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Short communication

Development and application of a novel immobilized marine microalgae biofilter system for the treatment of shrimp culture effluent

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ABSTRACT

Removal of excessive nutrients is essential for aquaculture wastewater treatment to protect receiving waters from eutrophication and for potential reuse of the treated water. This semi pilot-scale wastewater treatment system consisting of an agar-alginate algal blocks (AAAB). It was tested for removal of nutrients in shrimp farm wastewater. Aquaculture wastewater (90 days old *Litopenaeus vannamei* cultured water) was treated with a novel biofilter that was filled with a marine *Picochlorum maculatum* immobilized in alginate blocks. The removal rates of nutrients (phosphate, nitrate, nitrite and ammonia) were evaluated at each cycle for ten cycles. The results showed that the nitrite (89.6%) and ammonia (98.5%) were removed effectively while -phosphate (57%) and nitrate (46.4%) was removed less effectively.

1. Introduction

Although recently a large number of studies have reported the use of freshwater microalgae to treat various wastewater [1–4], only a few number of studies were available on nutrients removal by marine microalgae and no report was available on *Picochrloum macultum* except for our previous work [5]. Compared to free living microalgae cells, immobilized microalgal cells can provide several advantages: for example, the immobilized microalgae can simplify wastewater treatment, because the entrapment of living cells can increase the life of cells and microalgae can maintain their metabolic activities for long periods [6]. de-Bashan and Bashan [7] reported that immobilization is an efficient method to separate microalgae from culture medium when they are used in tertiary wastewater treatment. Dinesh Kumar et al. [8] reported that the *Chlorella marina* embedded in alginate beads removed 90% of nitrate (IC–1 μmol L^{-1}) and 60% of phosphate (IC-0.1 μmol L^{-1}) from aqueous solution in 24 h of retention time. Adam et al. [9] also reported that *Tetraselmis* sp-embedded beads removed 41% of nitrate (IC-0.192 μmol L^{-1}) and 18% of silicate (IC-0.103 μmol L^{-1}) from tannery wastewater-at pH 7 with 3 h of retention time. While there are advantages of using microalgae beads for wastewater treatment, there are various challenges as well. These include the adequate control of pH, stocking density of beads, algal cell concentration in beads, and algal cell leakage from the beads [9,10]. For example, extremely high beads concentration reduce the light penetration in wastewater, enhances self-shading effects and the settling of beads at the bottom of the treatment tank. In order to overcome such problems, the present study developed a novel biofilter system with some minor modification from Lee et al. [11], that uses a chain type immobilized marine microalgae to treat shrimp aquaculture wastewater. There are several advantages of this system such as 1) pH adjustment not required, because the pH of the wastewater didn't affect the entrapped microalgal cells, 2) it does not need to control bead density or algal cell concentration, the reason behind this is, AAAB didn't damage due to over loading of algal cells in the blocks, and 3) it can be deployed at lab and outdoor farms to treat the wastewater, easy to handle and moreover, this system is eco-friendly.

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Table 1
Mean and standard deviation (SD) of nutrients removal (PO_4^{3+}, NO_3^-, NO_2^-, NH_3^+) under different cycles by agar-alginate immobilized *Picochlorum maculatum* blocks.

Cycles	Phosphate (μmol L^{-1})		Nitrate (μmol L^{-1})		Nitrite (μmol L^{-1})		Ammonia (μmol L^{-1})	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
0	0.348 ± 0.000	0.348 ± 0.000	3.715 ± 0.000	3.715 ± 0.000	3.985 ± 0.000	3.985 ± 0.000	12.295 ± 0.000	12.295 ± 0.000
1	0.329 ± 0.001	0.333 ± 0.001**	3.692 ± 0.003	3.705 ± 0.004*	3.948 ± 0.009	3.968 ± 0.006	11.270 ± 0.023	11.070 ± 0.033*
2	0.311 ± 0.003	0.319 ± 0.002	3.545 ± 0.151	3.552 ± 0.057	3.930 ± 0.005	3.918 ± 0.003*	8.412 ± 0.043	8.673 ± 0.012*
3	0.304 ± 0.002	0.313 ± 0.002	3.399 ± 0.010	3.363 ± 0.031	3.925 ± 0.008	3.732 ± 0.021**	6.184 ± 0.013	6.261 ± 0.071
4	0.292 ± 0.004	0.285 ± 0.001	3.399 ± 0.003	3.342 ± 0.011**	3.912 ± 0.004	3.150 ± 0.024***	5.274 ± 0.022	4.146 ± 0.037***
5	0.275 ± 0.003	0.259 ± 0.004	3.393 ± 0.004	3.196 ± 0.004***	3.896 ± 0.004	2.404 ± 0.013***	5.920 ± 0.053	2.560 ± 0.110**
6	0.268 ± 0.003	0.239 ± 0.002**	3.391 ± 0.004	2.749 ± 0.019***	3.836 ± 0.005	1.545 ± 0.007***	6.154 ± 0.109	1.824 ± 0.028***
7	0.272 ± 0.004	0.219 ± 0.003**	3.400 ± 0.005	2.530 ± 0.016***	3.808 ± 0.009	0.733 ± 0.015***	6.613 ± 0.028	1.380 ± 0.124***
8	0.287 ± 0.003	0.198 ± 0.002**	3.410 ± 0.008	2.339 ± 0.016***	3.749 ± 0.008	0.651 ± 0.030***	6.955 ± 0.023	0.764 ± 0.044***
9	0.294 ± 0.002	0.171 ± 0.007**	3.413 ± 0.006	2.148 ± 0.005***	3.714 ± 0.004	0.525 ± 0.008***	7.250 ± 0.044	0.513 ± 0.010***
10	0.299 ± 0.005	0.147 ± 0.005**	3.423 ± 0.006	1.991 ± 0.004***	3.715 ± 0.004	0.414 ± 0.004***	7.587 ± 0.034	0.176 ± 0.008***

Results are shown as mean± SEM. Each nutrients (μmol L^{-1}) were compared with that at control (paired *t*-test, two tailed), n = 3.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

2. Materials and methods

2.1. Culture of microalga

The water sample was collected from the Palk Bay region of Muthukuda coast (Lat. 9° 51′ 48″ N; Long 79° 7′ 15″ E), Tamil Nadu, Southeast coast of India. This region pooled with vast amount of nutrients from the wastewater released by nearby shrimp culturing ponds. Isolation and identification of microalgae was done by agar plating technique [12]. A culture of the *Picochlorum maculatum* (Accession number – KJ754560) cells were cultured according to standard method [5] in sterilized seawater enriched with Walne medium [13].

2.2. Construction of biofilter with agar alginate algal blocks

Picochlorum maculatum cells were immobilized in round blocks prepared according to Lee et al. [11] with minor modification. Agar-alginate block consisting 2.5 cm in diameter and 1.25 cm thick composed with 15 g agar and 5 g sodium alginate in 1000 mL of double distilled water. The *P. maculatum* cells (111,200 cells/ml) were added evenly after autoclaving of agar-sodium alginate mixture. The algal cell viability, analyzed by cells were subjected to high temperature for a prolonged time i.e 10–15 min. Algal cells were counted again and the concentration of living cells was slightly decreased from 111200– 98300 cells/ml.

Autoclaved agar-alginate mixture was poured in a tray with a thickness of 2 cm. After solidification of agar-alginate mixture, a round mold was used to cut the blocks from flattened agar-alginate mixture. The *P. maculatum* immobilized agar-alginate blocks (2.5 cm diameter + 1.25 cm thick) were connected using polyethylene wire. The blocks on the wire, polyethylene button was placed in front and back of the each block for the support (Fig. 1a–d). In each blocks eight holes (0.2 m in diameter) were punched by using plastic straw. Agar with sodium alginate without microalga was kept as control. In the agar blocks, the polyethylene wire was inserted into a transparent tube contain 2.6 cm in diameter. Overall, 63 blocks were attached and the total length of string was 5.5 m and the same number of blocks and length were maintained for control too. The nutrients removal experiment using AAAB lasted for a day. The control biofilter was also similarly designed. The aquaculture wastewater (90 days old *Litopenaeus vannamei* cultured water containing pH – 8.41, salinity – 37psu, PO_4^{3+}- 0.35 μmol L^{-1}, NO_3^- – 3.72 μmol L^{-1}, NO_2^- – 3.99 μmol L^{-1}, $NH3^+$ – 12.29 μmol L^{-1}) was collected from Parangipettai, India. It was kept in a plastic tank with a capacity of 10 L and placed at a higher position of about 60 cm height from the collection tank. One side of the agar-alginate blocks tube was connected to the upper tank and the end of the tube was inserted in to a collection tank (10 L capacity). The wastewater flow rate was adjusted to 80 mL/min (to AAAB is fully immersed with wastewater) for maximum nutrient adsorption. Once the wastewater was passed to bottom tank completely the lower part of the blocks and bottom tank were shipped to a higher position and wastewater was filtered with the same method. The procedure was continued up to 10 cycles (5 for lower part and 5 for higher part). To know the highest value of adsorption rate the repeated cycles were carried out. The detailed schematic representations of treatment plant were given in Fig. 2. The wastewater has not been sterilized and experiment was carried out at temperature between 22 and 35 °C. Simultaneously the control and experimental test was carried out for three times Fig.3.

2.3. Evaluation of nutrients removal

Initial and final concentrations of nutrients in wastewater were analyzed according to Strickland and Parsons [14] and Jenkins and Medsken [15] and for detailed methodology see our previous publication [5]. The percentage of nutrients removal was calculated using the following equation:

$$\%\,\text{of removal} = \frac{\text{ICWW} - \text{FCEC}}{\text{ICWW}} \times 100$$

ICWW- initial concentration of wastewater; FCEC- final concentration of each cycle

2.4. Statistical analysis

The control and experimental values were subjected to paired *t*-tests. Significant levels for all analyses were set to $p < 0.05$. All statistical calculations were done using SPSS version of 16.0.

3. Results

The observed initial concentration of pH, salinity, phosphate, nitrate, nitrite and ammonia concentration in the shrimp waste water were 8.41, 37 psu, 0.35, 3.72, 3.99 and 12.29 μmol L^{-1}, respectively. The density of *P. maculatum* cells that were used for immobilization were 1, 11, 200 cells/ml. When the cells were viewed under the microscope, significant morphological and color changes were observed in the agar alginate immobilized *P. maculatum* cells. The effect of agar-alginate *P. maculatum* block on nutrients removal with paired '*t*' test was summarized in Table 1.

Fig. 3a showed that the phosphate removal efficiency of *P. maculatum* agar-alginate immobilized blocks. Both control and