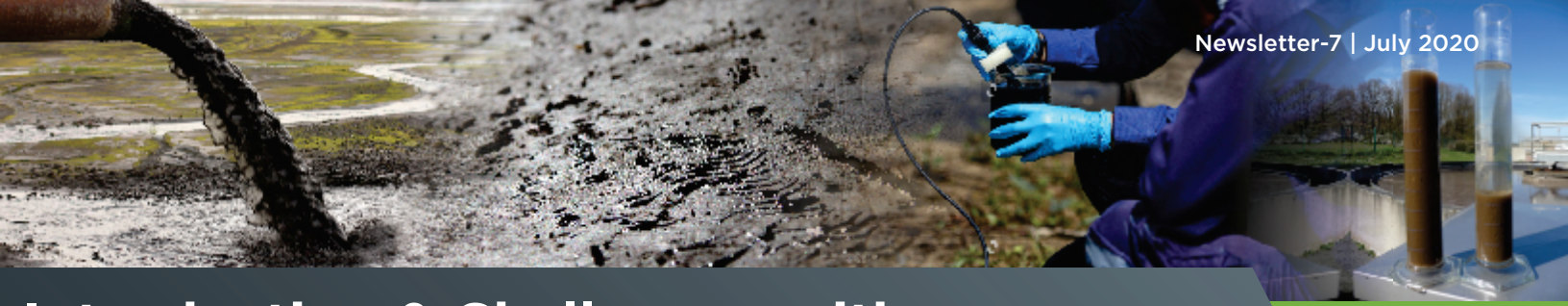


Introduction & Challenges with Activated Sludge Respiration Inhibition Test

Objective of the Test

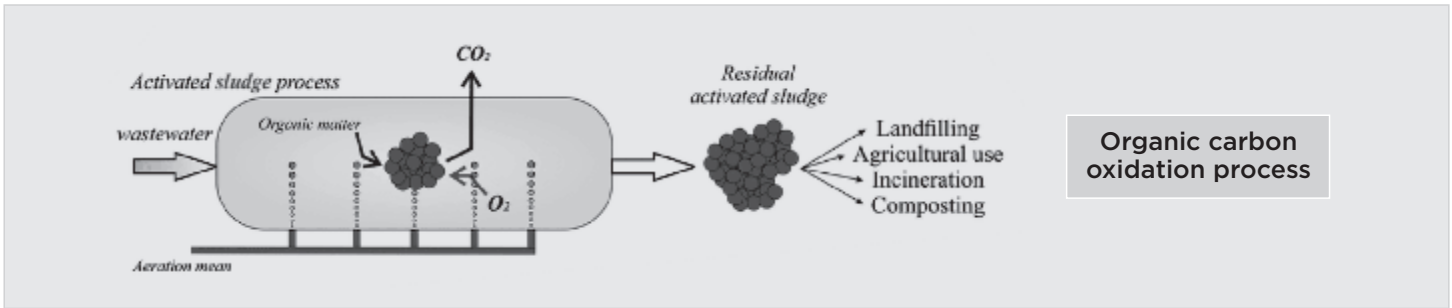
The objective of the Activated Sludge Respiration Inhibition Test is to provide a rapid screening method to assess the effects of a test chemical on the respiration rate of microorganisms found in the activated sludge of the aerobic stage of wastewater treatment plants[2].

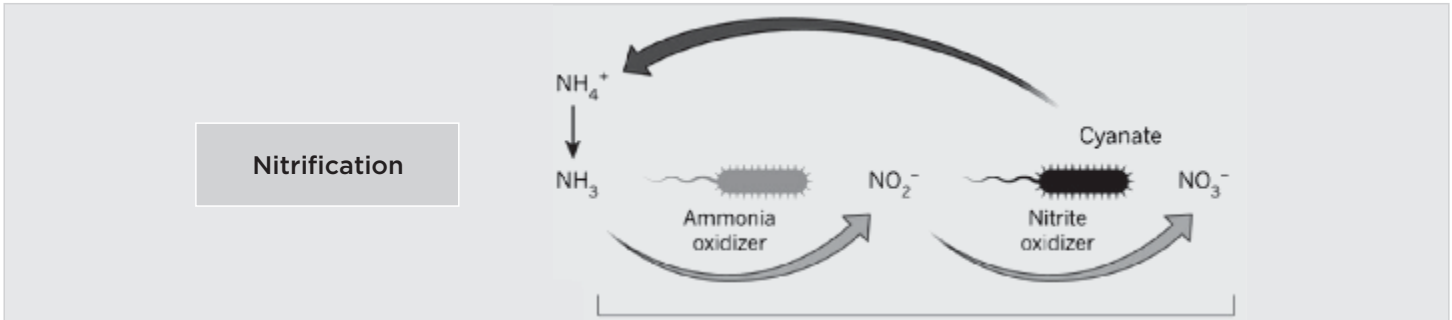
Introduction

The activated sludge process is considered as a standard method for the biological treatment of wastewaters. Outcomes of the test may also serve as an indicator of suitable non-inhibitory concentrations of test chemicals to be used in the biodegradability tests[2]. For most purposes, the method to assess the effect on organic carbon oxidation processes alone is adequate. However, in some cases, an examination of the effect on nitrification alone or both processes separately are needed for the interpretation of the results and understanding the effects.

The inhibitory effects of a test chemical on both processes: organic carbon oxidation (heterotrophic) and ammonium oxidation (nitrification) may be determined, and the EC_{50} values may be calculated[2]. Nitrification in the aerobic treatment of wastewater is a necessary step for removing nitrogen compounds through the denitrification to gaseous products from wastewaters. Some test results show that the test chemical has a more significant effect on the oxidation of ammonium than on general heterotrophic oxidation. Therefore, performing additional testing using a specific inhibitor of nitrification and by measuring the oxygen uptake rates in the presence and absence of an inhibitor, e.g. N-allylthiourea (ATU) is quite necessary[2].

Three oxygen uptake rates, e.g., total, heterotrophic, and nitrification, may be calculated using the nitrification inhibitor. The sensitivity of activated sludge, required to be checked with a suitable reference compound (a known respiration inhibitor, e.g., 3,5-dichlorophenol), by parallelly exposing with the test compound.





International test guidelines

Three test guidelines of OECD, EPA and EC are available to perform the test. The OECD and the EC test guidelines have defined these two processes: organic carbon oxidation (heterotrophic) and ammonium oxidation (nitrification), while EPA guideline has defined only one process: organic carbon oxidation.

Test procedure

The sludge microorganisms are exposed to a different concentration of the test chemical in geometric series for 30 min and/or 3 hours and the respiration rates of samples are measured using oxygen electrode. A negative control (without test chemical), positive control (with known respiration inhibitor), abiotic control (to check any abiotic degradation of test chemical) and nitrification control (with known nitrification inhibitor) are run parallelly. The inhibitory effect of the test chemical on the respiration rate in the test vessels is expressed as a percentage of the mean respiration rate of the controls.

The test design is divided into three portions, as mentioned below and may be performed according to the test item's chemical nature.

1. Range finding test
2. Definitive test
3. Limit test

As the test results, 50% effect concentration related to oxygen consumption (EC_{50}) and 95% confidence limit is calculated using appropriate statistical methods (e.g. probit analysis).

Challenges

Activated sludge from a sewage treatment plant is normally used as the microbial inoculum for the test[2]. It is quite challenging to get suitable sludge due to the sensitivity of the test organisms, and hence it is required to collect from the diverse sources. The sensitivity of the test organisms is the most critical variable for improving monitoring with biological test organisms. The test ensures the detection of toxic pollutants and contaminants at lower concentrations to enhance the sensitivity of test organisms. This allows protection of microorganisms within the activated sludge process at the wastewater treatment plant. Some trial experiments may be required to check the sensitivity of sludge collected from diverse sources.

A few challenges faced during the study/experiment are:

1. Difficulties in obtaining sludge with adequate sensitivity and low nitrification value.
2. Maintaining the adequate oxygen uptake rate in control samples
3. The coefficient of variation of oxygen uptake rate in control replicates below the limit.

Other critical parameters, such as the water solubility, vapour pressure, volatility, adsorption characteristics and purity of the test chemicals, also play an important role. These parameters directly or indirectly impose an effect on the EC_{50} value and the inhibition rate. Reference substance concentration range is also important to obtain the EC_{50} value between 2 to 25 mg/L (3, 5-dichlorophenol). The information on the test chemical composition and purity may help define the strategy to perform activated sludge test.

JRF has experience and expertise to undertake the test, as per the three guidelines referred above.

References

1. EC, 2008, COUNCIL REGULATION (EC) No 440/2008 of 30 May 2008, laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), C.11. Biodegradation: Activated sludge respiration inhibition test, Official Journal No L 54, 01/03/2016.
2. OECD, 2010: The Organisation for Economic Co-operation and Development Guidelines for the testing of chemicals, OECD 209, Activated Sludge, Respiration Inhibition Test (Carbon and Ammonium Oxidation, adopted by the Council on July 22, 2010.
3. US EPA, 2008: The United States Environmental Protection Agency (EPA), fate, transport and transformation test guidelines, OCSPP 850.3300, Modified Activated Sludge, Respiration Inhibition Test (EPA 712-C-014), January 2012.

About the Author:

Arun Raithatha

M. Sc. (Chemistry), Senior Scientist, Dept. of Environmental Fate & Metabolism, Jai Research Foundation

For over 20 years in the industry, Arun has a vivid experience, viz., leadership, training, designing, conducting, and reporting of the environmental fate studies. He is adept in conducting a higher tier metabolism, biodegradation, sorption studies and identifying key metabolites using ^{12}C and ^{14}C test compounds, as per the GLP requirement, for the registration of a compound in compliance with OECD, EPA, and EU guidelines. He has participated in the training conducted at the Bhabha Atomic Research Centre (BARC) on the use and handling of radioactive substances in the field of research and he is a certified Radiological Safety Officer.