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# Phytoremediation to Remove Nutrients and Textile Dye Effluent Using Seagrass (*Cymodocea rotundata*)

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**Abstract:** Leaching of excessive amounts of nutrients, primarily phosphorus (P) and nitrogen (N), is a main cause of surface water pollution and eutrophication. It is recognised as being detrimental to the overall health of the water ecosystem. In this study, seagrass (*Cymodocea rotundata*) was used as biosorbent to remove excessive N and P from aqueous solution and dye from the textile wastewater. The study revealed that the seagrass *C. rotundata* has a great potential in removing N and P from aqueous solution at a short biosorption equilibrium time of 60-120 minutes. The high biosorption rate (N- 9.84µmol/l and P- 0.76µmol/l) was noticed in the pH 5 and 6 for N and P, respectively. Furthermore, seagrass; *C. rotundata* results the significant reduction in dye at 0.1 gram biomass concentration in 120 minutes duration from the textile wastewater.

**Key words:** *Cymodocea rotundata* · Seagrass · Biosorption · pH · Nitrate · Phosphate · Textile Wastewater

## INTRODUCTION

Over the past 40 years, antipollution laws have greatly reduced discharge of toxic substances into our coastal waters. Estuarine and coastal ecosystems receive increasing amounts of nutrients as a consequence of anthropogenic loading [1-4]. Increased nutrient loading is widely acknowledged to impact the structure and function of coastal systems [5]. Nutrient over-enrichment leads to algal blooms, reduced dissolved oxygen in the water column and decreased fish stocks [6-8]. Increased nutrient inputs to the water column can also adversely affects seagrass survival and production through stimulation of growth in phytoplankton, epiphyte and macroalgal communities [9-11]. This effort, however, has focused largely on point-source pollution of industrial and municipal effluent. No comparable effort has been made to restrict the input of nitrogen (N) from municipal effluent or to control the flows of N and phosphorus (P) that enter waterways from dispersed or nonpoint sources such as agricultural and urban runoff or as airborne pollutants. As a result, inputs of nonpoint pollutants, particularly N, have increased dramatically [11]. Nonpoint pollution such as N and P contaminants now represent the largest pollution problem concerning the coastal waters of the world. Nutrient pollution is the common thread that links an array of problems along the nation's coastline, including eutrophication, harmful algal blooms, dead zones; fish kills, shellfish poisonings and loss of seagrass and kelp beds, coral reef destruction and even accounts for death of marine mammals and seabirds. More than 60 percent of our coastal rivers and bays in every coastal state of continental India are moderately to severely deteriorate by nutrient pollution [11].

Nitrate and phosphates removal can be carried out by adsorption, ion exchange, electrodialysis, reverse osmosis, microbiological treatment (denitrification), chemical treatment (coagulation), etc. Amongst them, adsorption is simpler and effective for nitrate removal. Biological biomass is considered to be stable and hence considered as one of most promising adsorbents. Biological biomass and alginate is the polymer matrix structures having ions and a functional group along with alkyl chain present in it [12].

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Industrial effluent is a mixture of various textile dyes. Amongst various pollutants in effluents dye is one of the major pollutants. A dye is a colored soluble substance having affinity towards the substrate to which it is being applied. It is released as an effluent from textile, leather, paper and other industries and causes water and land pollution, if disposed in improper way. Dye polluted water has negative impact on human health [13]. Dyes cause turbidity and interfere with photosynthesis of the phytoplankton thereby interfering with physical characteristics of the water [14]. Some earlier workers have studied the nutrient removal potential of live seagrass [15-21] and dead seagrass [22, 23]. In this study, the kinetics of nitrate and phosphate uptake and colour removal from the dye wastewater by seagrass (*Cymodocea rotundata)* powder was attempted.

## MATERIALS AND METHODS

**Preparation of Biosorbent:** The healthy seagrass (*Cymodocea rotundata)* leaf was collected from Palk Bay, Muthukuda coast (Lat. 9° 51' 48'' N; Long 79° 7' 15'' E) Southern India. The seagrass (*Cymodocea rotundata)* biosorbent powder was prepared as described in Mithra *et al.* [24]. The seagrass was washed twice with running tap water and then thoroughly rinsed with Milli-Q water to remove particulate materials and salts from the surface. Then the biomass was shade dried followed by oven drying at 60°C for 24 hours. Dried biomass was ground by using mortar and pestle and subsequently sieved to a particle size of 500 – 850µm and then stored in desiccator for nutrient biosorption experiments.

### Experimental Setup

**Nutrients Adsorption:** Nutrient stock solutions were prepared by the dilution of corresponding salts namely sodium nitrate (N) and Di potassium hydrogen phosphate (P) in double distilled water. Then these stock solutions were diluted (10%) with double distilled water for the preparation of working solutions. The initial concentration of nitrate and phosphate working solutions were 10µmol/l and 0.1µmol/l, respectively. The pH of each solution was adjusted between 5 and 8 using diluted $NH_4OH$ and HCL and pH was estimated using pH meter (ELICO, INDIA). The nutrient adsorption efficiency of the seagrass powder was done at three different concentrations viz., 0.1 g, 0.5 g and 1.0 g. The experiments were conducted in a conical flasks maintained at 30°C in shaker for various time intervals (30, 60, 90 and 120 minutes) at 120 rpm. At the end of the experiment, the flasks were removed from the shaker and the solution was separated from the biomass by filtering through Whatman (47mm) filter paper. Then the final concentration of the nutrients was estimated. The amount of phosphate in the sample was measured using Shimadzu Model-2450 spectrophotometer as per the standard protocol described by Strickland and Parsons [25]. Nitrate was estimated by using the method of Jenkins and Medsken [26].

**Dye Removal:** The textile effluent was collected from the dye factory located at Karur, Tamil Nadu, India. The decolorization experiment was made as described in Telke *et al.* [27]. Fifty ml of textile wastewater was taken in 250 ml conical flasks. The seagrass biosorbent powder was used at different concentrations (0.1, 0.5 and 1.0 grams) and kept in rotary shaker (120 rpm). The dye effluent was drawn at regular intervals (30, 60, 90 and 120 minutes) for analysis of dye concentration. Suspended particles from the conical flasks were removed by centrifugation at 7,000 rpm for 20 min. Decolorization was monitored by measuring the absorbance of the supernatant at (according to λ max) 490 nm using Hitachi U-2800 Spectrophotometer. Percentages of decolorization of dry cell weight at different time intervals were measured. All decolorization experiments were performed in triplicate. The rate of decolorization was calculated as follows.

$$\text{Decolorization (\%)} = \frac{(IA) - (OA)}{(IA)} \times 100$$

Initial absorbance (IA) = Absorption at 0 hour of incubation.

Observed absorbance (OA) = Absorption at the time (30, 60, 90 and 120 minutes) of incubation.

**Data Analyses:** Statistics were performed using Origin Pro 8 software. ANOVA with post-hoc test analyses based on Turkey and fisher test was used to compare the differences between two groups. Parameters with a probabilistic value of <0.05 was considered statistically significant. For Two-way ANOVA comparison was made on nutrient reduction based on the time and pH.

## RESULTS

**Nutrient Biosorption:** The nitrate ($NO_3^-$) concentration decreased significantly during the experiments, suggesting that seagrass possesses good adsorbent capacity (Fig. 1). The initial concentration of nitrate was 10µmol/l. Among the three different biomass concentration studied (1.0, 0.5 and 0.1 grams), the maximum (9.84µmol/l) nitrate removal was obtained at pH

Table 1: Nitrate and phosphate removal from the aqueous solution using seagrass *Cymodocea rotundata* with reference to time and pH

| Nutrients | Source of Variation | DF | Sum of Squares | Mean Square | F | P-value | P<0.05 |
|---|---|---|---|---|---|---|---|
| Nitrate | pH | 3 | 7.80 | 2.60 | 19.75 | 4.45 | IS |
| | Time | 3 | 1.53 | 0.51 | 3.87 | 0.01 | S |
| Phosphate | pH | 3 | 1.49 | 0.49 | 50.82 | 7.13 | IS |
| | Time | 3 | 0.10 | 0.33 | 3.45 | 0.02 | S |

DF- Degrees of freedom; S- Significant; IS – In-Significant

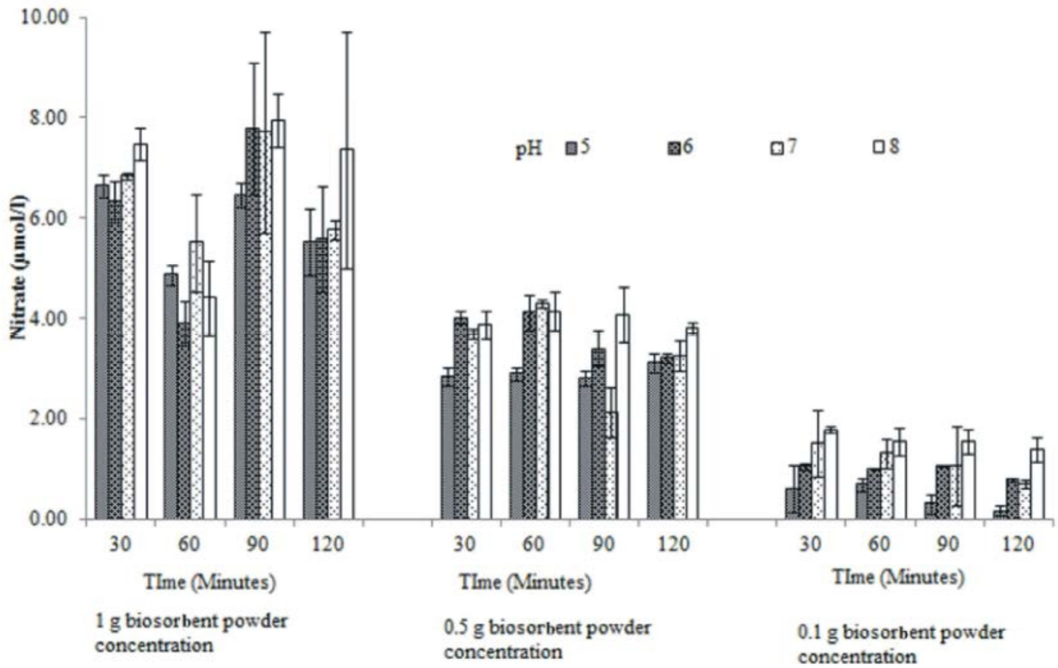


Fig. 1: Effect of pH, retention time and *C. rotundata* biosorbent concentration on nitrate removal

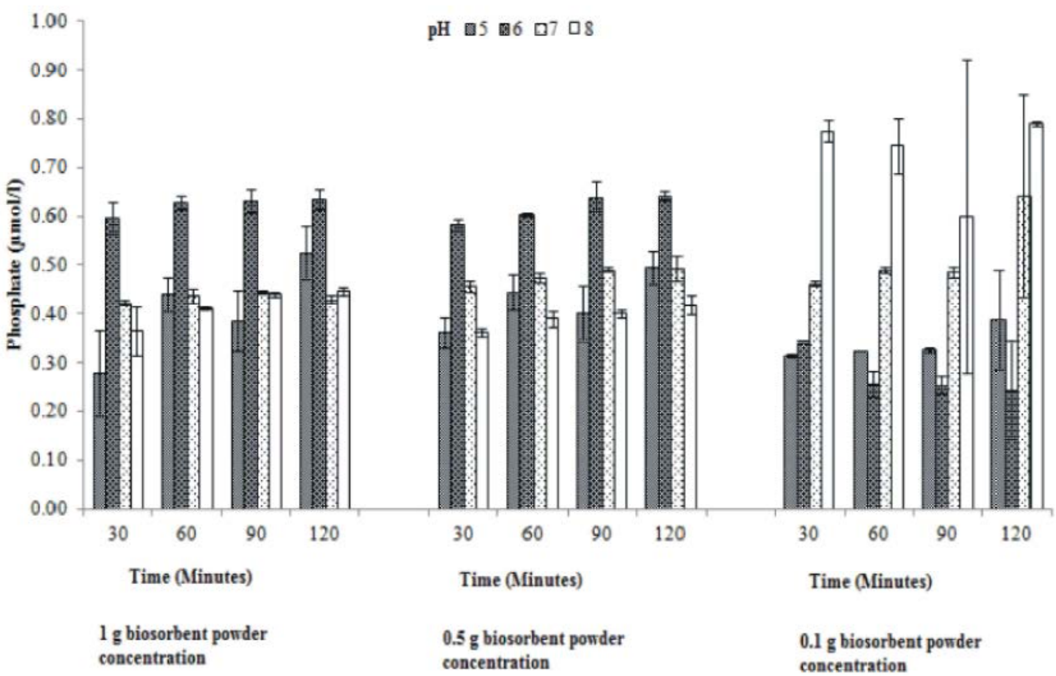


Fig. 2: Phosphate removal with emphasis on pH, retention time and biosorbent concentration

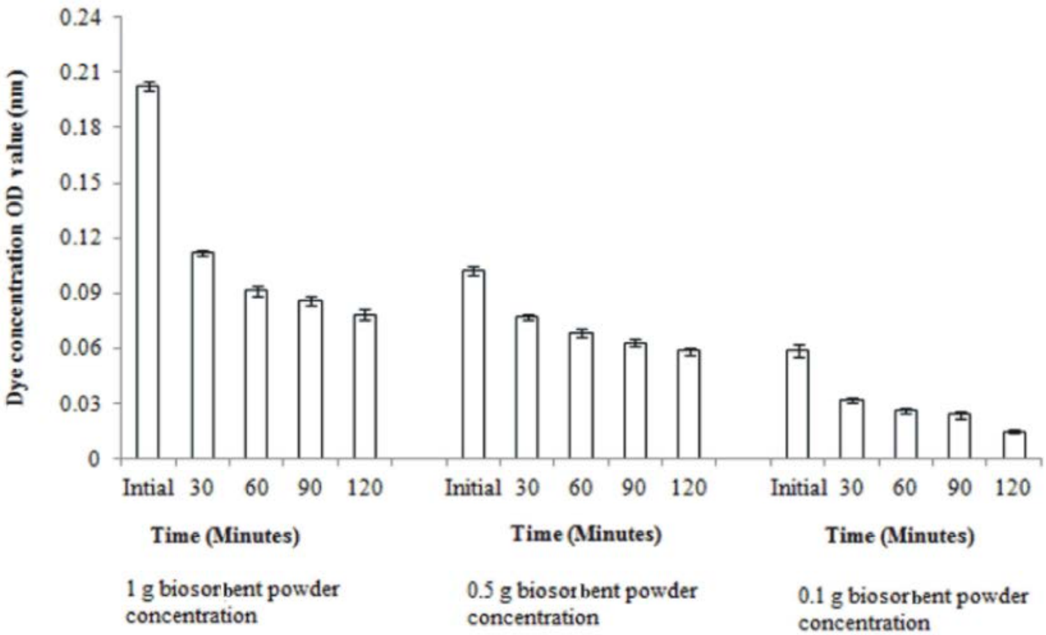


Fig. 3: Effect of retention time and algal biosorbent concentration on colour removal from dye wastewater

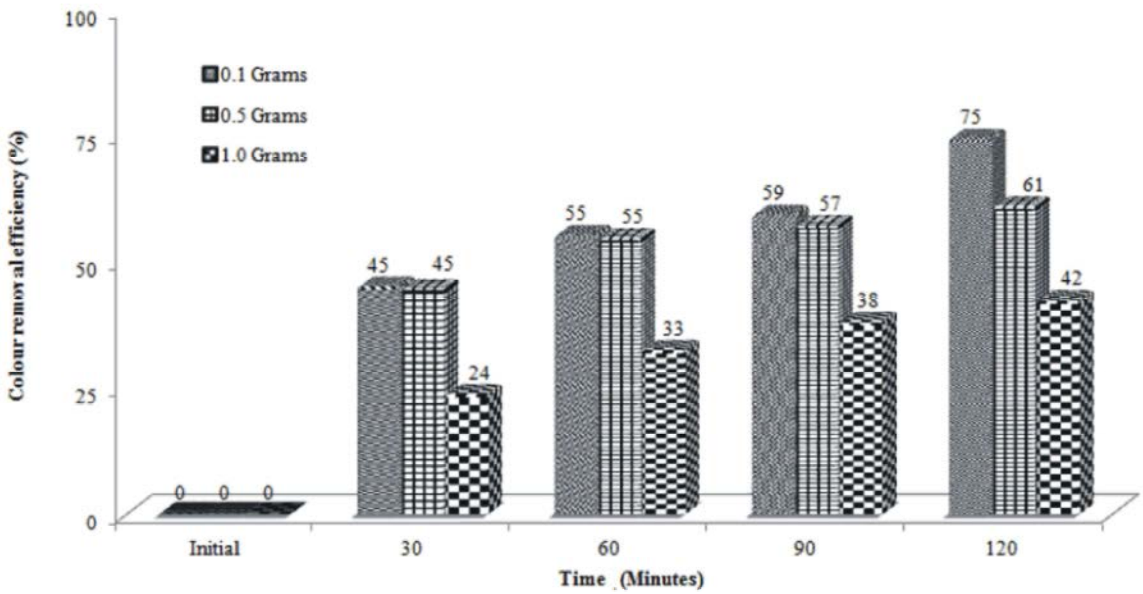


Fig. 4: Colour removal efficiency (%) of seagrass with reference to retention time and biosorbent concentration

5 in 0.1 g biomass concentration. Currently this is considered as the best nitrate reduction among the pH and retention time tested. The second best nitrate reduction (9.70µmol/l) was noticed at same pH (5) and biomass concentration (0.1g) in 90 minutes incubation period. The minimum nitrate removal was observed in pH 8 at 90 minutes incubation and 1 g biomass concentration where the nitrate reduction recorded 2.05µmol/l. The order of removal of nitrate at 0.1 g biomass concentration were 98.43% (pH 5) > 92.95% (pH 7) > 92.17% (pH 6) > 86.29% (pH 8), respectively. The differences among the two tested factor of nitrate were highly significant ($p<0.05$) with time (Table 1).

Similar experiments were conducted for phosphate removal using seagrass (*C. rotundata)* with effect of contact time, pH and biomass concentration. The lowest reduction (0.21µmol/l) of phosphate was observed at pH 8 in 120 minutes incubation, while the maximum

(0.81±0.02µmol/l) was observed in pH 6 at 120 minutes incubation with 0.1 g biosorbent concentration. The second best (0.76±0.02µmol/l) phosphate removal was recorded at 30 minutes incubation in pH 6 with 0.1 g biosorbent concentration. The recorded phosphate removal based on the pH was in the order of pH 6 (80.60%) > pH 5 (63.76%) > pH 7 (54.36%) > pH 8 (41.77%), respectively. The present experiment expressed the potential phosphate removal at pH 6, 120 minutes incubation with 0.1g biosorbence (Fig. 2).

**Decoloration:** The decoloration experiments were conducted to determine the colour removal efficiency of seagrass (*C. rotundata)* biosorbent powder at different concentrations (0.1, 0.5 and 1.0 g) at different time intervals (30, 60, 90, 120 minutes). Seagrass (*C. rotundata)* showed good colour removal efficacy (75%) at 120 minutes incubation and 0.1g algal concentration from the dye effluent (Fig. 3). Among the four different time points, 120 minutes showed high colour removal. Similarly, among the three biomass dosages tested, 0.1g biomass showed high dye removal while least dye removal was achieved in 1 g of biomass. The present observation revealed that high biomass dosage proportionately reduced the colour removal rate.

## DISCUSSION

The optimum pH and time duration is the necessary factor for adsorption process. Only at optimum pH the nutrients get attached to the seagrass. The present experiment showed that pH 5 for nitrate and pH 6 for phosphate aided well for their removal. Finally, pH 5 and 6 exhibited good adsorbing capacity of nutrients from the aqueous solutions using seagrass (*C. rotundata)* biosorbent powder. Similarly, the extension of incubation also enhanced the adsorption process. Among the different time intervals (30, 60, 90 and 120 minutes) studied, 120 minutes revealed the maximum nitrate and phosphate removal efficiency. Therefore, this study indicates that pH and time intervals are key factors for the adsorption process.

The effect of pH on nutrient biosorption has been studied by many researchers and the results demonstrated that the pH values can affect biosorption. The results in fungi biomass [28, 29] and in algae biomass [30-37] have already been reported. Researchers have explained the ability of seagrasses to take up inorganic nitrogen from the sediment and in the water column [38, 39] for production of energy.

As the pH increases, the removal of nutrients from the solution decreases. The decreasing trend in nutrient removal with increasing pH is dependent on surface hydrolysis reactions [40]. The increase in biosorption levels with a decrease in pH can be explained by the influence of negative charges that are dependent on the dissociation of functional groups [41]. The pH dependent nutrient uptake can largely be related to the functional groups present in algae and also on the nutrients and metal chemistry in solution [42]. At lower pH values, the concentration of hydrogen protons in the solution far exceeds that of ions and hence, these protons compete with the nutrients in forming a bond with the active sites (functional groups) on the surface of the algae, leaving the nutrients free in solution. The bonded active sites thereafter become saturated and thus inaccessible to other cations. When the pH increased, the competing effect of hydrogen protons decreased and the positively charged ions took up the free binding site. A resultant increase in the sorption capacity or removal efficiency was observed by Ajjabi and Chouba [43].

Industrial effluent is not stable and it varies widely depending upon the process practiced. Adaptation of biological organisms to varying pH and incubation conditions makes them more suitable for the degradation of industrial effluents [44]. The present study indicates the potential of seagrass as a biosorbent with increased decolorization and increased incubation time (Fig. 3). The seagrass, which is capable of decolorizing the mixture of textile dyes, is important in treatment of textile effluent. Seagrass also decolorized the dye effluent variably at different time points and biomass concentration. Concentration of reactive, disperse and direct dyes with decolorization efficiency varied from 24 to 75% (Fig. 4). Similar correlation between increased incubation time and dye degradation was noted by previous workers [45-46]. It is clear that the decolorization was due to the adsorption of color to cells [47].

The effect of biomass concentration on the decolorization of dye was studied using different dosage range (0.1, 0.5 and 1g). High biomass concentrations can exert a shell effect that protects the active sites from pollutants [48]. However, pollutant removal was found to decrease with increasing biosorbent dosage, probably due to the complex interaction of several factors [49]. This may be explained as a partial cell aggregation that takes place at high biosorbent concentrations causing a decrease of the active sites. Early workers [50-51] also observed this trend in their biosorption experiments with the use of *Sphingomonas paucimobilis* biomass.

It has been suggested that electrostatic interaction between cells can be a significant factor in the relationship between biomass concentration and pollutants sorption. In this connection, lower biomass revealed maximum colour removal and minimum colour removal was found in higher biomass dosage. The present study suggests that *C. rotundata* might be the potential organism for the degradation of textile dyes and textile industry effluent.

## CONCLUSION

The present study concluded that the *C. rotundata* showed significant result on excess nutrients and colour removal of dye. It is clearly understood that the species can be considered as a potential candidate for phytoremediation of the domestic and textile industrial effluent. As seagrass (*C. rotundata*) is precious species, it is important that the plant be strictly confined in the remediation system so that we can make full use of its nutrient scavenging ability without bringing unnecessary damage to the ecosystem. More studies on how a variety of aquatic plants perform in different waters (with different nutrient ranges, pH, electrical conductivity) under different environments (temperature, solar radiation, etc.) are needed for employing an appropriate plant to achieve maximum purification of the water.

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