blood cells suspended in saline and also by Copley[1]. The error may not occur with all organisms and may vary with techniques and workers. It can be allowed for by standardising techniques and be minimised by reducing the number of pipetting operations. Preferably the error can be eliminated by using a pipette with two graduation marks the distance between them being less than the distance between the lower graduation mark and the tip of the pipette. With dropping pipettes the drops should be delivered from a full pipette.

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REFERENCE

1. Copley, *Transactions of the Faraday Society*, September, 1959.

After Mr. Porter presented the paper there was a DISCUSSION. The following points were made.

B. subtilis spores had shown similar behaviour to that described for *P. spinulosum*. A thoroughly wetted tube eliminated the error with the mould spores.