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 multi-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 successional 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 to examine 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 model 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. 1) 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 ℓ. 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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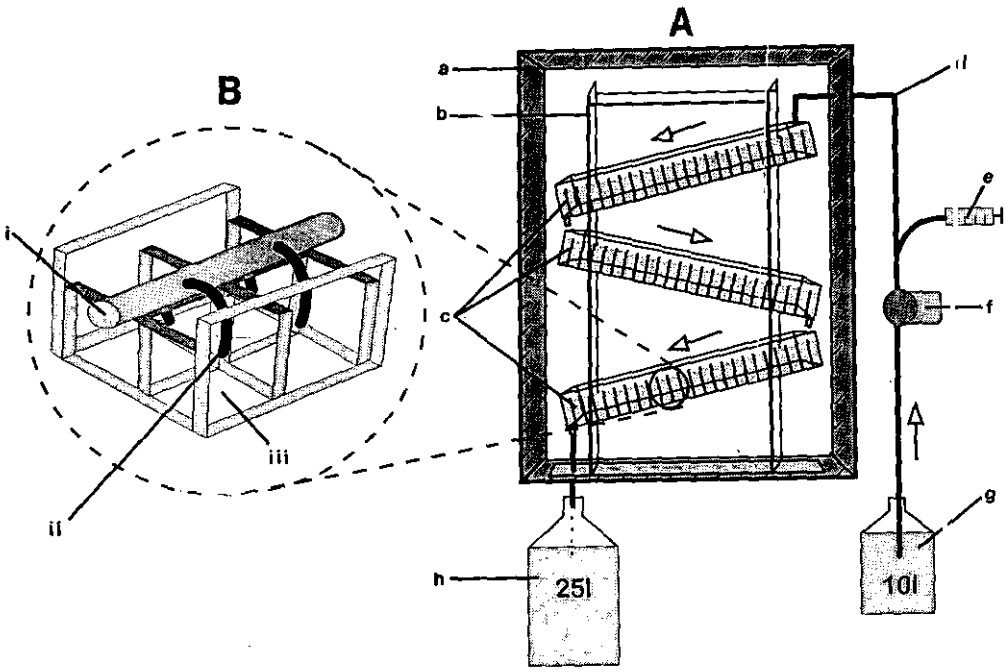

Figure 1A
Diagram illustrating side view of the multi-stage laboratory model consisting of:
a - insulated dark box;
b - steel frame;
c - unit blocks of 2 x 25 chambers;
d - silicone tubing (i.d. 2.8 mm);
e - flow meter;
f - flow inducer;
g - influent medium
h - effluent reservoir

Figure 1B
Three-dimensional view through two adjacent channels illustrating the system of inter-connected tubing used to aerate each chamber with
i - central hose pipe (i.d. 15 mm);
ii - irrigation tube (i.d. 3.5 mm);
iii - chamber (36 x 36 x 95 mm)

ambient liquid temperature of 20°C ± 2°C.

The model was operated as a continuous open-flow system with the influent medium pumped (Watson-Marlow 503U) into the first chamber of each channel. The medium flowed from one chamber to the next in weir fashion down the course of the model system.

An industrial blower (Regenair R2103,Gast Corp.) was used to avoid oxygen limitation by bubbling air into each chamber via a system of interconnected irrigation tubes (i.d. 3.5 mm) attached to a central hose pipe (i.d. 15 mm)(Fig. 1a). The air was first bubbled through a water trap to humidify it and thus minimise liquid loss in the chambers.

Inoculum

To provide an inoculum for the model, water samples (200 mℓ) were taken from various points along the Umzinduzi River in the Pietermaritzburg, KwaZulu/Natal area. Samples were taken during the wet season and were taken from regions of slow- and fast-flowing waters, from the sediment/water interface and from the water column. The samples were pooled and subjected to an enrichment/isolation step in order to provide a representative diverse microbial population for the study. The enrichment/isolation was made in a continuous chemostat culture (1 ℓ) subjected to a dilution rate of 0.01 h^{-1}. After seven days the inoculum was added to the model. Prior to sampling, the model system was allowed to establish a steady state over a period of one month.

Medium

A modified non-selective growth medium, R_2A (Reasoner and Geldreich, 1985) was used which contained (mg·ℓ^{-1} of distilled water): yeast extract, 10; proteose peptone, 10; casamino acid, 10; glucose, 10; soluble starch, 10; sodium pyruvate, 6; urea, 15; NaCl, 5.2; $CaCl_2$, 3; $MgSO_4$, 1.5; and $K_2HPO_4.3H_2O$, 21 with the pH adjusted to 7.3 . This medium was used both in the enrichment/isolation stage and to maintain the isolated association within the model. A flow rate of 10 mℓ·h^{-1} was chosen to facilitate temporal and spatial separation of the component species within the system. The overall dilution rate for the whole system was 0.011 h^{-1}, while the dilution rate for each individual chamber was 0.82 h^{-1}.

Chemical analyses

The model was operated for one month prior to chemical analysis. Samples (20 mℓ) were taken from Chambers 1, 3, 5, 10, 15, 20, 25, 30, 40, 50, 60 and 70.

Organic carbon catabolism

Organic carbon catabolism was monitored by dissolved organic carbon (DOC mg C·ℓ^{-1}) assay, measured with an infrared spectrophotometer (Skalar CA-10)(*Standard Methods*, 1989).

Nitrification

The process of nitrification was traced by the determination of ammonia, nitrate and nitrite (mg N·ℓ^{-1}). The dissolved ammonia concentration was determined with an ammonia electrode (Orion 95-12) in conjunction with a pH meter (Crison). Concentrations in aqueous samples (5 mℓ) were determined by comparison with a prepared ammonium chloride standard calibration curve (concentration range 0.1 to 10 mg·ℓ^{-1}).

Nitrate and nitrite analyses were made with a high pressure liquid chromatograph (Waters 600E) fitted with an IC-Pak anion column and conductivity detector (Waters 431). Borate/gluconate eluent (borate/gluconate concentrate, 20 mℓ; n-butanol, 20 mℓ; acetonitrile 120 mℓ made up to 1 ℓ with Milli-Q water) was used at a flow rate of 1.2 mℓ·min^{-1}. To prepare borate/gluconate concentrate: sodium gluconate, 15 g; boric acid, 18 g; sodium tetraborate decahydrate, 25 g; and glycerine, 250 mℓ were dissolved and diluted to 1 ℓ with Milli-Q water. The concentrations in each sample (100 µℓ) were determined by comparison of the mean peak areas with nitrite (5 to 15 mg·ℓ^{-1}) and nitrate standards (5 to 15 mg·ℓ^{-1}).

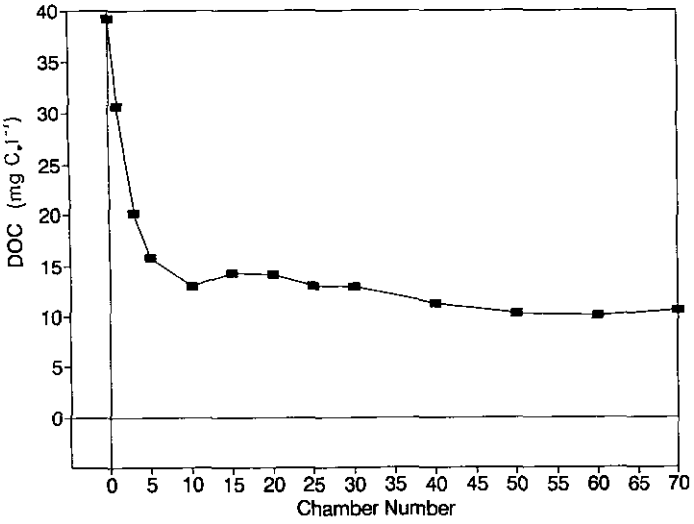

Figure 2
Residual dissolved organic carbon concentrations in discrete chambers of the multi-chamber model prior to perturbation with phenol

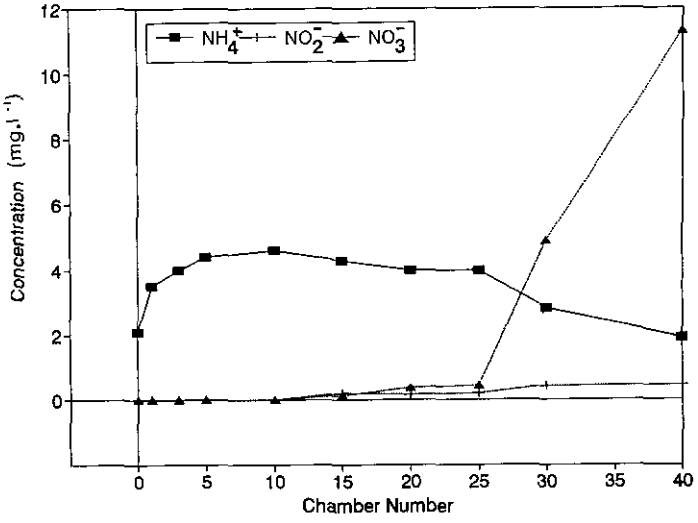

Figure 4
Course of nitrification after a continuous two-week perturbation of phenol (0.21 mM) to Chamber 1 of the multi-chamber model

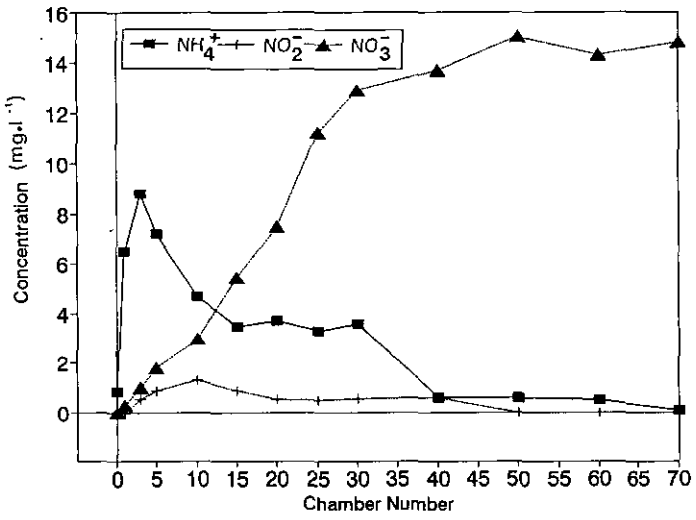

Figure 3
Course of nitrification along the model prior to perturbation with phenol

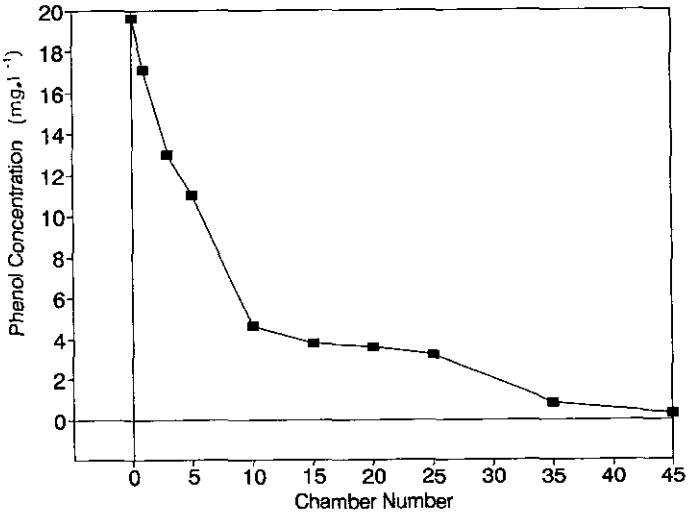

Figure 5
Residual phenol concentrations after a continuous two-week perturbation of phenol (0.21 mM) to Chamber 1 of the multi-chamber model

Phenol

Phenol analyses were made with the same HPLC fitted with a C_{18} Novapak column and UV-absorbance detector (Waters 486). A methanol:ethanol: 0.01% (%$_v$) phosphoric acid (40:15:45) eluent with 10 mℓ of PIC A reagent (Waters) was used at a flow rate of 1 mℓ·min^{-1}. Phenol concentrations of 20 µℓ samples were determined by UV-absorbance at 214 nm by mean peak area comparison with suitable phenol standards (5 to 100 mg·ℓ$^{-1}$).

Results and discussion

The data presented here indicate initial findings from a single experimental channel and illustrate the spatial separation of organic carbon degradation and nitrogen transformation along the course of the model.

The organic carbon was catabolised early within the system (Fig. 2). This correlated with increased microbial biomass viewed in the first 3 to 5 chambers of the model.

Figure 3 illustrates the course of nitrogen transformation. An initial increase in ammonia concentration indicated that ammonification occurred early and resulted from the deamination of amino acids and the breakdown of urea. The subsequent decrease in ammonia concentration with a concomitant increase in nitrate indicated that populations of nitrifying bacteria had established. Nitrite did not accumulate in any appreciable concentrations due to its subsequent oxidation to nitrate during the nitrification process.

In a preliminary perturbation study, the addition of 20 mg·ℓ$^{-1}$ phenol (0.21 mM) to the influent medium effected a population shift in nitrification activity over a two-week period (Fig. 4). Nitrate assimilation occurred from Chamber 25 onwards. From Fig. 5 it was evident that nitrification began only after the residual

phenol concentration had been reduced to less than 4 $mg \cdot \ell^{-1}$ (34μM) in the model, thus indicating the sensitivity of nitrification to phenol. Phenol removal can be attributed to various abiotic and biotic factors such as biodegradation, volatilisation and absorption.

From the results it was apparent that low concentrations of phenol must be reached before nitrification can proceed. The ability of a natural ecosystem to catabolise or displace a perturbant compound through biotic and/or abiotic means is thus an important consideration in any ecotoxicological impact assessment.

Replicate channels were found to follow similar trends in terms of organic carbon degradation and nitrogen transformation. Variability between and within channels needs to be addressed and requires further investigation. These variables will be the focus of a future publication.

The value of the model lies in the fact that it incorporates ecologically relevant elements which adds to the extrapolative value of information gained during ecotoxicological testing. It is suitable for both acute and chronic testing of pollutant compounds and may also be used in biodegradation studies. It also holds potential for evaluating toxicological impacts on waste-water treatment processes. The main disadvantages of such a system arise from its operational complexity, its impractical application in screening large numbers of compounds, and its lack of standardisation. Future work will determine whether the value of the information gained outweighs these practical disadvantages.

Conclusions

Within the model a representative microbial association responsible for catabolism of natural molecules was established. Successional changes in the interrelated processes of organic carbon catabolism and nitrogen transformation were separated in space and time. This then facilitated examination of individual functional groups in the presence and absence of phenol without violating the integrity of the microbial association. The model system described has thus proved to be effective for examining ecotoxicological impacts on aquatic environments.

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