METHOD STATEMENT

Determinand:
Respiration inhibition test

Matrix:
Sample Type: wastewater treatment works samples

Principle of Method:
The purpose of the respiration inhibition test is to assess the effect of a test substance on microorganisms by measuring the respiration rate (oxygen consumption) under defined conditions in the presence of different concentrations of the test substance.
The respiration rate of the bacteria of the activated sludge is the rate at which they are using energy for various life processes. This can be measured as the rate at which heat is produced, but is easiest to measure indirectly from the rate at which oxygen is consumed. Any decrease in energy expenditure - or respiration rate – has a direct correlation with toxic effects on these life processes. For wastewater treatment works, it is essential that the bacteria and other microorganisms of the activated sludge are maintained in as healthy a state as possible, in order to maximise the rate at which they biodegrade waste organic molecules. The state of health of the microorganisms can be easily monitored from their rate of respiration.
The oxygen consumption of an activated sludge fed with a standard amount of synthetic sewage feed is measured immediately. The respiration rate (oxygen consumption) of the activated sludge in the presence of various concentrations (at least four) of the test substance under otherwise identical conditions is also measured. The inhibitory effect of the test substance at a particular concentration is expressed as a percentage of the mean respiration rates compared to the respiration rate of the control. This is known as the percentage inhibition. An EC_{50} value is calculated from the percentage inhibition at two different concentrations. The respiration rate is the rate of respiration of both carbonaceous and nitrifying bacteria also known as the total respiration rate.
The EC_{50} value should be regarded merely as a guide to the likely toxicity of the test substance either to activated sludge sewage treatment, or to wastewater microorganisms.
The study design is in general accordance with OECD 209 guideline.

Sampling and Sample Preparation:
In the laboratory the activated sludge should be kept at 20°C and constantly aerated. Place 300ml of the activated sludge into each side of the split flask and place into the holding area of the Strathtox. This will maintain the sludge at 20°C for the duration of the test. The activated sludge should also be kept aerated and this is achieved by the built in aeration pump which can be switched on or off by using the buttons on the front of the machine.
Add 3ml of the nitrifying sludge feed into the activated sludge into each side of the split flask. Add 3ml of the nitrifying sludge inhibitor to the left hand side only indicated by the red marker. This should be done 30 minutes before any analysis is carried out.
If required by the client the samples should be neutralised to between pH 6.0 and pH 8.0 before testing. Adverse pH conditions will reduce the respiration rate of the activated sludge but the client may not consider this a component of toxicity. The samples should be neutralised by addition of small amounts of 1M hydrochloric acid or 1M sodium hydroxide as required until the pH is within the correct range.

Interferences
Samples with a high BOD or COD may not be suitable for this method as the oxygen uptake may be too rapid to produce a linear trace.

Performance of Method:

Range of Application:

The results are reported as EC_{50}

Results should be reported according to guidance of this method.

Limit of Detection:

The limit of detection cannot be calculated for this method

Uncertainty of measurement:

Uncertainty of measurement cannot be calculated for this method.

References:

OECD Guidelines for the testing of chemicals 2010