METHODS FOR TOXICITY TESTING OF XENOBIOTICS IN WASTEWATER TREATMENT PLANTS AND IN RECEIVING WATER BODIES

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Abstract. This paper focuses on conventional and advanced promising methods for the evaluation of toxicity originated by xenobiotics in WWTPs and surface waters. A concise review about toxicity tests applied to activated sludge and to receiving water bodies was presented. Advantages or limitations of the methods were discussed. Both heterotrophic and nitrifying activity were considered. Experimental results on the application to activated sludge of conventional toxicity tests (based on respirometry and bioluminescence) and advanced methods (based on the direct quantification of viable or dead bacteria by using flow cytometry) were presented in this paper. The advantage of respirometry and flow cytometry is related to the use of bacteria present in WWTPs, without need of using pure bacterial strains different from activated sludge.

Keywords: activated sludge; bioluminescence; flow cytometry; heterotrophic activity; inhibition; nitrification; respirometry; surface water; toxicity; xenobiotics

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1. Introduction

The presence of toxic xenobiotics in the sewerage reaching a wastewater treatment plant (WWTP) may cause (A) a partial inhibition of activated sludge, reducing the rate of biological kinetics and worsening the effluent quality and (B) a further impact on the quality of the receiving water body when refractory xenobiotic compounds are not degraded in the WWTP.

The bacteria inhibition induced by toxic chemicals may be caused by the formation of a stable complex with an enzyme, a receptor site or others, or by physico-chemical changes in pH, redox, etc. Among effects produced by the exposure of microorganisms to harmful chemicals, possible actions are:

- damage or strong permeabilization of cell structure, followed by cell lysis;
- inhibition of cell metabolic activity, such as respiratory activity, intracellular enzyme activity or membrane potential;
- blocking of cell replication.

The effect of toxicants against single environmentally relevant bacteria species such as *Vibrio fischeri* was widely investigated, but many contributes indicate that the correct evaluation of the inhibitory effect should be performed directly against the mixed bacteria populations present in activated sludge or surface waters. Furthermore the same microorganisms present in different natural environments can differ significantly in their physiological status; while mixed populations inside the activated sludge can undergo an acclimatization process, when xenobiotics enter WWTPs habitually.

This paper focuses on the alternatives for measuring toxicity on activated sludge in WWTPs and surface water, comparing advantages and limits of the methods. Some experimental results are described and compared: a simplified method (based on respirometric techniques), an advanced approach (based on flow cytometry) and a conventional bioluminescence bioassay (based on *Vibrio fischeri*). The common xenobiotic compound 3,5-dichlorophenol was tested in the proposed methods.

2. Methods for Toxicity Testing on Activated Sludge in WWTPs

A considerable number of different tests have been proposed in the scientific literature for assessing toxicity on biological treatment systems. A review on toxicity methods was published in 2000 by Water Environment Research Foundation (WERF, Love and Bott, 2000), where the criteria for influent wastewater toxicity monitoring methods and suggested research needs were addressed. Recently Ren (2004) described the most recent research and developments in methods for assessing wastewater toxicity on activated sludge.