*Technical note*

# A multi-stage laboratory model for determining the impacts of anthropogenic substances on a microbial association found in aquatic ecosystems

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## Abstract

A mulli-stage model designed to examine fundamental carbon and nitrogen cycling processes inherent within aquatic ecosystems is described. A microbial association capable of organic carbon catabolism and nitrification was established within the model prior to separation of successkmal metabolic events but with retention of microbial association integrity. Organic carbon catabolism, ammonification of organic nitrogen and the process of nitrification readily differentiated in time and space. Following these separations, the ecotoxicological impacts of perturbant compounds on the association were assessed. A preliminary study with phenol (0.21 mM) showed that nitrification was inhibited until phenol concentrations were significantly lowered (<34µM) through removal by biotic or abiotic means.

## Introduction

Micro-organisms are increasingly used in toxicological bioassays to determine potential environmental impacts of xenobiotic compounds (Blessing and Submuth, 1993). The advantages of using micro-organisms as test species include their ubiquitous nature, short life cycles, rapid response to changes in environment, stability and ease of culturing, and the significant role they play in ecosystem dynamics (Bitton and Dutka, 1984).

In addition to standardised single species testing protocols, several workers have advocated the use of microbial communities to assess potential impacts of pollutant compounds (Cairns et al, 1992). The rationale for using microbial communities for impact assessments is that they provide information not available from standard single species tests. In particular, such approaches can incorporate ecologically important elements such as species interactions and energy flow. These elements can be used to determine the end points of testing and will give closer approximations to events as they would occur *in situ* (Cairns et al., 1992).

To incorporate these dimensions, laboratory model ecosystems have increasingly found application in ecotoxicological studies to determine potential impacts of anthropogenic substances on aquatic ecosystems (Portcella et al., 1982; Freitsch, 1991; Scholz and Muller, 1992). Ranging in size and complexity, laboratory models have sought to provide simple analogues of natural ecosystems in which inherent and characteristic structural and functional properties can be simulated (Wimpenny, 1988).

In this paper we describe the configuration of a continuous flow model system, used to culture a representative microbial association responsible for fundamental cycling processes inherent to aquatic ecosystems, namely, the degradation of organic substances and nitrogen transformations under aerobic conditions. The laboratory model was specifically designed to determine the impacts of priority pollutants on such cycling processes. Phenol was chosen as a representative model molecule.

To incorporate spatial and temporal heterogenic components into the model, so that changes in space and time could be physically differentiated, a multi-stage system was chosen. Separation of species habitat domains, of the isolated microbial association, with retention of overlapping activity domains, was a priority .The model described here was adapted from one designed toexamine self-purification (Freitsch, 1991). Previous use of this model type has allowed successional changes of microbial associations occurring during self-purification processes to be elucidated (Freitsch, 1991).

## Materials and methods

### Laboratory mode! configuration

The basic overall design of the multi-stage system was adopted from a model system used by Freitsch (1991). The model described here differs in configuration, length and arrangement.

The model (Fig. l)consisted of four identical channels, 3 m in length and 36 mm wide, each consisting of 75 chambers. The chamber vessels had an operational volume of 122 ml (36 x 36 x 95 mm) and, thus, the total volume for each channel was 9.15 *(*. The channels were constructed from 5 mm Plexiglass and built in 6 unit blocks each consisting of 2 x 25 chambers. The units were supported by a steel framework and were arranged in tiers (Fig. 1). Each unit was angled at 15° to create a weir flow effect to ensure mixing of nutrient medium within the individual chambers.

### Operational criteria

To limit the number of variables, the model system was operated under conditions of constant darkness, temperature and aeration. Thus, the model was housed within an insulated dark box (1.2 x 1.6 x 0.6 m) constructed from masonite boards lined with polystyrene sheets (20 mm thick). Three thermostatically controlled heating elements (60 W) situated within the box were used to achieve an

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