

METHOD STATEMENT



Determinand:

Enumeration of Algae using the Utermohl inverted Microscope technique

Matrices validated:

Treated and Surface Waters

Principle of Method:

After the sample is taken it is fixed with Lugol's iodine solution. The sample is then pressurised, and a representative portion of known volume is transferred to a sedimentation tube. After a suitable settling period, which is dependent on volume, any algae cells in the sample are directly identified and enumerated using an inverted microscope.

The method is a direct enumeration method but will use a multiplication factor, based upon the volume of sample analysed, number of microscope fields of view used and the area of each field to calculate the final result.

Sampling and Sample Preparation:

Samples should be taken in PET bottles. (Preferably green/amber)

Fix the sample with Lugol's Iodine. 10-20ml per litre.

(by agreement Clients may fix the sample prior to transit to the lab.)

Shake the sample.

Loosen the bottle cap and squeeze the bottle sides to expel any air headspace. (Take care not to lose any of the sample which may contain the more buoyant cells).

Re-tighten the bottle top.

Drop the sample onto a hard floor from a height of around 1.2m.

* The sudden change in pressure will collapse any air vacuoles in the cells, rendering them amenable to sedimentation.

Re-introduce an air headspace and shake the sample well.

(NOTE samples known to hold high numbers of cells, especially Blue-Green algae should have this process repeated.)

Unfixed samples should be refrigerated and immediately sent to the lab. Fixed samples can be sent in at room temperature and stored until analysed.

Interferences:

High sample debris will interfere with light microscopy.

Performance of Method:

The Limit of Detection has not yet been determined for this method.

References:

The Enumeration of Algae, Estimation of Cell volume, and use in Bioassays 1990

A key to common British algae - E.G. Bellinger

A guide to Freshwater algal Flora – John, Whitton and Brookes