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Research article

Potential of cyanobacterial biofilms in phosphate removal and biomass production

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ABSTRACT

Four cyanobacterial biofilms, raised from cyanobacterial mats and dominated by *Phormidium* and *Oscillatoria* spp., were successfully grown attached to polyester mesh discs, and were tested for their probable application in PO_4^{3-}-P removal from domestic sewage and other nutrient enriched wastewaters. Biofilm # 2, dominated by *Phormidium fragile*, best removed PO_4^{3-}-P; nevertheless, some of it also grew outside the substrate making harvesting difficult. Other biofilms also efficiently removed PO_4^{3-}-P from the medium in the following order: Biofilm # 1 > Biofilm # 3 > Biofilm # 4. Their growths were restricted to discs and are therefore better candidates as they can be efficiently harvested after PO_4^{3-}-P removal. PO_4^{3-}-P removal was primarily due to its uptake during growth of the biofilm rather than because of precipitation as pH of the medium remained <8.5. NO_3^--N concentration in the medium determined PO_4^{3-}-P removal efficiency of the test biofilms and therefore optimum N:P ratio is necessary for optimizing PO_4^{3-}-P removal. The test biofilms could also efficiently remove NO_3^--N from the medium.

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1. Introduction

Cultural eutrophication, which triggers excessive plant and phytoplankton growth in water bodies, is a global problem (Smith, 2003). Of the various nutrients implicated in cultural eutrophication, nitrogen and phosphorus are the most important ones (Paerl, 2008). Furthermore, phosphate is of far greater importance because many cyanobacteria, such as, *Anabaena*, *Aphanizomenon*, *Nostoc*, etc., which grow abundantly in eutrophic waters, are nitrogen fixers and hence do not depend on nitrogenous compounds for their growth (Smith, 1983). Phosphate-bearing detergents are inter alia responsible for enhanced phosphate concentration in domestic sewage. Hence it is extremely important to remove phosphorus from domestic sewage and other wastewaters so that waterbodies do not suffer from eutrophication. Advanced wastewater treatment strategies can be employed for phosphate removal from wastewater (Kumar et al., 2007); however, they are expensive and hence not commercially viable. High-rate oxidation pond, a phytoplankton-based commonly used secondary method of wastewater treatment, may remove from wastewater some of the phosphorus, if algal biomass is harvested after wastewater treatment (Oswald et al., 1957).

Potential in removing phosphate from aqueous medium or wastewater has been tested for phytoplankton like *Chlorella*, *Scenedesmus, Arthrospira, Oscillatoria,* etc. (Lee and Lee, 2001; Aslan and Kapdan, 2006; Cai et al., 2013; Ji et al., 2013; Song et al., 2014; Valderrama et al., 2002). However, difficulty in harvesting tiny phytoplankton after phosphate removal is a major constraint. Therefore, use of phytoplankton for phosphate removal on a large scale does not seem to be a good proposition. To obviate the problem of biomass harvesting, immobilized algae and cyanobacteria have been tested. However, the commonly used immobilizing agent alginate is costly and not stable at extreme pH values (de-Bashan and Bashan, 2010). In this context, naturally growing and self-immobilized cyanobacterial biofilms and mats seem to be good option because of their widespread distribution (Bender and Phillips, 2004) and ease in harvesting. However, these self-immobilized communities remain little explored for their nutrient removal potential in comparison to comprehensively studied phytoplankton.

The main aim of the present study has been to grow

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cyanobacterial biofilms, raised from cyanobacterial mats, attached to an artificial substratum and to use them for PO_4^{3-}-P removal in batch system. Four cyanobacterial mats were collected locally and grown in the laboratory, and PO_4^{3-}-P removal potential of biofilms developing from them was assessed. Since nitrogen concentration may also limit the growth of algae in natural waters (Larsdotter, 2006), an effort was also made to study PO_4^{3-}-P removal by the test biofilms at varying concentrations of NO_3^--N. The potential of the test mats in removing NO_3^--N was also assessed.

2. Materials and methods

2.1. Cyanobacterial mats

Four cyanobacterial mats, collected from man-made ponds located in areas close to the campus of the Banaras Hindu University, Varanasi, or Ghazipur, a nearby district, India, were used in the present study. Species of *Phormidium* or *Oscillatoria* were the dominant organisms in all the tested mats which were identified using morphological criteria as per Desikachary (1959) and the web resource "AlgaeBase" (http://www.algaebase.org). Mats were designated as Mat # 1, 2, 3 and 4 for the experiment. Before use in PO_4^{3-}-P and NO_3^--N removal studies, the test mats were acclimatized to grow in BG 11 culture medium (Hughes et al., 1958). The culture medium was prepared using analytical grade salts.

The test mats grew in the laboratory forming a thin microbial film that was attached to the bottom of the culture vessel. In due course of time, the film detached from the bottom and became buoyant, subsequently increasing in thickness and entrapping air bubbles. The cyanobacterial biofilm needed at least two weeks growth for sufficient increase in thickness (~1 mm) so that it could be called as proper mat. Test mats were able to grow floating covering the entire surface area of the medium. The test mats could also very well grow attached to polyester mesh discs (size: 3.46 cm^2; 42 mesh cm^{-2}) which could float on the surface of the culture medium. The test mats, especially Mat # 4, were fragile but their handling became easier when they were allowed to grow colonized onto polyester mesh discs. In the present study, polyester mesh discs were used for all the experiments.

2.2. Experimental design

As already mentioned above, polyester mesh disc was used as the artificial substrate to facilitate the colonization and growth of mat-forming organisms. In all the experiments, artificial substrates were inoculated with mat microorganisms. For this purpose, the test mats were homogenized in BG 11 medium containing 713 μg l^{-1} of phosphorus, which is 1/10th of the recommended phosphate concentration in this medium, and an inoculum of the mat was prepared. The concentration of phosphorus in the mat inoculum was reduced because of short duration of experiments and the intention to have uniform inoculum conditions for all the experiments. Thoroughly washed and autoclaved mesh discs were immersed in mat inoculum for 24 h and gently shaken 4–5 times to facilitate the colonization and growth of the mat-forming organisms.

All the experiments in the present study were carried out for a time period of 5 days, which was not adequate for mat formation. Instead of a proper mat, the substrates showed a thin biofilm of the mat forming organisms on its surface after 5 days of growth. For this particular reason, this community has been referred to as biofilm in the present study. Throughout the text, the biofilms developing from Mat # 1, Mat # 2, Mat # 3 and Mat # 4 have been referred to as B # 1, B # 2, B # 3 and B # 4, respectively.

An experiment was conducted to study the effect of different initial PO_4^{3-}-P concentrations (1–10 mg l^{-1}) in the BG 11 medium on the growth behavior and phosphate removal capacity of the biofilms. BG 11 medium has been used for PO_4^{3-}-P removal studies because its composition resembles that of many nutrient enriched wastewaters. The concentrations of PO_4^{3-}-P selected for the study are within the range reported for domestic wastewaters (Forster, 2003; Torit et al., 2012; Ji et al., 2014). PO_4^{3-}-P concentration in the medium was varied using its salt dipotassium hydrogen phosphate; the other constituents of the medium were exactly as per the recipe given by Hughes et al. (1958). In all the treatments potassium ion concentration was maintained using KCl solution. The experiment was performed in 100 ml flasks each containing 20 ml BG 11 medium with specified PO_4^{3-}-P concentration. One mat inoculum-containing disc (initial biomass ~0.5 μg chl *a*) was added to each flask. The pH of the medium was adjusted to 7.8 using 0.1 N HCl or NaOH. Each flask was gently shaken 4–5 times every day. The culture flasks received 72 μmol m^{-2} s^{-1} of photosynthetically active radiation (400–700 nm) from cool daylight fluorescent tubes in a 12 h light and 12 h dark cycle at 27 °C. Every day, the culture flasks (4 replicates) of each concentration of PO_4^{3-}-P for every mat were sacrificed for estimating residual phosphate phosphorus concentration in the medium and biomass (chlorophyll *a*).

To study the effect of variations in the concentration of NO_3^--N on PO_4^{3-}-P removal and biomass production, an experiment was carried out keeping the concentration of PO_4^{3-}-P constant, i.e., 5 mg l^{-1} in the medium. The concentration of NO_3^--N varied from 5 to 40 mg l^{-1}. Analytical grade KNO_3 has been used for varying NO_3^--N concentration in the medium. Where ever necessary, KCl was used to maintain the concentration of potassium ions in the medium. The culture flasks were inoculated and incubated under conditions already described above. After 5 days of incubation, all the flasks were harvested for biomass estimation as well as for measuring residual NO_3^--N and PO_4^{3-}-P concentrations in the medium.

2.3. Growth measurement

The specific growth rate (μ) of the test biofilms was calculated using the following equation:

$$\mu\left(\text{day}^{-1}\right) = \ln X_2 - \ln X_1 / t_2 - t_1,$$

where X_2 and X_1 are the biomass in terms of chl *a* in mg l^{-1} at time t_2 and t_1, respectively.

The relative performance of the test biofilms was assessed by determining yield coefficient (Y, mg chl *a* mg^{-1} nutrient) for phosphate as well as nitrate (Aslan and Kapdan, 2006) from the slope of the plot between nutrient utilized and corresponding biomass achieved after five days of growth.

2.4. Chemical and statistical analyses

In the present study, the biomass of biofilm was measured in terms of chl *a*, which was estimated by extracting the pigment in chilled methanol (Lichtenthaler and Buschmann, 2001). For this purpose, biofilm-colonized discs were put in methanol in a freezer for 24 h. Sometimes, especially in the case of B # 2 which showed the growth of microalgae and cyanobacteria outside the mat, it became necessary to harvest all the biomass by centrifugation at 6000 rpm for 10 min. For quantification of chl *a* in methanol extract, the equation given by Lichtenthaler and Buschmann (2001) has been used. Phosphate removal by the test biofilms was measured by estimating the residual PO_4^{3-}-P concentration in the medium following the ascorbic acid method whose detection limit is

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