

Full Length Research Paper

## Removal of phosphate and nitrate from aqueous solution using seagrass *Cymodocea rotundata* beads

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The aim of the present study was the removal of phosphate and nitrate by sodium alginate seagrass (*Cymodocea rotundata*) beads from aqueous solutions. The adsorption characteristics of phosphate and nitrate on the seagrass beads were optimized under different operational parameters like adsorbent dosage, initial concentration of phosphate and nitrate, retention time and pH. The results showed that 71% of phosphate and 62% of nitrate removal was obtained using this seagrass beads. It is concluded that seagrass beads is a relatively efficient, low cost and easily available adsorbent for the treatment of nutrients rich wastewater.

**Key words:** Biosorption, seagrass, *Cymodocea rotundata*, nutrients, beads.

### INTRODUCTION

Large-scale production of wastewater is an inevitable consequence of all contemporary societies. Most wastewaters are usually hazardous to human health and the environment and must be treated prior to disposal into rivers, lakes, seas, and land surfaces. Secondary treatments of domestic and agro-industrial wastewater still release large amounts of phosphorus and nitrogen. These nutrients are directly responsible for eutrophication of rivers, lakes and seas (Lau et al., 1997; Trepanier et al., 2002) and disposal of partially treated wastewaters produce a constant threat to dwindling freshwater resources on a global scale (Montaigne and Essick, 2002). Prior to discharging wastewater into water bodies,

removing excessive nutrients is usually obligatory, even though it is not performed in many cases, especially in developing countries. The wastewater treatment industry presently uses several methods to remove phosphorus and nitrogen (Duenas et al., 2003) and other pollutants. Some are used in large-scale treatment facilities and a few are experimental projects and used on a small-scale basis (from a process-engineering viewpoint) (Stratful et al., 1999; Van Loosdrecht et al., 1997). In this context, the current study examined the biosorption capacity of seagrass beads on nutrients (phosphate and nitrate) removal from the aqueous solution. Various methods have been carried out on nutrient removal (Azhar et al.,

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2009, 2010a, 2010b, 2011). Several adsorbents like clay and zeolite (Azhar et al., 2011, 2004), limestone (Celik et al., 2001; Aziz et al., 2004), carbon-zeolite composite (Ehret et al., 2001), silica-carbon-calcium carbonate (Farrah and Preston, 1985), microbes (Zeng et al., 2011), marine microalgae (Dinesh Kumar et al., 2013), seaweed (Mithra et al., 2012) and seagrass (Vasanthi, 2012) have been used for biosorption of nutrients. The use of algae in biotechnology has been increased in recent years, these organisms being implicated in food, cosmetic, aquaculture and pharmaceutical industries (Borowitzka and Borowitzka, 1988). Cell immobilization techniques have been developed in order to solve the pollution related problems. The use of immobilized algal cells in water purification processes has been reported long ago (Robinson et al., 1988), as algae form part of the organisms fixed in percolating filters of wastewater treatment plants. But at the end of the sixties of the past century, novel techniques for immobilizing biocatalysts in general (from enzymes to whole cells) began to spread in the literature (Papageorgiou, 1987), and the use of immobilization techniques diversifies. Immobilized algae have been used for biomass obtaining and macronutrient removal. The extremely high accumulation capacity of some of these organisms for potentially dangerous substances (Maeda and Sakaguchi, 1990) has been also exploited for bioremediation techniques applied on polluted waters (especially involving metals; Greene and Bedell, 1990). Therefore, the present attempt has been made on the use of seagrass *Cymodocea rotundata* beads for the removal of nutrients from the aqueous solution. Further, the attempt was also made in the optimization of conditions like pH, incubation time, biomass concentration and initial nutrient concentration on the removal of nitrogen and phosphorus from aqueous solution.

## MATERIALS AND METHODS

### Collection of seagrass

The healthy and fresh seagrass *C. rotundata* was collected from Palk Bay, Muthukuda (9° 51' 48" N; 79° 7' 15" E) Southeast coast of India. The biosorbent powder was prepared according to Bishoni et al. (2004). The seagrass was washed twice with running tap water and then thoroughly rinsed with distilled water to remove particulate matter and adhering salts from the surface. Then the biomass was shade dried and later oven dried at 60°C for 24 h. Dried biomass was subsequently ground with mortar and pestle and sieved to a particle size of 500-850 µm and then stored in desiccators for nutrient biosorption experiments.

### Preparation of seagrass beads

The seagrass beads were prepared according to Santos et al. (2002). To prepare 100 ml of alginate solution with the required alginate concentration, the alginate was first carefully dissolved by stirring in 70 ml of distilled water. In the remaining 30 ml of distilled water, 3.5 g of NaCl were dissolved to obtain 3.5 g/l salinity final

solution. When the alginate was completely dissolved, the two parts were mixed in magnetic stirrer. Cation solutions were prepared in nanopure water. Beads were prepared by adding alginate solution drop wise by means of 20 ml of syringe (0.8 mm × 40 mm needle, Braun, Melsunger, Germany) into the cation solution, from a height approximately one drop per second. Beads were kept stirring in the cation solution for 45 min to allow complete hardening of the alginate and washed several times with filtered (0.45 µm) natural seawater to eliminate the remaining cation.

### Preparation of nutrient stock solutions

The nutrient stock solutions were prepared by diluting the corresponding salts namely, sodium nitrite and di potassium hydrogen phosphate in distilled water. The stock solutions were diluted with distilled water for the preparation of working solutions. Different initial concentration (10, 1 and 0.1 µmol/l) of working solutions of nitrate and phosphate was prepared. All the nutrient stock solutions (100 ml) were taken in 250 ml conical flasks. The pH of each solution was adjusted from 5 to 9 using diluted sodium hydroxide (NaOH) and hydrochloric acid (HCL) and pH were estimated by using pH meter (ELICO, INDIA).

### Adsorption of nutrients by seagrass beads

To the nutrient stock solutions, different densities of seagrass beads were added to the conical flasks. Then the flasks were maintained at 30°C in shaker for various time intervals namely, 30, 60, 90 and 120 min at 200 rpm. At the end of the experiment, the flasks were removed from the shaker and the solution was separated from the biomass by filtration through Whatman filter paper (47 µm) using Millipore filtering apparatus. Then the final concentration of the nutrients was estimated. The amounts of nutrients in the sample were measured spectrophotometrically using Shimadzu Model-2450 as per the standard protocol described by Strickland and Parsons (1972). Nitrate was estimated according to Jenkins and Medsken (1964).

### Effect of initial concentration

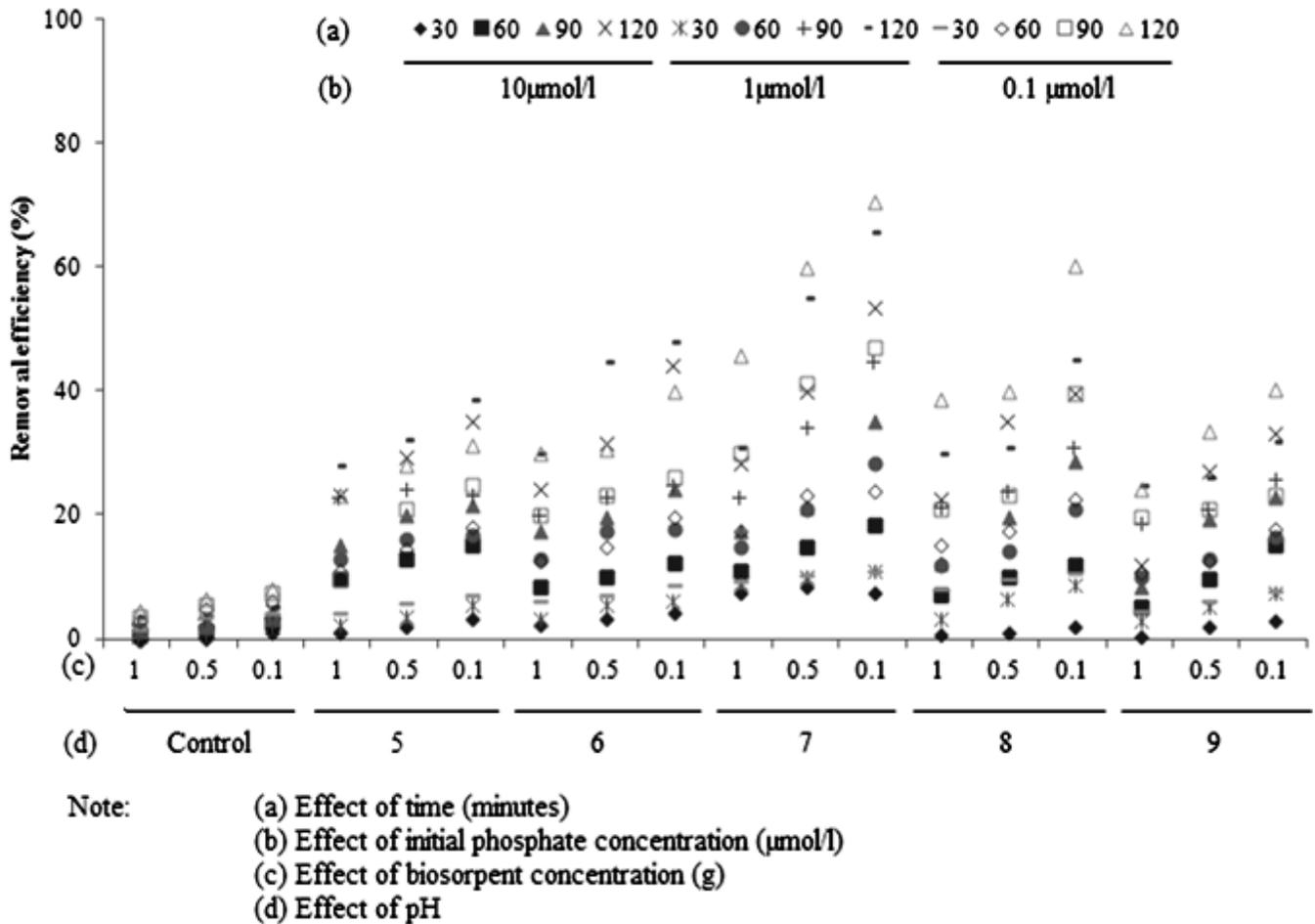
Three different concentrations 0.1, 1 and 10 µmol/l were used to study the effect of different initial concentration for their nutrient removal capability.

### Effect of pH and time interval

The different pH viz., 5, 6, 7, 8 and 9 were used for the nutrients removal experiment using seagrass beads. The pH of each solution was adjusted using diluted NaOH and HCL (ELICO, INDIA). Different time interval such as 30, 60, 90 and 120 min were maintained to standardizing the incubation time for nutrients removal. The 250 ml conical flasks filled 200 ml of aqueous solution and beads were kept on the rotary shaker at 30°C for 200 rpm. At the end of the experiment, the flasks were removed from the rotary shaker and the solution was separated from the biomass by filtration through Whatman filter paper (47 µm). Then the concentrations of the nutrients were estimated.

### Effect of biomass dosage

Different seagrass biomass dosages such as 0.1, 0.5 and 1 g were used for the beads preparation and subsequent adsorption processes to optimize the biomass dosage for nutrients removal.



**Figure 1.** Effect of pH, initial concentration, retention time and biomass dosage on phosphate adsorption.

### Calculation

The removal (adsorption) efficiency was calculated using the following equation:

$$\text{Removal efficiency} = 100 (C_0 - C_e) / C_0$$

Where,  $C_0$  = concentration of the sample solution before treatment;  
 $C_e$  = concentration of the sample solution after treatment.

## RESULTS AND DISCUSSION

### Optimization of the pH

The hydrogen ion concentration (pH) is one of the most critical controlling parameters in adsorption processes. This is due to the competitive effect of the  $\text{H}^+$  and also due to the fact that the pH affects the ionisation of the functional groups on the surface of the sorbent material (Wahab et al., 2010). Phosphate removal by *C. rotundata* beads was observed at pH values ranging between 5 and 9. Figure 1 shows that as the solution pH increases in the range of 5–9, the removal efficiency of phosphate also

increased gradually and attains a maximum value (70.9%) when the pH value was 7. However, when the pH increases to 8 and 9, the removal efficiency drops dramatically (60.3 and 40.3%). Similarly, for nitrate removal, the effect of pH was investigated by maintaining values between 5 and 9 and the result is presented in Figure 2. In case of nitrate the equilibrium sorption capacity was found to be minimum at pH 5 (1.1%) and maximum at pH 8 (61.8%).

The nitrate adsorption was highly pH dependent, which affects the surface charge of the beads; the overall results were confirmed by those of previous workers (Chatterjee et al., 2009). The adsorption of phosphate onto the biosorbent depends on initial solution pH (Figure 1). The phosphate adsorption was low in pH 5; when the pH increased from 5 to 7 the phosphate adsorption also proportionally increased. For further increases in pH from 7 to 9 however, adsorption of phosphate onto the biosorbent was found to decrease (Figure 1), suggesting the existence of an optimum pH for the maximum phosphate adsorption. This result was consistent with the proposed predominant adsorption mechanism that the optimum pH for phosphate removal by the seagrass beads surface should be around

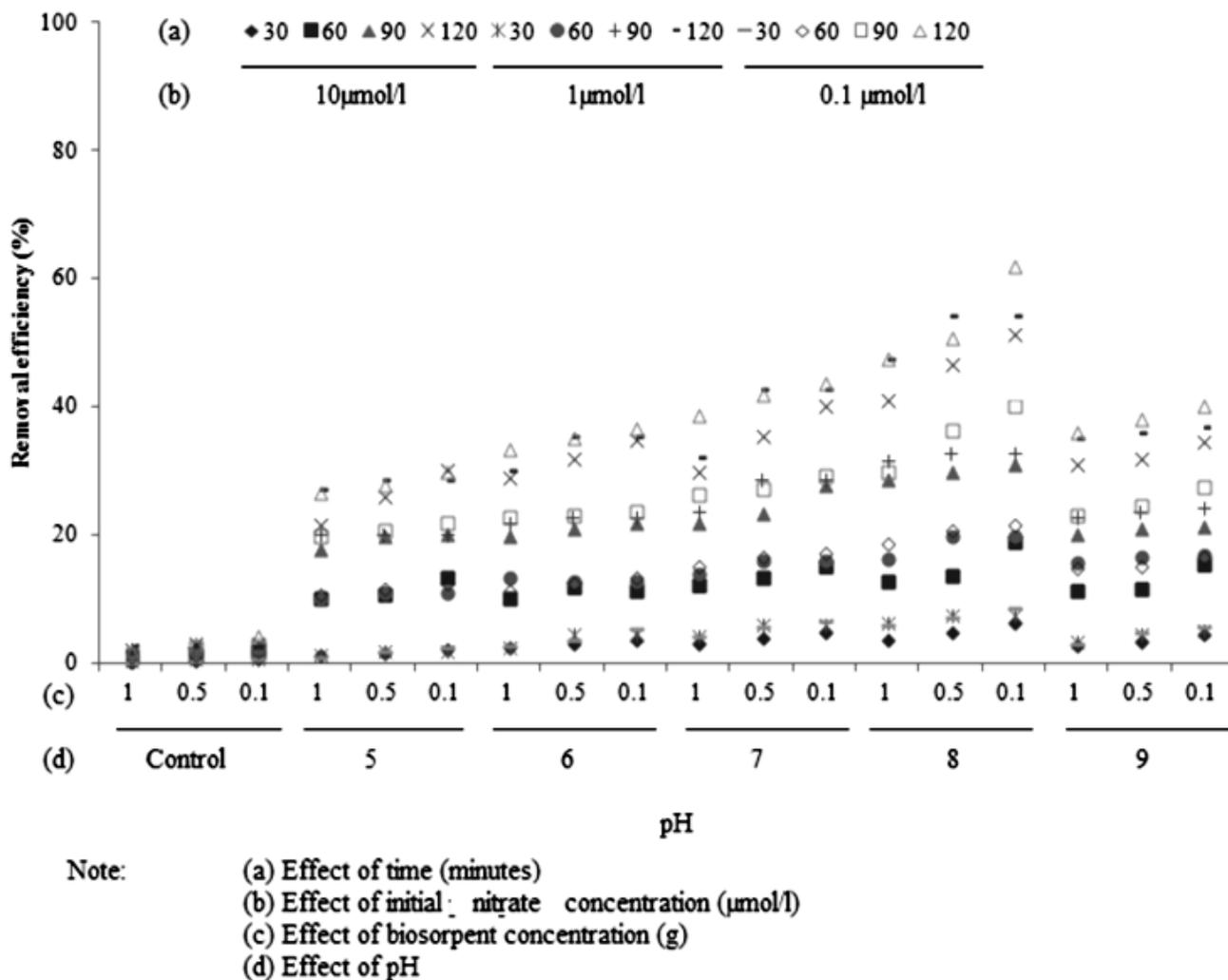


Figure 2. Effect of pH, initial concentration, retention time and biomass dosage on nitrate adsorption.

7 at which almost all phosphate exists in the form of  $\text{H}_2\text{PO}_4^-$  (that is, mononuclear adsorption). If solution pH is higher than the optimum value, polynuclear interactions may be triggered to consume more adsorption sites. Similar results were also reported by some earlier workers (Kumar et al., 2010; Yao et al., 2011) who studied the pH effect on phosphate removal from aqueous solution by using other carbon-based adsorbents.

### Optimization of the time

The equilibrium time required for the biosorption of phosphate and nitrate using *C. rotundata* beads were studied at various initial concentrations and various biosorbent concentrations at different time intervals. Figures 1 and 2 show that removal efficiency sharply increases with increase in time and attains maximum removal in phosphate and nitrate at 120 min incubation

period (Mithra et al., 2012; Dinesh Kumar et al., 2013). The nutrients adsorption increased rapidly during the first 30 min and remained up to 120 min. Such rapid biosorption process has been correlated with the characteristics of the biomass, and its other parameters interact with the aqueous solutions (Ingleton and Simmons, 1996). The contact time is an important consideration for the treatment of wastewater and accordingly, the contact time is fixed as little higher than the respective equilibrium times for each biosorbent (Popuri, et al., 2007; Yan-kui, et al., 2006). Our experiment showed that the 120 min duration was found suitable for maximum adsorption of phosphate and nitrate from the aqueous solution using *C. rotundata* beads.

### Optimization of the initial concentration

When determining the sorption capacity of a filtered material

with an agitation experiment the initial concentration of the solution greatly impacts on the sorption results. In general, the sorption increases with increased initial concentration of the species as agreed by some earlier workers (Taneva, 2012; Rahman et al., 2012). Initial concentrations of both nutrients were varied namely, 10, 1 and 0.1  $\mu\text{mol}$ . In our experiment, we observed that removal capacity was increased with decreased nutrients concentration. The maximum removal of 70.9% phosphate through optimized pH (8), time (120 min) and biomass dosage (0.1 g) from the aqueous solution were observed at 0.1  $\mu\text{mol/l}$  and minimum removal of 53.7% was observed at 1.0  $\mu\text{mol/l}$ .

In the case of nitrate removal, the optimized time and biomass dosage was found as 120 min and 0.1 g, respectively, but pH 7 was varying from phosphate. The maximum (61.8%) and minimum (51.2%) removal of nitrate was noticed at 0.1  $\mu\text{mol/l}$ . The removal efficiency was found increased when the initial nutrient concentration decreased. This may be attributed to the saturation of binding sites on the biomass surface leading to increased number of unabsorbed ions from the aqueous solution, because of the lack of binding sites on the biomass at higher concentrations than 0.1  $\mu\text{mol/l}$  (Akçelik et al., 2008).

### Optimization of the amount of biosorbent

The effect of the amount of adsorbents on the removal of phosphate is given in Figure 1. The increasing biomass dosage in beads reduced their efficiency in nutrients removal and caused cell leakage. Initially the removal efficiency was rapidly increased due to the rate of adsorption site. With the decrease in adsorbent amount, adsorption sites become profuse compared to phosphate current in the solution. It shows that maximum removal efficiency was 70.9% when the amount was 0.1 g. The phosphate removal at 0.5 g and 1.0 g biomass dosage in beads were recorded to be 60.1 and 45.9%, respectively. In the case of nitrate, minimum removal (47.5%) was recorded at 1.0 g and maximum removal (61.8) was observed at 0.1 g. The removal efficiency at 0.1 g biomass dosage in beads was increased nearly 14.3% than 1.0 g biomass dosage in beads. The 0.1 g biomass dosage revealed higher removal efficiency. But further increases in biosorbent concentration causes a decrease in percentage of removal. This trend could be due to the formation of biosorbent aggregates at higher biomass concentrations, which in turn could reduce the effective surface area available for the biosorption. With the increasing dose of biosorbent, the value of removal was decreased. The explanation for this behavior can be that adsorption sites remain unsaturated during the adsorption reaction since the number of sites available for adsorption increase by increasing adsorbent dose (Nuhoglu and Oguz, 2003; Zou et al., 2006).

Increasing seagrass powder entrapped within the beads

did not cause any significant improvement in nutrient removal in the present study as agreed by earlier workers (Chevalier and Noue, 1985; Lau et al., 1997). On the contrary, super concentrated cell stockings in the beads posed serious leakage problem (Robinson et al., 1986; Lau et al., 1997). Lukavsky et al. (1986) reported that nutrients such as ammonia and phosphate could first be adsorbed on the surface of the bead, then penetrate slowly into alginate and be continually sorbed into cells. Super concentrated cell stockings may restrict to some extent the nutrient diffusion through the alginate pores (Jimenez-Perez et al., 2004).

### Adsorption isotherms

Adsorption isotherms, Langmuir and Freundlich were used to describe the adsorption data for a range of adsorbate concentrations. These isotherms relate adsorption density,  $q_e$  (uptake of adsorbate per unit weight of adsorbent) to equilibrium adsorbate concentration in the bulk fluid phase,  $C_e$ . The Langmuir isotherm treats surface sites analogous to dissolved complexing ligands. It is derived by combining sorption equilibrium constant with a mass balance on the total number of adsorption sites. The Langmuir isotherm is valid for monolayer adsorption onto a surface containing a finite number of identical sites. The model assumes uniform energies of adsorption onto the surface and no transmigration of adsorbate in the plane of surface. The Langmuir isotherm is represented by the following equation:

$$C_e/q_e = 1/Q_0b + C_e/Q_0 \quad (1)$$

Where:  $C_e$  is the equilibrium concentration (mg/L);  $q_e$  is the amount adsorbed at equilibrium time (mg/g);  $Q_0$ , and  $Q_0b$  are Langmuir constants related to adsorption capacity; and energy of adsorption, respectively.

In Langmuir plots for adsorption, the linear plots of  $C_e/q_e$  versus  $C_e$  (Figure 3) confirm that the adsorption follows the Langmuir isotherm model. Langmuir constants,  $Q_0$  and  $Q_0b$  were determined from the slope and intercept of the respective plots.

The essential characteristics of a Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor or equilibrium parameter,  $R_L$ , which is given by the equation

$$R_L = 1/1+bC_0 \quad (2)$$

Where,  $C_0$ , is the initial dye concentration (mg/L);  $b$ , is the Langmuir constant (L/mg);  $R_L$ , indicates the shape of the isotherm as shown in Table 1.

$R_L$  values of live and other pretreated biomass at different adsorbate concentrations were always less than one and more than zero thereby indicating favourable adsorption of adsorbates onto the adsorbent. In the

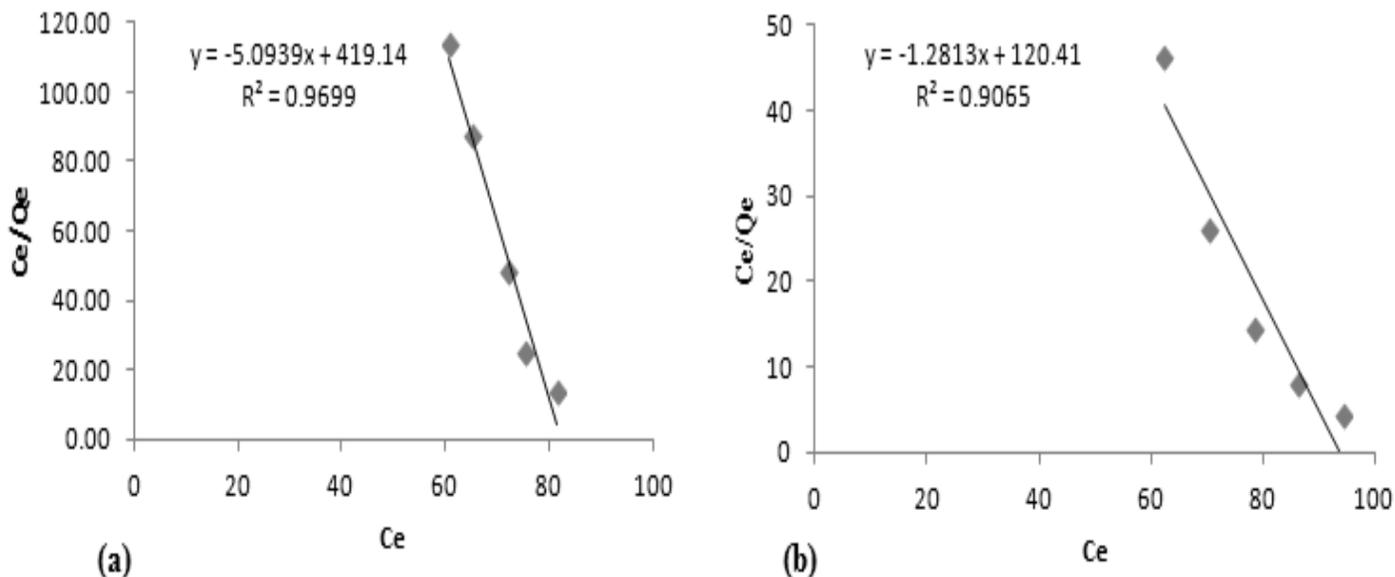


Figure 3. Langmuir adsorption isotherms of (a) phosphate and (b) nitrate on *Cymodocea rotundata* beads.

Table 1. The shape of isotherm.

R <sub>L</sub>	Type of isotherms
R <sub>L</sub> >1	Unfavorable
R <sub>L</sub> = 1	Linear
0< R <sub>L</sub> <1	Favourable
R <sub>L</sub> = 0	Irreversible

present study it was observed that R<sub>L</sub> values of phosphate removal was 0.99 whereas for nitrate it was reported as 0.998.

Freundlich equation can be derived by assuming that the free energy of adsorption decreases logarithmically as adsorption density increases. The Freundlich equation is used for heterogenous surface energies in which the energy term, Q<sub>0</sub>, in the Langmuir equation varies as a function of the surface coverage, q<sub>e</sub>, strictly due to variation in the heat of adsorption.

$$q_e = K_f C_e^{1/n} \tag{3}$$

Rearranging the equation (3) gives

$$\log_{10} X/m = \log K_f + 1/n \log_{10} C_e \tag{4}$$

Where, C<sub>e</sub>, is the equilibrium concentration (mg/L); X/m, is the amount adsorbed at equilibrium time (mg/g); K<sub>f</sub> and n are Freundlich constants.

N gives an indication of favourability and K<sub>f</sub> [mg/g (L/mg)<sup>n</sup>], the capacity of the adsorbent. This Freundlich adsorption isotherm is applied for the adsorption of phosphate and nitrate removal. Linear plots of log C<sub>e</sub> versus log X/m,

Freundlich plots (Figure 4) showed that the adsorption of adsorbates followed Freundlich isotherm model, suggesting that the average energy of adsorption decreases with increasing adsorption density. From the slope and intercept of the plots, the values of n and K<sub>f</sub> were calculated. The values of n in the range of 1 to 10 denote good adsorption of the adsorbate onto the adsorbent. In the present study, the n values of phosphate were 0.05 and nitrate was about 0.08 whereas K<sub>f</sub> values of phosphate were 1.51 and nitrate in the range of 7.94 for adsorption of nutrients studied onto *C. rotundata* beads. In the present work we have found that 0.1 g biomass containing beads efficiently removed nitrate and phosphate from the aqueous solution compared to other biomass dosage (0.5 g and 1.0 g).

### Conclusions

The amount of phosphate adsorption on the *C. rotundata* beads increases with decreased biomass content in the aqueous solution, which indicates that the adsorption depends upon the availability of binding sites for phosphate. Also, the equilibrium of phosphate and nitrate adsorption was attained at about 120 min retention time, while the highest percentage of nutrients adsorption occurred at the pH range 7-8 (8 for phosphate, 7 for nitrate). And lastly, the nutrient's adsorption was found to increase with the decreased initial concentration.

### Conflict of interests

The authors did not declare any conflict of interest.

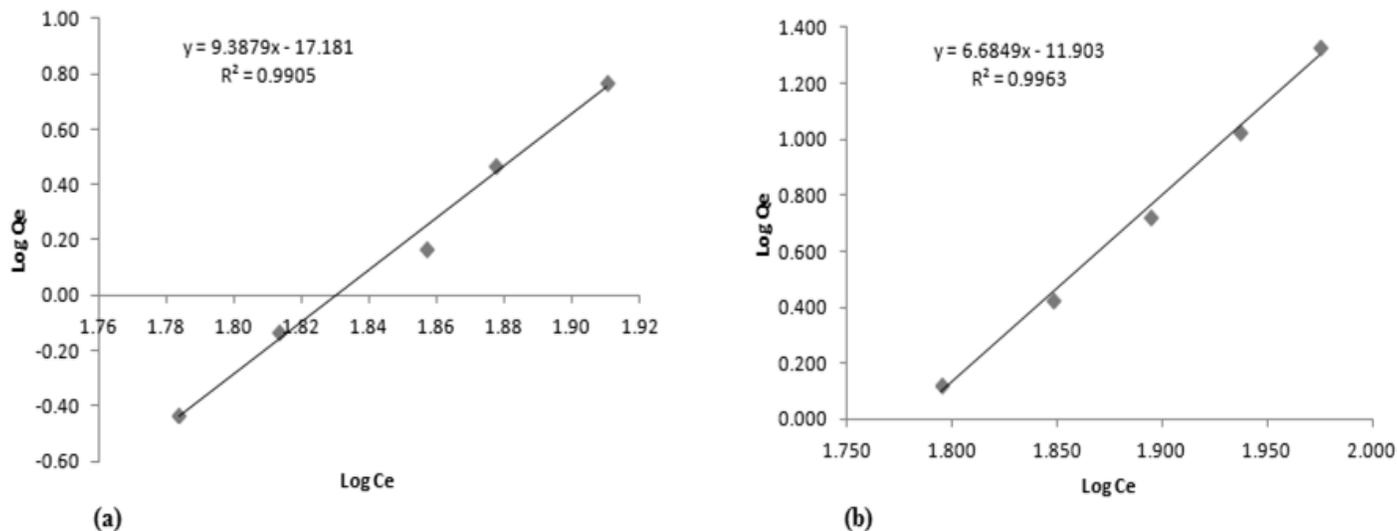


Figure 4. Freundlich adsorption isotherms of (a) phosphate and (b) nitrate on *Cymodocea rotundata* beads.

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