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**Research Article**

**Effect of Immobilized  
Microalgae *Scenedesmus  
pecsensis* on Reduction of  
Physicochemical  
Characteristics in Sugar  
Mill Effluent**

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**Abstract**

Environmental pollution has been recognized as one of the major problems of the modern world. Sugar industry is one of the most important agro based industries in India and is highly responsible for creating significant impact on rural economy in particular and countries economy in general. The sugar industry is playing an important role in the economic development of the Indian sub continent, but the effluents released produce a high degree of organic pollution in both aquatic and terrestrial ecosystems. They also alter the physico-chemical characteristics of the receiving aquatic bodies and affect aquatic flora and fauna. In the present study, the effect of Microalgae isolates on the reduction of physico-chemical characteristics in sugar mill ef-

fluent was investigated. The sugar mill effluent was bioremediated by using the immobilized microalga *Scenedesmus pecsensis*. Maximum reduction of physicochemical characteristics was observed in the microalga *Scenedesmus pecsensis* in sugar mill effluent.

**Keywords:** Sugar mill effluent, Physico-chemical properties, *Scenedesmus pecsensis* and Bioremediation.

**INTRODUCTION**

Sugarcane effluents contain sugar and other carbohydrate organic compounds. It will create the immediate oxygen demand by these effluents cause rapid depletion of dissolved oxygen of receiving streams and produce anaerobic conditions. Therefore, sugarcane effluents render the water totally unfit for fish and other aquatic life. The effluents which are coming from sugar industry if directly used for agricultural practices, i.e. irrigation purpose, will affect the soil fertility as well as plant growth and seed germination.

While large amount of wastewater released in the open water stream or any aquatic region it will create rapid plant growth followed by increased activity by decomposed and a depletion of the oxygen level is called eutrophication. In this circumstance we require cost efficient and environmental friendly bioremediation techniques. Bioremediation of wastewater using natural biosorbents can be an efficient alternative method. Among the various biological organisms using bioremediation, microalgae providing promising results in elimination of nutrients and other pollutants. Nowadays, mobilization and immobilization microalgal bioremediation techniques are actively practiced. The main advantage of immobilization technology, which entraps the microalgae cells into a matrix,

solves the harvest problem. Higher nutrient removal efficiency was observed in the immobilized bioremediation techniques than the freely suspended cells of the same algal species<sup>1</sup>.

The microalga *Scenedesmus peccensis* immobilized into Na-alginate was investigated in this study. We examined the maximum nutrients removal rate. Therefore, this study is aimed at investigating the potential of sodium alginate immobilized *S. peccensis* beads for the removal of excessive nutrients in sugar cane effluent.

## MATERIALS AND METHODS

### Collection of wastewater:

The sugar cane effluent sample for the present experiment was collected from the outlet of a sugar cane industry located at Sethiyathoppu at Cuddalore District in Tamil Nadu. The effluent was screened through coarse and fine screens to remove floating solids. The physico-chemical characteristics of the effluent sample were analysed using standard methods by APHA<sup>2</sup>, Strickland and Parsons<sup>3</sup> and Jenkins and Medsken<sup>4</sup>. The effluents were stored at 4°C to avoid changes in its characteristics.

### Microalgae culture

The microalga *Scenedesmus peccensis* was cultured in special air conditioned room. The stock cultures were kept in 1 and 2 litres culture flasks.

### Preparation of immobilized marine algal beads

The algal beads were prepared followed by Santos *et al.*<sup>5</sup>. To prepare 100 ml of alginate solution with the required alginate concentration, the alginate was first carefully dissolved by stirring in 100 ml of distilled water. When the alginate was completely dissolved, the two parts were mixed in a magnetic stirrer. Growing marine algal cells were taken out from the culture room and added algal culture into alginate solution. Cation solutions were prepared in nanopure water. Beads were prepared by adding drop wise the alginate solution, by means of 20ml of syringe (0.8mm×40mm needle, Braun, Melsunger, Germany) into cation solution, from a height approximately one drop per second. Beads were kept stirring in the cation solution for 45 minutes to allow complete hardening of the alginate and washed several times with filtered natural seawater

to eliminate the remaining cation.

### Analysis of nutrients:

#### Nitrate-Nitrogen (NO<sub>3</sub><sup>-</sup>-N)

About 2 ml of wastewater sample was taken in a beaker then 1 ml of brucine sulphanic acid reagent, and 10 ml of sulphuric acid solution was added and stirred gently for 5 min. Beaker was covered with watch glasses and kept in dark for 10 min. yellow colour developed, 10 ml of distilled water was added and kept in dark condition for 30 minutes. Absorbance concentration was measured at 410 nm. Absorbance was plotted against nitrate concentration.

#### Reactive silicate (SiO<sub>4</sub>-Si)

About 25 ml of wastewater sample was taken in polythene beaker then 1 ml of molybdate reagent was added after 5 -10 minutes 1ml of oxalic acid and 1 ml of ascorbic acid were added. Swirled during the reagent additions and kept the sample for 30 minutes. Absorbance concentration was measured at 810 nm. Absorbance was plotted against silicate concentration.

#### Phosphate-Phosphorous (PO<sub>4</sub>-P)

Dissolved reactive phosphate was measured by the treatment of water sample with acidified molybdate reagent to give phospho-molybdate complex which is then reduced to a highly colored blue compound (phosphomolybdenum blue). Absorbance of this color was measured after 10-30min, against a standard blank in 1 cm quartz cuvette at 880 nm.

#### Nitrite-Nitrogen (NO<sub>2</sub>-N)

Nitrite was determined after diazotising it with sulphanilamide and coupling with N (1-Naphthyl)-ethylene diamine-dihydrochloride. The absorbance of the pink color complex developed was measured after 5-10 min. against a standard blank at 543 nm, using 1 cm cuvette.

#### Ammonia-Nitrogen (NH<sub>3</sub>-N)

Ammonia-nitrogen was determined by the indophenol blue method based on the principle that in a moderately alkaline medium, ammonia was allowed to react with hypochlorite in catalytic amounts of nitroprusside to form indophenol blue.

The formation of monochloramine requires a pH between 8 and 11.5. The resultant blue complex was measured at 630 nm by spectrophotometer.

### Total phosphorous and total nitrogen

The determination of total phosphorous and total nitrogen (TN) of effluent water sample was oxidized with the help of strong oxidizing agent (alkaline perusulfate) by autoclaving in closed condition. The organic forms of phosphate and nitrate and also their inorganic forms in lower oxidation states are finally oxidized to inorganic phosphate and nitrate respectively.

### Process parameter optimization of immobilized algal beads for removal of pollutants:

#### Experimental procedure

Microalgal beads were prepared as previously explained. Empty beads were used as control. The experiments were conducted with 250 ml of conical flasks with 200 ml of sugar cane effluent and 100 numbers of immobilized microalgal beads were inoculated into a effluent flask and incubated for predetermined duration. At the end of the experiment, the effluent was separated from the immobilized microalgal beads through filter paper. The amount of nutrients in the sample were measured spectrophotometrically using Shimadzu Model-2450 as per the standard protocol.

## RESULTS AND DISCUSSION

### Characteristics of untreated sugar cane industry effluent

The sugar cane industry wastewater temperature is 23.5°C, highly dark colored and alkaline condition with strong and objectionable odour that presents significant disposal or treatment problem. Physico-chemical characteristics of wastewater are given in Table 1.

The application of microbial consortia presents considerable advantages over the use of pure cultures degradation of waste water. Although, most studies have used bacteria, fungi and algae for the biodegradation of organic pollutants, recent studies have indicated that in addition to providing oxygen for aerobic bacterial biodegraders, microalgae can also biodegrade organic pollutants directly either as free cells or as immobilized compo-

site <sup>6,7</sup>. This is supported by the present results that green microalgae provide a reliable alternative for the elimination of nutrients from the sugar cane wastewater.

**Table 1. The physico-chemical characteristics of untreated wastewater**

S. No.	Parameter	Value
1.	pH	5.0
2.	Colour	Dark brown
3.	Conductivity ( $\mu$ S)	2,156
4.	Salinity (ppm)	1
5.	Dissolved oxygen ( $\text{mg L}^{-1}$ )	6.1
6.	Temperature ( $^{\circ}\text{C}$ )	23.5
7.	Total suspended solid ( $\text{mg L}^{-1}$ )	0.098
8.	COD ( $\text{mg L}^{-1}$ )	30.67
9.	Calcium ( $\text{mg L}^{-1}$ )	0.6
10.	Magnesium ( $\text{mg L}^{-1}$ )	0.4
11.	Phosphate ( $\mu\text{mol/l}$ )	12.63
12.	Nitrate ( $\mu\text{mol/l}$ )	53.20
13.	Ammonia ( $\mu\text{mol/l}$ )	10.11
14.	Silicate ( $\mu\text{mol/l}$ )	37.91
15.	Nitrite( $\mu\text{mol/l}$ )	2.21
16.	Total Phosphorus ( $\mu\text{mol/l}$ )	3.37
17.	Total Nitrogen ( $\mu\text{mol/l}$ )	8.51

### Effect of algal densities in beads on nutrients removal using immobilized microalgal beads:

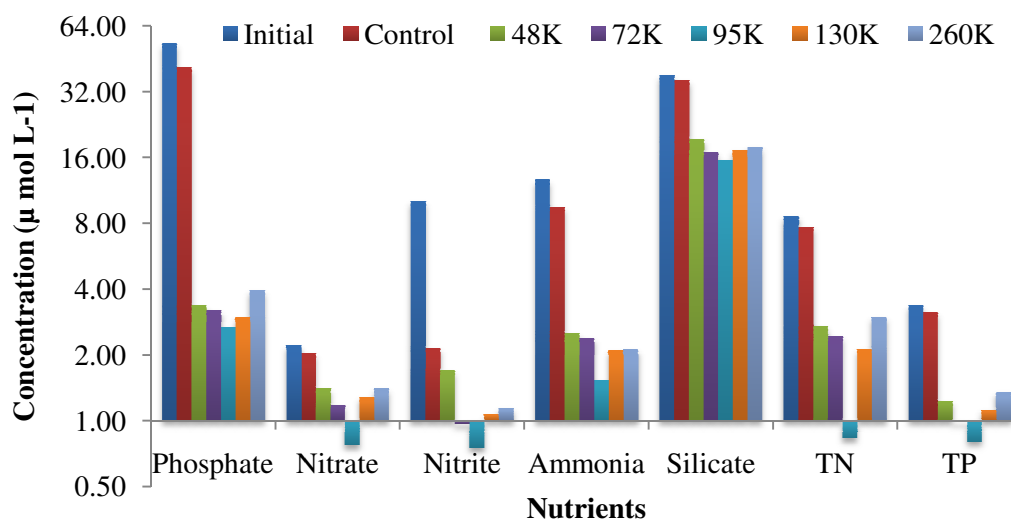
The beads density 100 numbers was chosen for further biosorption experiments (Fig. 1). The concentration of the all the nutrients decreased differently during the present experiments in different algal cell densities, indicating that algal cell densities possess important role on biosorption.

The significant decrease in the concentration of nutrients was observed at all the tested algal cell densities such as 48000cells/ml, 72000cells/ml, 95000cells/ml, 130000cells/ml and 260000cells/ml. The maximum nutrients removal was obtained at 95000cells/ml containing chamber which was reduced to 2.67 from 12.63 $\mu\text{mol L}^{-1}$  (phosphate), 53.20 to 5.77 $\mu\text{mol L}^{-1}$  (nitrate), 10.11 to 0.75 $\mu\text{mol L}^{-1}$  (nitrite), 10.11 to 1.53 $\mu\text{mol L}^{-1}$  (ammonia), 37.95 to 15.59 $\mu\text{mol L}^{-1}$  (silicate), 3.38 to 0.80 $\mu\text{mol L}^{-1}$  (total phosphorus) and 8.52 to 0.83 $\mu\text{mol L}^{-1}$  (total nitro-

gen). The lowest nutrients removal rate has been found in 260000cells/ml for phosphate, total phosphorus and total nitrogen and 48000cells/ml for nitrate, nitrite, ammonia and silicate removal. After optimization of pH, retention time, beads density for nutrients removal by using *S. pectusensis* beads, the percentage of removal has been increased in all nutrients like phosphate, nitrate, nitrite, ammonia, silicate, total phosphorus, and total nitrogen and percentage are 94.98%, 64.94%, 92.58%, 87.90%, 58.92%, 90.36%, 76.33% respectively.

Increasing the algal cell densities (beyond the limit or moderate cell densities) in beads reduced their

efficiency in removal nutrients and caused cell leakage (monitored in the experiment high stocking algal cells in beads 130000cells/ml and 260000cells/ml). Increasing algal cells entrapped within the beads from beyond the limit didn't cause any significant improvement in pollutants removal in the present study as agreed by earlier workers<sup>8,9,10</sup>. In the present work, we found that 95000 cells/ml of algal cells containing beads efficiently removed the nutrients from the sugar cane wastewater compared to other algal cell density (48000cells /ml, 72000cells /ml, 130000cells/ml and 260000cells/ml).



(Fig. 1): Effect of Algal cell densities in beads - on nutrients removal from sugar cane wastewater using *S. pectusensis* beads

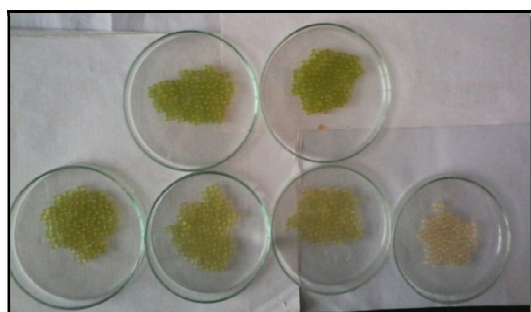


Fig: 2 Immobilized Microalgae

### Summary and Conclusion

The present result on nutrients (phosphate, nitrate, nitrite, ammonia, silicate, total phosphorus, and total nitrogen) adsorption rate of immobilized microalgae indicated that nutrients were significantly reduced by immobilized microalgae *S. pectusensis* beads. The present study clearly indicated that the microalgae *S. pectusensis* immobilized beads showed outstanding performance in nutrients reduction in sugar cane effluent.

Therefore it is concluded that the immobilized mi-

croalgae *S. pectusensis* beads can be used as bioremediant agent in the effluent treatment plants for sugar cane wastewater in particular for the removal of excess nutrients for the conservation of nature and natural resources. Furthermore, the effluent treated microalgae are being utilized as feedstock for production of bio fertilizers, biofuel and aqua feed for zero waste management and low cost treatment.

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