The Pharma Innovation Journal 2019; 8(9): 128-133

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(9): 128-133

www.thepharmajournal.com
Received: 04-07-2019
Accepted: 08-08-2019

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Phycoremediation of dairy effluent by using microalgal consortium

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Abstract
Aim: The present research work was carried out to study the phycoremediation of dairy effluent by using algal consortium.
Methodology: The effluent sample was collected in a plastic container from the dairy industry. The effluent sample was treated with mixed algal consortium inclusive of *Scenedesmus dimorphus, Scenedesmus acutus, Desmococcus* sp., *Chlorella vulgaris, Chlorella conglomerate, Chroococcus* sp., and physicochemical analysis like pH, BOD, COD TSS, TDS, Dissolved oxygen, total hardness, total alkalinity, calcium and magnesium, phosphate and chloride were evaluated.
Results: The analysis of dairy effluent observed a significant decrease in the BOD (57%), COD (54%). TSS, Alkalinity (80%), Chloride (80%), Calcium (75%) and Magnesium (27%) after treating with the algal consortium.
Conclusion: The results of the study showed mixed algal consortium to be useful to reduce various physicochemical parameters in wastewater and could be potentially used for the treatment of dairy wastewaters.

Keywords: Algal consortium; microalgae; phycoremediation; BOD; COD, TDS, TSS

Introduction
India ranks second in the top milk producing countries in the world accounting for 60.6 billion kg following the US at 91.3 billion kg. India is the largest producer of milk in the world with production of 155.5 million tons (2015-2016) as per the data gave by NDDB (National Dairy Development Board) and DAHDF (Department of Animal Husbandry, Dairying and Fisheries).
Dairy industry produces wastewater rich in organic matter and thus lead to the creation of odorous and high COD high BOD-containing water. It also contains soluble organics, suspended solids, trace organics which releases gases, causes taste and odour problems, imparts colour and turbidity and promotes eutrophication. In dairy wastewater, nitrogen originates mainly from milk protein and also present in organic nitrogen from such as proteins, urea and nucleic acids. Phosphorus is found mainly in inorganic forms such as orthoactive phosphorus (po_3^{-4}) and polyactive phosphorus (p_2o_4) [7]. A significant amount of Na, Cl, K, Ca, Mg, Fe, CO, Ni, Mn are also always present in dairy wastewater. The presence of a high concentration of Na and Cl is due to the use of a large amount of alkaline cleaner in the dairy plant [1].
The disposal of dairy wastewater without or with partial treatment can cause serious pollution in the ecosystem and groundwater, causing health and hygiene hazards to life. The dairy effluent is predominantly organic in nature due to its biodegradable constituents. Microalgae can grow well in these effluents because of its high nutrition content. The use of microalgae culture in wastewater treatments offer a modern solution of tertiary treatment of wastewater together with the production of potentially valuable biomass, which can be used for several purposes such as biogas and biofuel production, composting, animal feed or in aquaculture and production of chemicals.
A significant reduction in total phosphorus, total nitrogen and COD was reported in dairy wastewater treated with algae in both indoor and outdoor conditions [2, 3]. Microalgae can be used to reduce the inorganic and organic load of wastewaters at a minimal cost [4]. The dairy wastewater treated by algae can be used for growing algae for biofuel production [5]. Most of the studies have concentrated on the use of a single algal species for reducing the organic load of dairy waste water. Therefore the present study was focused on using mixed algal consortium as a means of phycoremedy to remove reduce the pollutants in dairy wastewater.

Methodology
Sampling area
Samples were collected from dairy industries for the analysis of physicochemical parameters. Twenty litres of samples was collected in a clean container. The sample container was sealed tightly after collection. The sample transported safely to the laboratory and stored in a cold room at 4ºC for estimation of physicochemical parameters and morphological studies.

Analysis of sample
The collected dairy effluent was analyzed for the various physicochemical parameters like pH, temperature, total dissolved solids, total suspended solids, total alkalinity, dissolved oxygen, chemical oxygen demand, magnesium &calcium, phosphate, and chloride content. Dissolved oxygen, biological oxygen demand, chemical oxygen demand, chloride were analysed by adopting the standard procedure of volumetric analysis. Dairy wastewater parameter was examined on the 0 day, 5^{th} day and 7^{th} day of phycoremediation.

Morphological identification of algae
The dairy effluent was treated with mixed algal consortium inclusive of the following algae *Chlorella vulgaris, C. conglomerate, Scenedesmus dimorphus, Scenedesmus acutus, Desmococcus sp*. The morphological identification of the algae was carried out at Phycospectrum Environmental research centre using a phase contrast microscope.

Physico-chemical analysis
Total Solids: 25ml of the sample was shaken vigorously and filtered in a pre-weighed dish and then evaporated the sample to dryness at 103º-105 ºC on a steam bath. The evaporated sample was dried in an oven for about an hour at 103º-105 ºC and transferred to a desiccator for final cooling in a dry atmosphere and recorded for constant weight.

Calculation

$$\text{Total solids (mg/L)} = \frac{(A-B)}{\text{Sample volume ml}} \times 1000$$

Where, A = Initial weight of the filter, B = Final weight of the filter

Total Suspended Solids: 25ml of the sample was shaken vigorously and filtered into a glass fibre disk fitted to a suction pump. The filter was carefully removed from the filtration apparatus by the help of forceps and dried for an hour at 103º-105 ºC in an oven. Then the filter was cooled and weighed for constant weight.

Calculation:

$$\text{TSS (mg/L)} = \frac{(A-B)}{\text{Sample volume ml}} \times 1000$$

Total Dissolved Solids: The difference in the weights of Total solids (A) and Total suspended solids (B) expressed in the same units gives Total Dissolved solids (TDS).

Total Hardness: 50 ml of sample was taken in a conical flask. 1-2 ml of buffer solution and 1-2 drops of EBT indicator will be added into the flask. The solution turns wine red. The sample will be titrated against standard EDTA Titrant. The sample will be titrated up to the endpoint until the colour turn from wine red to blue and notice the Titrant reading.

Calculation: Hardness (mg/L) = EDTA used (ml) × 1000 of sample.

Total alkalinity: 10ml of water sample was taken and added 2 drops of phenolphthalein indicator. The solution turns pink and titrated with the dilute H_2SO_4. The endpoint come with short disappear of the pink colour volume of diluteH_2SO_4 will be noticed. In the same flask, 2-3 drops of methyl orange will be added and the colour of the solution turns yellow. Further titration continued and anew endpoint reached when a solution in the flask is just turned to pink.

Calculation: Alkalinity = total H_2SO_4 × 0.02N × 1000× 10ml of the sample

Chlorides: 10 ml of water sample was taken in a conical flask, and 2 drops of potassium chromate indicator was added and titrated against standard silver nitrate till silver dichromate ($AgCrO_4$) starts precipitating.

Calculation

$$\text{Chlorides} = \frac{(A-B)\,(N)\,(35.45)}{\text{Sample was taken in ml}}$$

Where,
A - Volume of silver nitrate consumed by the sample
B - Volume of silver nitrate consumed by the blank
N - Normality of silver nitrate.

Dissolved oxygen: Manganous sulphate and 2ml of potassium iodide were added and sealed. The BOD bottle was shaken in the upside down reaction for at least 6 times. The brown precipitates were allowed to settle down. At this stage 2ml of concentrated sulphuric acid was added, and mixed well until the brown precipitates dissolve. 50ml of the sample was taken in a conical flask and titrated with 0.01N sodium thiosulphate. Using starch as an indicator, 2 drops of the starch solution was added to the conical flask which changes the colour of the contents from pale to blue. It was titrated again with Sodium thiosulphate till the blue color disappears.

Calculation

$$\text{Dissolved oxygen mg/L} = \frac{(0.1)\,(1000\text{ml of sodium thiosulphate})}{50}$$

Biological oxygen demand: The pH of the water sample was adjusted to neutrality using 1N acid or 1N alkali solutions. The 50ml of water sample was filled in 4 BOD bottles without bubbling. 2ml of Manganous sulphate and 2ml of alkaline iodide were added to the bottle and sealed. This was mixed well and the precipitate was allowed to settle down. Conc. Sulphuric acid was added and mixed well until all the precipitates dissolve. The dissolved oxygen content in the two of the 4 bottles by the titration method. The mean of the two readings (D_1) was taken and the remaining two bottles were incubated at 27 ºC in a BOD incubator for 2 days. The oxygen concentration in all the two incubated samples was estimated. The mean of the two readings (D_2) was taken.

Calculation

$$\text{BOD mg/L} = \frac{8 \times 1000 \times N}{V}$$

Chemical oxygen demand: 50ml of the sample was taken in a conical flask and 10ml of potassium dichromate solution, was added. To it, a pinch of each silver sulphate and mercuric sulphate and 30ml of concentrated sulphuric acid was added to the conical flask. The flask was connected to a condenser, The sample was mixed before heating and the flask was kept into reflux for a minimum 60 ºC for 2 hours. The flask was cooled and diluted to about 100ml by adding distilled water. 2-3 drops of ferroin indicator solution were added and titrated against ferrous ammonium sulphate solution. At the endpoint, the blue-green colour of contents changed to reddish blue.

Calculation

$$\text{Chemical oxygen demand} = \frac{(R-T) \times N \times E \times 1000}{\text{The volume of sample (mL)}}$$

Where
T = Volume of titrant (FAS) used against sample (mL)
B = Volume of titrant used against blank (mL)
N = Normality of ferrous ammonium sulphate
E = Equivalent weight of oxygen.

Calcium and magnesium: 10 ml of the water sample and 0.2 ml of sodium hydroxide was added flask. One pinch of Murexide solution was added to the sample and titrate with the 0.1N EDTA solution until the pink colour changes to dark purple.

Calculation

$$\text{Calcium (mg/L)} = \frac{T \times E \times 1000}{V}$$

Magnesium (mg/L) = (TH - Ca)

Calcium where,
T = titrate value in ml
E = mass in mg of calcium ions equivalent to 1ml EDTA solution
V = volume of the sample.

Magnesium where,
TH = titrate value of total hardness
Ca = titrate value for calcium

Phosphates: 1ml of the potassium dihydrogen phosphate was taken in the Nessler's tube and diluted with 40ml of distilled water. Then 8ml of mixed reagent was added to the tube. The control sample was prepared with 40 ml of distilled water and 8ml of mixed reagent. The OD value was measured. 40ml of the effluent sample was taken in a conical flask and added the 8ml of potassium persulphate autoclave at 15mintues. After cooling then added the 8ml of mixed reagent was added to the flask. Then the OD was measured.

Calculation

$$\text{Phosphate (mg/L)} = \frac{\text{Absorbance of sample x Conc. of Std X 1000}}{\text{Absorbance of Std. x Sample taken}}$$

Result

Characteristics of dairy wastewater: The colour of the treated effluent with algae changed from grey to green on the 7th day. The change in colour may be due to the growth of algae utilizing organic matter in the effluent that has made the water clear.

a. Raw effluent sample **b.** Treated effluent sample

Physico – chemical analysis

Various parameters such as alkalinity, TDS, TSS, BOD, COD, Hardness, calcium, magnesium, phosphate were measured.

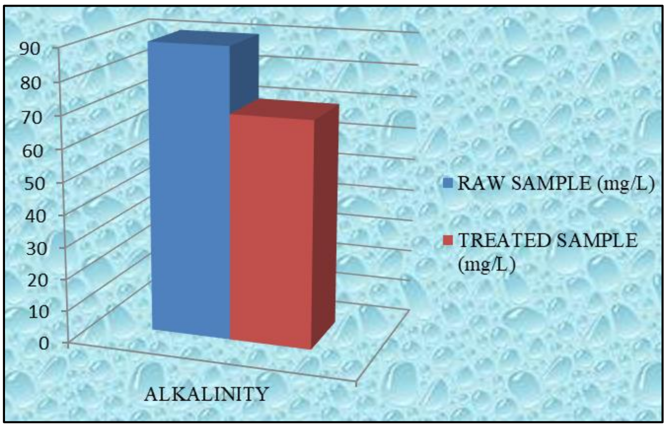

Chart 1: Alkalinity

The dairy industry effluent discharge contains a high amount of carbonate ions. High alkalinity can cause nuisance problems. If alkalinity is less it changes the pH level in water systems and makes the water corrosive. It indicates the ability of water to neutralize acids from wastewater. The alkalinity was reduced from an initial 90mg/L to 70mg/L after treatment [chart:1]. Total suspended solids affect the intensity of water. Suspended solids are the cause of suspended particles inside the water body influencing turbidity and transparency. A high concentration of suspended solids can cause many problems for stream health and aquatic life. High level of TSS will increase water temperature and decrease dissolved oxygen in the water. Total suspended solids of the effluent decreased upon treatment with microalgae from initial 3.92mg/l to 0.32 mg/l.

Biological oxygen demand: Biological oxygen demand is a measure of the quantity of oxygen used by microorganism in the oxidation of organic matter. If the BOD is low, there is an abundance of oxygen which leads to good water quality. The biological oxidation is a very slow process. During oxidation, organic pollutants are oxidized by a certain microorganism into carbon dioxide and water using dissolved oxygen. BOD will vary by region. In the present study BOD levels were reduced from 130mg/L to 74 mg/ L after treatment [chart:2 a].

Chemical oxygen demand: The chemical oxygen demand is a measure of water and wastewater quality. The COD test is used to measure the number of organic compounds in a water sample. It measures the capacity of water to consume oxygen during the decomposition of organic matter and the oxidation of inorganic chemicals such as ammonia and nitrate. Water with high COD indicates the presence of organic waste. The results of the current study show COD levels reduced from 260mg/L to 140mg/L of the treated sample [chart:2 b].

Calcium hardness: There was a significant reduction in calcium. Calcium reacts with water to form calcium hydroxide and produce hydrogen gas. Calcium carbonate doesn't react with pure water, but it does react with carbonic acid, that is water with dissolved carbon dioxide, to produce soluble calcium bicarbonate. The carbonate ions, which not only decrease the crystallinity of calcium phosphates and promote the formation of amorphous calcium phosphates but also compete with phosphates in precipitating with calcium to form calcite. The Calcium hardness was reduced from 200mg/L to 150mg/L [chart:2 c].

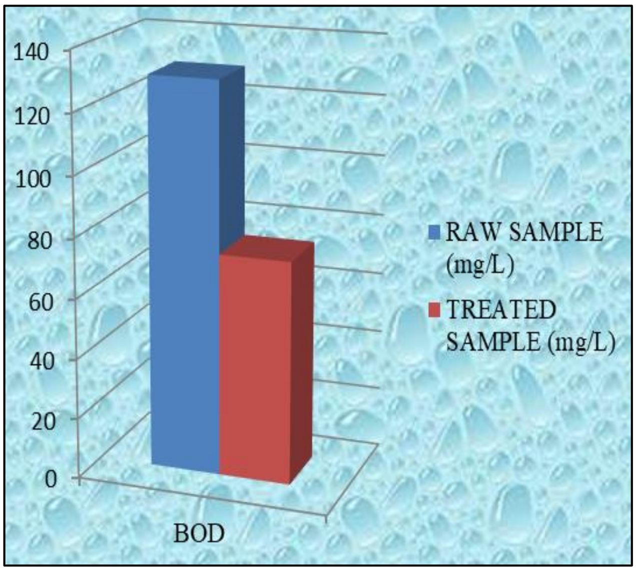

Chart 2a: BOD

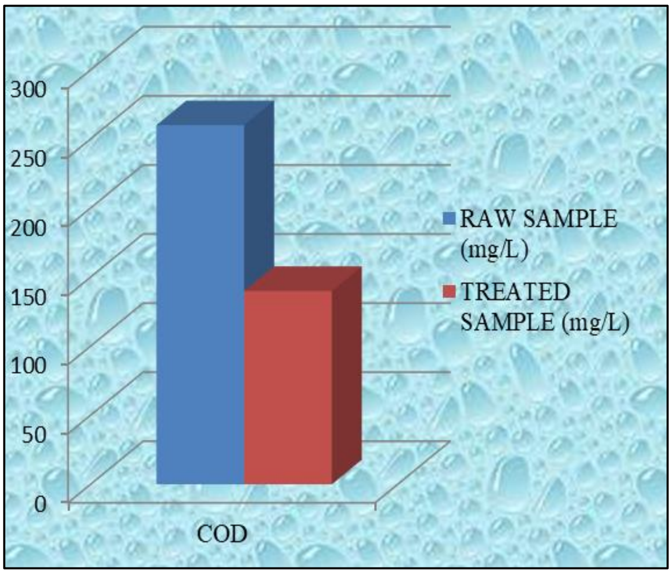

Chart 2b: COD

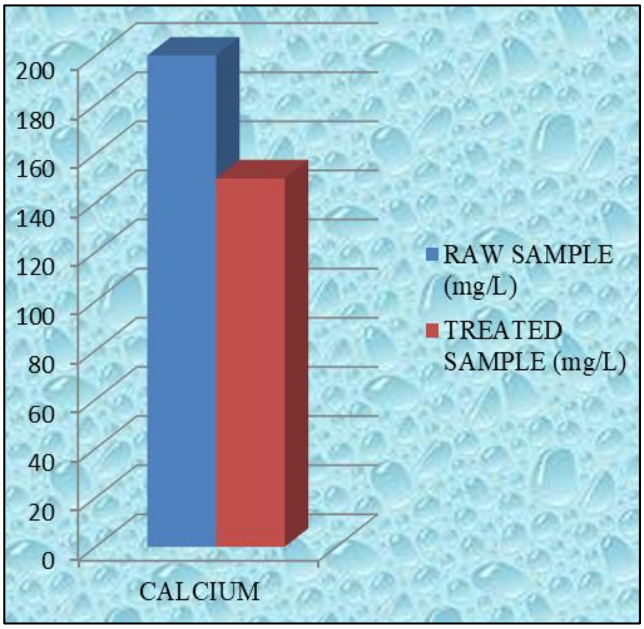

Chart 2c: Calcium

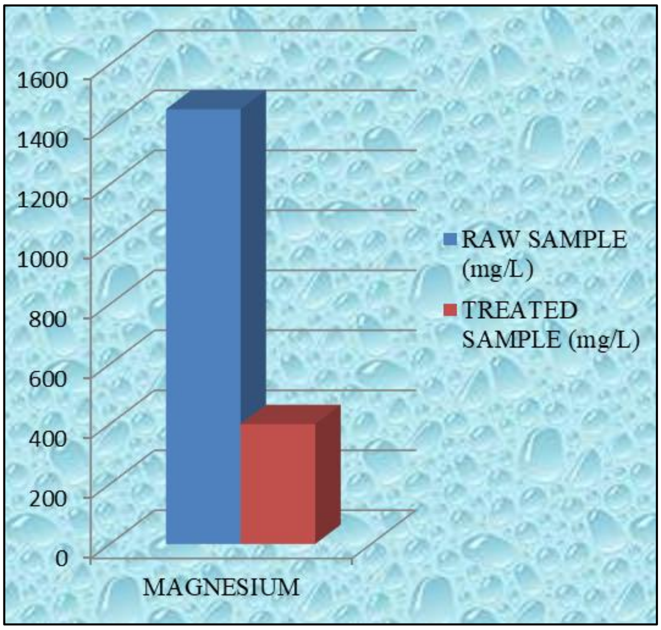

Chart 2d: Magnesium

Magnesium hardness: magnesium produces systemic poisoning although persistent over-indulgence in taking supplement and medicines can lead to muscle weakness, lethargy and confusion. The magnesium hardness was 1450 mg/L initially and after treatment, it was reduced to 400 mg/L [chart: 2 d].

Analysis of phosphate: The chlorides in the present study were reduced from 531.25mg/L to 425.4 mg/L. The Calcium hardness was reduced from 200mg/L to 150mg/L. Alkalinity was reduced from initial 90mg/L to 70mg/L after treatment

Discussion

The characteristic of effluent generally varies greatly within industries depending on the processes involved. In the dairy industry various operations like pasteurisation, preparation of butter, cheese, milk powder etc., produce wastewater rich in the organic matter thus leading to the creation of odorous and high BOD and COD containing water. The discharge of wastewater into the environment without any treatment poses a significant risk for public health and environmental pollution. So there is a need to treat the effluent before flow into the environment. Physicochemical analysis generally performed as per US EPA in dairy effluent includes pH, DO, TDS, TSS, BOD, COD and hardness. Algae are known to grow more rapidly in nutrient-rich water and are able to remove nutrients from wastewater successfully. In the present study, the effect of the microalgal consortium on physicochemical properties of dairy effluent was carried out.

The analysis of physicochemical characteristics of dairy wastewater by the traditional method was carried with an algal consortium based dairy wastewater treatment and their efficiency was checked. Various parameters lie BOD, COD, TDS, TSS, Calcium, Magnesium hardness were measured which showed differences before and after treatment. The morphological identification was made by phase contrast microscope and the algae were identified based on their morphological characteristics and found to be inclusive *Scenedesmus dimorphus, Scenedesmus acutus, Desmococcus* sp., *Chlorella vulgaris, Chlorella conglomerate, Chroococcus* sp.

A large number of pollutants impart colour and odour to the water making them unaesthetic. The removal of colour from wastewater is more important as they also contribute to major BOD load. In the present study colour of the effluent treated with mixed algal consortium changed from grey on the day of collection to green after 5 days of algal treatment. The change is colour may be due to the use of the nutrients present in the dairy effluent by the algal consortium. This finding is in concordance with Kotteswari *et al*., 2012 [6].

COD is used to measure pollution load in terms of quantity of oxygen required for oxidation of inorganic matter. The reduction of COD indicated that microalgae could utilize inorganic carbon in the wastewater as a source of energy and as a substrate for cell growth. In this study, the reduction of COD was from 260 mg/L to 140 mg/L (54 %). The reduction of COD in the present study is higher to the study reported by Kotteswari *et al.,* 2012 who reported a reduction of COD levels to 24.69% when the dairy effluent was treated with *Spirulina platensis* [6] and Gani *et al*., 2014 who reported a reduction of COD in dairy effluent after treatment with microalgae *Botryococcus* sp. to be 4.8% [7].

Presence of high BOD indicates the high quality of biological oxidizable organic matter in an effluent. High contents of BOD cause depletion in oxygen. In the present study, the BOD levels were reduced from 130mg/L to 74 mg/ L (57%). The reduction in BOD of the present study is much higher than the reduction level of 40.25% reported by Kotteswari *et al*., 2012 using Nostoc sp [6].

The total suspended solids affect the light intensity of water. Suspended solids inside the water body also influence turbidity and transparency. Total suspended solids of the effluent decreased upon treatment with microalgae from initial 3.92mg/l to 0.32 mg/l which might be due to the utilization of various nutrients by microalgae and there could have been a conversion of the total suspended solids in the effluent into dissolved materials for algal uptake and assimilation. Shivsharan *et al*., 2013 reported a reduction in TDS to 9% in treated sewage. The total dissolved solids is a measure of total inorganic salts and other substances that are dissolved in water [8]. The total dissolved solids remained the same after treatment.

Alkalinity is a measure of buffering capacity of the water. It is an important parameter which indicates the ability of water to neutralize acids from wastewater. In the present study, the alkalinity was reduced to 80% from initial 90mg/L to 70mg/L after treatment. This is significantly high when compared to Kotteswari *et al.*, 2012 who reported an 18% reduction in alkalinity of dairy effluent using Nostoc sp [6].

Presence of chlorides in water is attributed to dissolution of salt deposits discharge. The chlorides in the present study were reduced to 80% from 531.25 mg/L to 425.4 mg/L. The initial phosphate concentration was 1.42mg/L was reduced to 0.67 mg/L. the overall phosphate reduction percentage was 47%.

In the present study, the Calcium hardness was reduced to 75% from 200 mg/L to 150 mg/L however there was no significant reduction in Magnesium hardness observed. The hardness of water mainly depends on the calcium and magnesium salts. In the present study, the algal consortium could not show a significant reduction in hardness, despite reducing calcium to 75%. Hence there was no observable change in Total hardness found in the study.

In the present investigation, the performance of microalgal consortium in treatment of dairy wastewater was evaluated. Based on the results it is proved that algal consortium can be used to remove pollutants load and nutrients except for magnesium hardness in future research.

Conclusion

The result of the present study indicates that the treatment of dairy effluent by the algal consortium is very efficient and also proved to be cost-effective and effective eco-friendly treatment. The algal consortium played a vital role in reducing COD, BOD, TSS and alkalinity significantly. This study suggests the use of algal consortium as an alternative to the conventional physical and chemical methods of treatment of dairy effluents.

Acknowledgement

We are grateful to Prof. Dr. V. Sivasubramanian, Director, Phycospectrum Environmental Research Centre, Chennai for providing algal cultures and lab facilities.

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