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Treatment of Sugar Cane Industry Effluent using Immobilized Microalga *Scenedesmus pecsensis* : Responses From Different pH, Retention Time, Beads Density and Algal Cell Concentration in Beads

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ABSTRACT

Microalgae have ability to treat a wastewater by tertiary methods as already proved. Microalgae immobilization technology has been developed to reuse and improve the quality of treated effluent and also escape from the losing high value algal biomass. To do so, in this present study, *Scenedesmus pecsensis* has been immobilized using sodium alginate to evaluate the nutrients removal efficiency from the sugarcane industry (SCI) effluent with reference to pH, incubation period, number of beads, quantity of algae in beads and reusing ability of beads. The removal of nutrients were estimated with various pH (5 to 9), incubation period (30 to 120 minutes), beads density (50-150 numbers) and algal cell concentration in beads (40 k cells/ml, 72 k cells/ml, 95 k cells/ml, 130 k cells/ml and 260 k cells/ml) with control (beads without algae). The highest nutrients removal were achieved indifferent range of pH (7), retention time (120 minutes), beads density (100 numbers), algal cell density in beads (95000 cells/ml) and the percentage of removal was 94.98% (phosphate), 64.94% (nitrate), 92.58% (nitrite), 87.90% (ammonia), 58.92% (silicate), 90.36% (total nitrogen) and 76.33% (total phosphorus). Reusage of immobilized algal beads significantly increasing removal rate and the 92.10% of total nitrogen removal and 79.28% of total phosphorus removal were achieved at the 3rd cycle. Meanwhile, the beads damage were significantly induced the algal cell leakages while recycling of beads.

Keywords Sugarcane effluent, Microalga, *Scenedesmus pecsensis,* Algal beads, Reuse.

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INTRODUCTION

Bioremediation is the process of using bio-sources to recycle the waste in other forms that will reused by other bio-sources. In current scenario, the globe has facing various type of environmental pollution by different type of pollutants. Bioremediation by microorganism can continued up to their ability of conversion energy or utilization of harmful pollutants to obtain energy and valuable by production in the process (Tang et al. 2007). Microalgae are globally accredited as leading candidate for simple and natural treatment process for wastewater (Olguin 2003). Hence, many studies have been focused on nutrients removal efficiency of immobilized mocroalgae (Dinesh Kumar et al. 2013, 2016, 2018, Adam et al. 2015). Cells immobilization is innovative and alternative technology to solve the harvest problem and provide some other advantages on nutrients removal like increasing metabolic activity in algal cells (Ruiz-Marin et al. 2010). Maintaining algal cell viability is an important factor to improve nutrient consumption rate while bioremediation. Several researchers stated that the calcium alginate consider as adorable mediator for cell immobilization and also extending their viability duration in water column. Conversely, presence of chelating agents (citrate and phosphate) are vulnerable to alginate matrix which may affect the strength (Jimenez-Perez et al. 2004) and it has to be resolved with re-calcification of alginate beads (Smidsrod and Skjak-Braek 1990).

SCI effluents consists of variety of organic mixtures such as sugar and carbohydrates. It will proceed to oxygen demand and it may spread in effluents received in future and also the anaerobic conditions could make it to un-usable. This type of SCI effluent totally unfit for culturing practices like fish, prawn and others. Suppose the untreated SCI effluents applied directly for agricultural activities it will reflect on soil fertility, plant growth and seed germination. In this circumstances, immobilized algal bioremediation techniques contains vast interest. In this study, sodium alginate was used as immobilization polymer entrapping the fresh water microalga *Scenedesmus pecsensis*. Also we made an attempt an impact of changing pH, incubation period, number of beads, quantity of algae in beads besides reusing ability of beads on the nutrients removal efficiency of *S. pecsensis* beads in the SCI effluent.

MATERIALS AND METHODS

Collection of effluent

The untreated SCI effluent was collected from sugar cane industry outlet located at Sethiyathoppu, Cuddalore District, Tamil Nadu, India. The collected effluent was filtered through nylon mesh to remove small solids and particles. To avoid their changes of original nature, filtered effluent were stored at 4^oC. The physico-chemical characteristics in untreated and treated SCI effluent were estimated using standard methods (APHA 1998, Strickland and Parsons 1972, Jenkins and Medsken 1964).

Culture and immobilization of microalgae

The microalgae isolation, identification and cultivation were done according to Perumal et al. (2015). The microalgae immobilization was carried out according to Dinesh Kumar et al. (2013) with minor modification . In short, 100 ml of double distilled water (DDW) were carefully mixed with alginate powder by magnetic stirring. After complete mixing of DDW and alginate, the microalgae were added in to the mixture which were harvested in exponential phase. $CaCl_2$ solutions were made of nanopure water and used to making and hardening of beads. The alginate and algae mixture were added in drop wise from pre-determined distance using 20 ml of syringe (0.8 mm × 40 mm needle, Braun, Melsunger, Germany) into the $CaCl_2$ solution. Then the beads were allowed to 45 minutes in to $CaCl_2$ solution for hardening of beads surface. The beads were washed several times using tapwater which were filtered by 0.45 m pore size filter bag to eliminate the remaining $CaCl_2$ solution.

Experimental procedure

Microalgal beads were prepared as explained previously and beads without algae were used as control. The treatment were conducted in 500 ml conical flasks filled with 400 ml of SCI effluent and 100 numbers of immobilized algal beads were introduced into an effluent flask and incubated for predetermined duration. The treated SCI effluent were separated by filtration. The final concentration of nutrients in the treated sample were analyzed by UV spectrophotometer (Shimadzu Model-2450) according to methods explained previously.

pH, incubation time, beads density and algal cell concentration influences on nutrients removal

The treatment of SCI effluent were conducted by removing excessive nutrients with the help of immobilized algal beads. The experiment were conducted with changing pH and incubation time. The experimental pH was vary from 5 to 9 with control (without changing SCI effluent pH-4.9) for the removal of nutrients ion by immobilized algal beads. I normality NaOH and HCL were used to change the effluent pH. The various incubation time period was chosen (based on the earlier workers) for the removal of pollutants were 30, 60, 90 and 120 minutes respectively. After pre-determined incubation time, the effluent was separated from the algal beads by filtration. Then the treated effluent incorporated to estimate the nutrient removal rate. Second stage of experiment has been dealt with influence of different number of beads on nutrient removal from SCI effluent. This study was made with various numbers of beads viz., 50, 75, 100,125 and 150 with one control beads which did not have algae. For beads density experiment, the pH and incubation time were maintained which was given maximum nutrients removal rate in the previous batch experiment. Then the treated effluent were proceed to estimating the nutrient removal rate. The third stage experiment has been dealt with influence of quantity of algae in beads on nutrients removal rate from SCI effluent. To prepare the various quantity of algal cells containing beads, the centrifuged algae were dissolved in 10 ml deionized water. Then the algae and deionized mixture were mixed with sodium alginate for beads preparation. Themethods of beads preparation was already explained. The prepared beads contains different cell densities in single beads viz., 260 k cells/ml, 130 k cells/ml, 95 k cells/ml, 72 k cells/ml and 48 k cells/ml.

Desorption and reuse of beads on nutrients removal

To know the reusable efficiency of beads, desorption and reuse experiments were performed according to Revathi et al. (2017). After conducting first cycle of nutrients removal experiment, the algal beads were separated and treated with HNO_3 solution (100 mL, 1pH) for 60 minutes. After desorption treatment, the beads were washed twice with distilled water and then reused in a new adsorption experiment. In each cycles algal cell leakages were estimated by counting of algal cells in the effluent using counting chamber.

Table 1. The physico-chemical characteristics of untreated sugarcane industry effluent.

Parameter	Value
pH	5.0
Color	Dark brown
Conductivity (µS)	2,156
Salinity (ppt)	1
Dissolved oxygen (mg L^{-1})	6.1
Temperature (°C)	23.5
Total suspended solid (mg L^{-1})	0.098
COD (mg L^{-1})	30.67
Calcium (mg L^{-1})	0.6
Magnesium (mg L^{-1})	0.4
Phosphate (µmol/l)	53.20
Nitrate (µmol/l)	2.21
Ammonia (µmol/l)	12.63
Silicate (µmol/l)	37.95
Nitrite (µmol/l)	10.11
Total phosphorus (µmol/l)	3.37
Total nitrogen (µmol/l)	8.51

Three continuous adsorption and desorption cycles were conducted with triplicates.

RESULTS AND DISCUSSION

Features of untreated SCI effluent

The SCI effluent temperature was 23.5°C while collection, the color was appeared like highly dark contains strong odor. Water quality characteristics of untreated SCI effluent was given in Table 1. The researchers are stated that the using biological organism like bacteria, fungi and algae for the removal of pollutants from the effluent was resulted significantly. While treating effluent, the necessary oxygen for biodegraders provided by aerobic bacteria. The same time algae are have an efficient to remove the pollutants directly which ever as a mobilized or as immobilized forms (Fang et al. 2004).

Influence of pH and retention time on nutrients removal

The influence of pH and incubation time on nutrient removal has been examined by several investigators and their findings explaining that, if pH increased in the water column their pollution up taking ability also increased especially in fungi and algal biomass (Guibal 1992, Dinesh Kumar et al. 2013 and 2018).

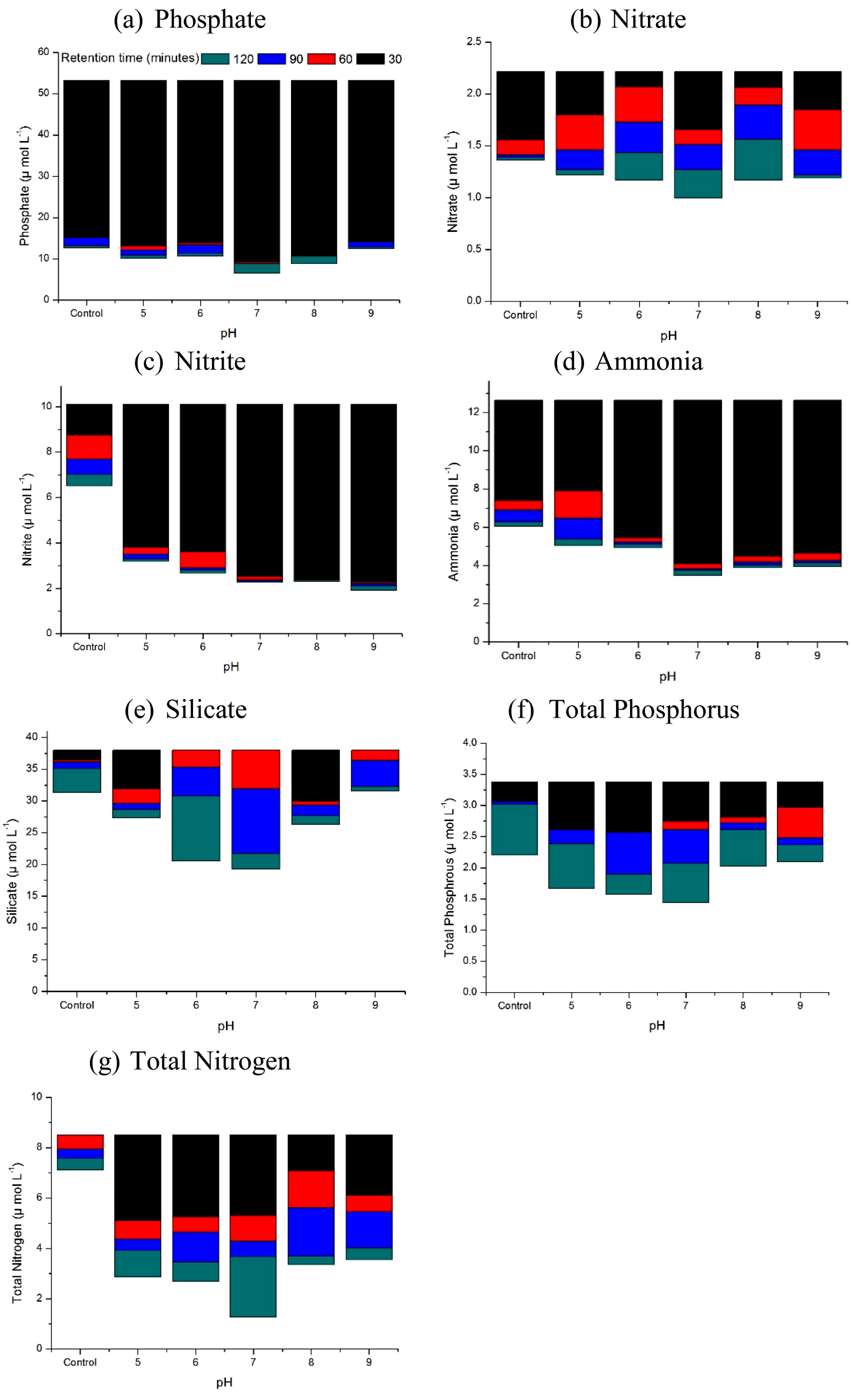

Fig. 1. Effect of pH and retention time on nutrients removal from sugarcane industry effluent using *S. pecsensis* beads.

While the treatment, the nutriens concentration from the SCI effluent was decreasing considerably at various pH (5-9). The highest nutrients removal was observed at pH 7 at 120 minutes of retention time while the minimum elimination was recorded at pH 9 in 120 minutes retention time (Fig. la-g). The recorded concentrations of highest nutrients removal at pH 7 during 120 minutes of retention time were as follows : Phosphate (53.20 to 6.53 µmol L^{-1}), nitrate (2.21 to 1.00 µmol/L^{-1}), nitrite (10.11 to 2.32 µmol/L^{-1}), ammonia (12.63 to 3.47µmol L^{-1}), silicate (37.95 to 19.23 µmol L^{-1}), total phosphorus (3.38 to 1.44 µmol L^{-1}) and total nitrogen (8.61 to 1.27 µmol L^{-1}). For testing effects of pH and retention time various levels ranging from 5-9 and 30 to 120 minutes were investigated. The early researchers explained that the, using algae on nutrients adsorption pH play a major role and it has to be consider for maximum nutrients adsorption (Dinesh Kumar et al. 2013, 2016, 2018 ,Mithra et al. 2012, Soumya et al. 2015, Vasanthy et al. 2015) Fig. 1a-g showed that the nutrients removal rate from SCI effluent using immobilized algae with reference to different pH and incubation time. The experiments reveals that there is strong relationship has been found between pH and rate of nutrients removal. It clearly explained that increasing pH from 5 to 7 triggering the removal rate in the level of three fold, if further extension of pH above7, the nutrients removal rate has been decreasing.

To enhance the adsorption range with an increase in pH (initial pH of SCI effluent was 5.0) can be play a major role via their negative charges which is available more numbers in the water column and may confide in the functional groups while segregation (Feng and Aldrich 2004). The pH associate nutrients binding can mostly be connected with functional assemblages available in algal surface and also on the chemical interaction between nutrients and effluents (Matheickal and Yu 1999). While the pH values going to low level, the hydrogen ions getting exceeds in their level. The exceeded protons may get strives with nutrients which already available in the water solution and it will form the bonds with the functional groups (active sites) on the surface of the microalgae. Finally this active sites which were bided with algae become inundated and therefore unapproachable to some other cations. The same time increasing pH may easy task to hydrogen protons and it will attracting their binders. Hence the nutrients available in the water column was charged positively when the pH level increase up to beyond the limit to bind the functional groups effectively and this statement also supported by Ajjabi and Chouba (2009). Incubation time is considered as one of the important factor in removing the nutrients from aqueous solution as well as wastewater. The optimizing experimental duration also increasing the nutrients removal efficiency of any kind of adsorbents. Hence, the present experiment inferred that the 120 minutes of incubation time resulted the highest nutrients removal. The reason may behind this, the valid strong binding forces are available between adsorbent SCI effluents. To reach the rapid equilibrium on the nutrient adsorption, diffusion should be accelerated mode on the outer surface with quick diffusion towards the intra particle matrix (Ho and Chiang 2003). Reducing incubation period showed that there is no significant enhancement on the elimination of nutrients from the water column. Hence, to increase the nutrients removal efficacy for further optimization experiments the optimized incubation time (120 minutes) and pH (7) were followed.

Influence of beads density on nutrients removal

In the present experiment beads density, proportionally removed nutrients from the sugarcane effluent (Fig. 2). The initial nutrients (PO^{3-}_4-53.20 µmol L^{-1}; NO_3^- -2.21 µmol L^{-1}; NO_2– -10.11 µmol L^{-1}; NH_3-12.63 µmol L^{-1}; SiO_4^{4-}-37.95 µmol L^{-1} ; TP-3.38 µmol L^{-1} and TN-8.52 µmol L^{-1}) were reduced significantly in different *S.pecsensis* beads densities. The maximum removal of nutrients was recorded with 100 numbers of beads (phosphate-88.67%, nitrate-59.73%, nitrite-81.31%, ammonia-75.30%, silicate-54.23%, total phosphorus-88.62% and total nitrogen-76.01%) whereas the least removal of nutrients was observed with 50 numbers for phosphate, silicate, total phosphorus and total nitrogen, 150 numbers of beads for nitrate and nitrite.

The moderate number of beads revealed that the highest nutrients removal efficiency than the high and low number of beads. Compared to various beads densities, 100 nos. showed good result. Several reports (Chen et al. 2003, Pakshirajan and Singh 2010) also concluded that adsorption of nutrients has been decreasing significantly when the number of beads increases beyond the limit by the reason of damaging beads gel membrane (Hannoun and Stephanopoulos 1986, Korgel et al. 1992). The higher density algal

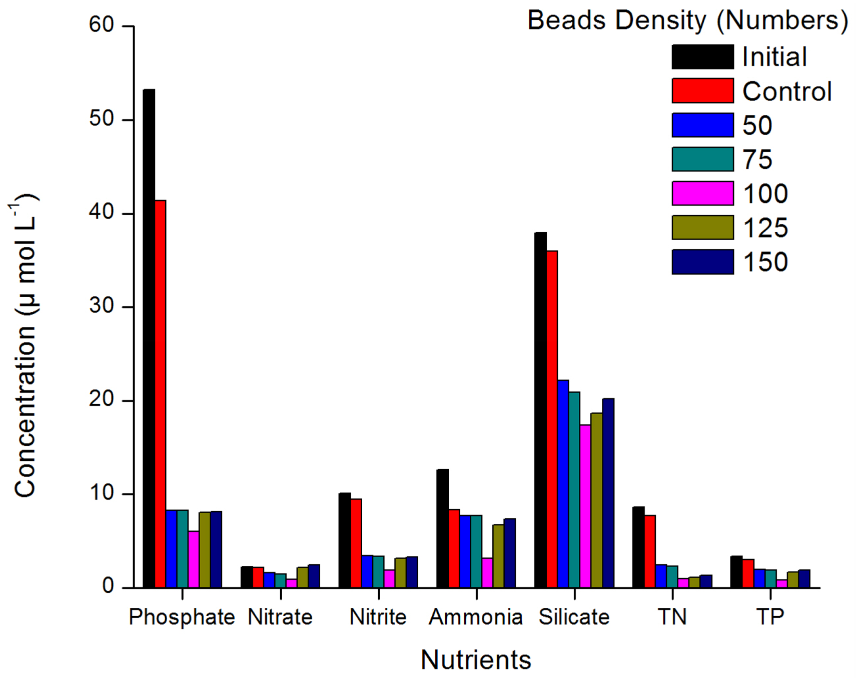

Fig. 2. Effect of beads density on nutrient removal from sugarcane industry effluent using *S. pecsensis* beads.

beads could be reduced the metabolic activity of cells and its leads to low nutrients removal rate. Due to heavy weight, the beads were settling in the bottom of the treatment column while the beads density were increased from the beyond the limit like 125 and 150. The same trend has been observed by Tam and Wong 2000) and Revathi et al. (2017) who stated that the rate of nitrate removal was slow when more beads used. The experiment also showed that both lower and higher number of beads (50, 75, 125 and 150) resulted the low nutrient removal while 100 numbers being optimum.

Influence of algal cell densities in beads on nutrients removal

As per the responses from previous experiments, pH 7, retention time 120 minutes and beads density 100 numbers were selected for algal cell density concentration in *S. pecsensis* beads experiment. In this experiment, the final concentration of all nutrients reduced inversely during varying algal cell densities used in *S. pecsensis* beads (260 k cells/ml, 130 k cells/ml, 95 k cells/ml, 72 k cells /ml and 48 k cells/ml), which indicating that the changing algal cell densities in beads play the major role on nutrients adsorption. The highest removal was observed in 95 k cells/ml containing beads and final concentration of PO^{3-}_{4} (2.67 µmol L^{-1}), NO_3^-(0.77 µmol L^{-1}), NO_2- (0.75 µmol L^{-1}), NH_3-(1.53 µmol L^{-1}) , SiO_4^{4-}(15.59 µmol L^{-1}), TP (0.80 µmol L^{-1}) and TN (0.83 µmol L^{-1}) were reduced from the initial concentration viz., 53.2, 2,21, 10.11, 12.63, 37.95, 8.61 and 3.38 µmol L^{-1} respectively (Fig. 3). Among the five different algal cell densities in beads tested, the lowest nutrients removal efficiency were observed in 260k cells/ml containing beads chamber especially for phosphate, TP and TN. The least removal of nitrite, nitrate, ammonia and silicate were observed in 48 k cells/ml containing beads chamber. In current experiment, the percentage of nutrients removal were significantly increase while implementing optimized parameters like pH (7), time (120 minutes), beads density (100 numbers) with 95 k cells/ml cell densities in beads. The percentage of increase varies from 88.67-94.98 (PO^{3-}_{4}), 59.73-64.94 (NO^{-3}), 81.31-87.90 (NO^{-2}), 75.30-87.90 (NH_3), 54.23-58.92

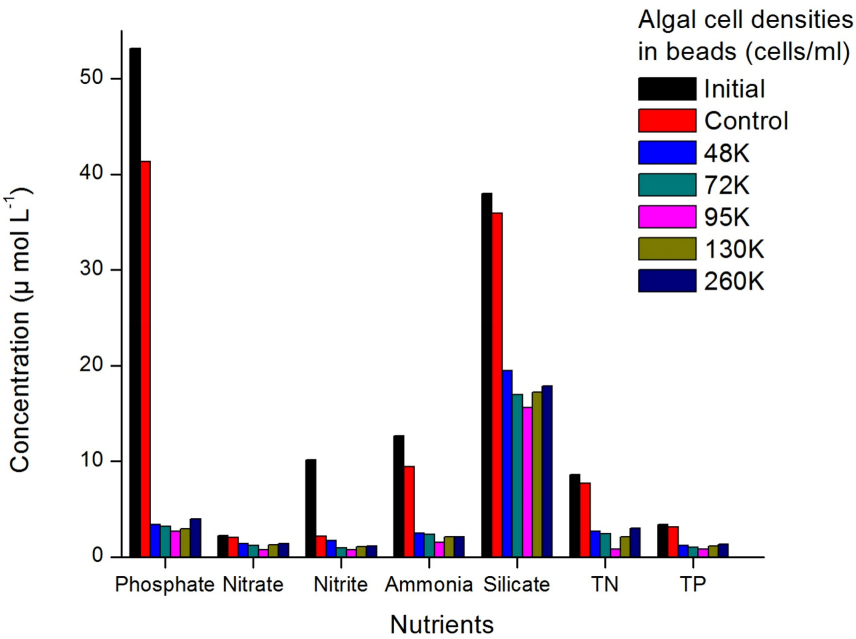

Fig. 3. Effect of algal cell densities in beads on nutrients removal from sugarcane industry effluent using *S. pecsensis* beads.

(SiO_3^{2}), 88.62-90.36 (TP), 76.01-76.33 (TN) . The nutrients removal efficiency were significantly increase while increasing algal cell densities in beads beyond the limit and these statement was supported by earlier workers (Dinesh Kumar et al. 2018, Adam et al. 2015, Lau et al. 1997). Abdel Hameed (2007) was proved that the stocking of medium concentration of algal cells in beads were removal phosphate (94%), nitrate (96%) and ammonia (100%) from the effluent efficiently than the lower cells stocking beads (67% , 36% and 75%). Malakootian et al. (2011) have confirmed that the removal rate increased with increasing algal dose in beads. Fan and Xu (2011) also reported increased pollutants removal when using increased algal densities. While the adsorbent dose increased simultaneously the nutrients removal rate also increased due to number of binding sites available in the adsorbent (Gok et al. 2010). But, the algal cells leakaging could be consider as a serous problem when the adsorbent doses increased, in the beads (Robinson et al. 1986, Lau et al. 1997) and damage the nutrients removal rate from the effluent with reference to algal cell concentration in the beads. If algal cell densities increased beyond the limit or moderate level in beads could be lose their nutrients removal efficiency due to leaking of algal cells. Earlier researchers also supporting our statement by the way of their conclusion like increasing algal cells in the beads from beyond the limit didn't make any positive improvement in removal of pollutants (Chevalier and de la Noue 1985, Lau et al. 1997, Soumya et al. 2015, Revathi et al. 2017). Likewise, the present study, we found that moderate algal cell stocking beads (95 k cells/ ml) had a higher nutrient removal rate than the other algal cell densities (48 k, 72 k, 130 k and 260 k cells/ ml) from SCI effluent.

Influence of beads recycling on nutrients removal

The successful recycled remediator only consider for commercial application. In the present study, HNO_3 used as desorption agent to remove pollutants from *S. pecsensis* beads. The 3 continuous recycling experiments were tested and 76.62% and 90.47% of TP and TN were removed in 1st cycle (Fig. 4). Significant increase of nutrients removal were observed in 2nd (1.78% and 1.05%) and 3rd (2.66% and 1.63%) cycle from the 1st cycle. The output of recycle experiment revealed that the *S. pecsensis* beads may offers to

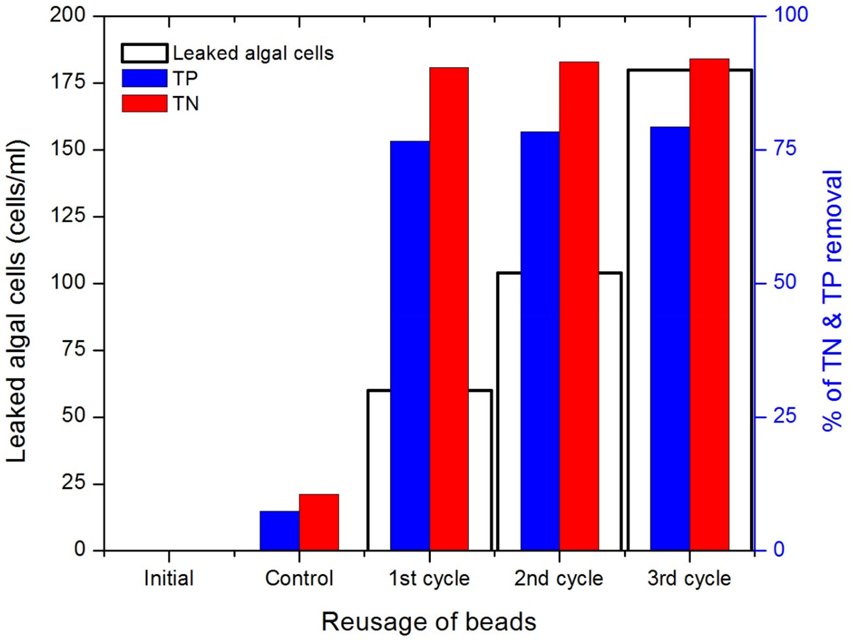

Fig. 4. Effect of reuse of *S. pecsensis* beads on nutrients removal from sugarcane industry effluent.

reuse up to three continuous cycles for eliminating nutrients. After 1st cycle the percentage of removal difference between 1st and 2nd (1.78% and 1.05%), 2nd and 3rd (0.88% and 0.58%) significantly decreased and it may due to leaking of algal cells from the beads with unstable condition of alginate. The results shows that the *S. pecsensis* have excellent recycling in continuous nutrients adsorption experiments.

CONCLUSION

The present study on nutrients adsorption rate of immobilized microalgae reveals that the nutrients were removed significantly by immobilized microalga *S. pecsensis* beads. Further, experiment on effect of different pH, incubation time, number of beads, quantity of algae on beads and algal beads reuse on nutrients absorption rate of *S. pecsensis* showed that maximum nutrients removal was obtained in the pH 7, 120 minutes of incubation time, 100 numbers of beads density and 95 k cells/ml of algal cell density in beads combination. Further results indicated that the nutrient reduction is dependent on the algal density in beads, pH, retention time and beads density. Therefore it is concluded that the immobilized microalgae *S. pecsensis* beads can be used as prominent bio-remediating agent in the effluent recycling units for SCI effluent in particular for the removal of excess nutrients for the conservation of nature and natural resources.

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