Exp.7: Estimating the Number of Sulphate Reducing Bacteria (SRB) by MPN Method.

If sulphate ions are present in an anaerobic environment, fermentation end products can be further transformed with the involvement of SRB.

SRB form a specialized group, which grows under anaerobic conditions and obtains energy from the oxidation of various organic compounds using especially sulphate as a terminal electron acceptor. The metabolic end product of the (dissimilatory) sulphate reduction is sulphide ion and, depending on the pH, hydrogen sulphide (H_2S).

SRB are more "restricted" than fermentative microorganisms in their spectrum of utilized organic compounds. Most of the fermentative microorganisms are able to transform very complex organic compounds and polymers, while the substrates for SRB are mainly various low molecular weight organic compounds, the final products of acetogenic fermentation (e.g. lactate, acetate and propionate).

SRB form a polyphyletic group and can be divided into four major taxon clusters: Gram-negative mesophilic SRB, Gram-positive spore-forming SRB, thermophilic SRB and thermophilic sulphate reducing Archaea. According to their metabolic characteristics, SRB can be divided in two main categories: incomplete oxidisers, those that oxidise organic substrates only to acetate (e.g. *Desulfovibrio, Desulfotomaculum*, *Desulfobulbus*), and complete oxidisers, those that oxidise organic substrates to CO_2 (e.g. *Desulfobacter, Desulfococcus, Desulfosarcina*).

Postgate's Medium B (PMB) is a differential culture broth, which is suitable for the cultivation of the members of the two most common SRB genera (*Desulfovibrio* and *Desulfotomaculum*). The differentiating effect of this medium is based on the appearance of black iron sulphide precipitation due to bacterial sulphate reduction.

Object of study, test organisms:

sulphate reducing bacteria

Materials and equipment:

Lake sediment sample from 2-5 cm layer below the surface,

Postgate's Medium B (PMB) broth

laboratory scales

sterile test tubes

pipette, sterile pipette tips

vortex mixer

microtiter plate

anaerobic chamber

Practice:

1. Measure 3.0 g sediment sample into 27 mL PMB broth. Homogenize the suspension for 5-10 minutes using a vortex mixer.
2. Pipette 0.3 mL of the obtained suspension into a test tube containing 2.7 mL PMB broth (label the degree of the dilution on the test tube).
3. Homogenize the further diluted sample and pipette another 0.3 mL of the obtained suspension into another test tube containing 2.7 mL PMB broth. Repeat the steps of the dilution until a six-member dilution series is obtained (label the degree of the dilution on each test tube).
4. Pipette 0.3 mL suspension from each member of the dilution series (including the first dilution) into the wells of a 96-well microtiter plate in five replicates (5 test-tube MPN method).
5. Place the microtiter plates inside the anaerobic chamber and incubate at 30°C for 2 weeks.

6. The number of SRB is estimated by the color change of the PMB broth (black precipitate of iron sulphide). Count the positive wells (black colored) on the plate, and by knowing the degree of the dilution, transform it using McCrady tables to obtain the MPN value.