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Phycoremediation of distillery effluent using *Spirulina platensis*

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Abstract
Phycoremediation is the process of removal of pollutants from waste water by using algae. Since algal culture is easily to grow, adapt and manipulate within the laboratory it can be used for remediation studies. Micro algae offer a low cost and effective approach to remove excess nutrients and other contaminants in biological waste water treatment. The present investigation was carried out to determine the bioremediation potential and bio mass production of *Spirulina platensis* using Distillery effluent. The studies showed that the maximum removal of colour, temperature (30^0c), pH (7.5), electrical conductivity (1.13 dSm^{-1}), total dissolved solids (400mgL^{-1}), COD (450mgL^{-1}), BOD (280mgL^{-1}), manganese (1.05mgL^{-1}), potassium (56.23mgL^{-1}) was recorded after 60 days of bioremediation experiments using *Spirulina platensis.* The parameters like dry weight, growth rate, lipid and protein content also measured. The study clearly indicated the effectiveness of micro algae for the removal of nutrients present in the distillery effluent and dry biomass obtained can be used as a feed for animals.

Keywords: Phycoremediation, distillery effluent, *Spirulina platensis*

Introduction
Microalgae offer a low-cost and effective approach to remove excess nutrients and other contaminants in tertiary wastewater treatment, while producing potentially valuable biomass, because of a high capacity for inorganic nutrient uptake (Muñoz and Guieyssea, 2006). Using microalgae in continuous treatment processes would be of great advantage, because most industries are in dire exigency for implementing cost effective continuous treatment processes. Algal species are relatively easy to grow, adapt and manipulate within a laboratory setting and appear to be ideal organisms for use in remediation studies (Sen *et al.*, 2013) [11].
The use of either naturally occurring or deliberately introduced microorganisms to consume and break down environmental pollutants (Sen *et al.*, 2013) [11], in order to clean a polluted site is called bioremediation. Distillery effluent is the waste water which contain high BOD and COD. It pollutes the land where it flows due to its various chemical elements
The chemical elements can be easily broken down with the use of micro algae. Micro algae are low in cost and it can easily broke down the excessive nutrients present in the waste water. *Spirulina* is a biomass of cyanobacteria that can be consumed by humans and other animals. The algae, *Spirulina* have the ability to reduce the BOD and COD in the distillery effluent. This study is based upon the utilization of *Spirulina platensis* in different dilution of anaerobically digested distillery effluent (ADDE) medium.

Materials and Methods
Collection of Culture
Spirulina platensis was obtained from Centre for Advanced Studies in Botany, University of Madras, Chennai and used throughout the study.

Culture multiplication
A *S. platensis* was auxenically grown in Zarrouk's medium. Firstly, we have transferred our culture in Zarrouk's broth from Zarrouk's agar slants. Culture were incubated in a culture room at temperature of 30±2 ºC and illuminated with day-light fluorescent tubes saving 4 Klux at a surface of vessels. During the process of growth the flask was shaken 3 to 4 time per day. The experiment was run in triplicates. All manipulation involving the transfer of culture in the liquid media or on agar plates were carried out under aseptic conditions on a laminar air flow.

Collection of Effluent
The effluent was collected from Mohan Breweries and Distilleries Ltd., Marie oulgaret, Puducherry.

Physico-chemical characterization of anaerobically digested distillery effluent

The anaerobically digested distillery effluent was analyzed for various physico-chemical characteristics. In the treated effluent, the biomass was separated by centrifugation at 12000xg for 15 minutes prior to analysis. The physico-chemical parameters such as colour, temperature, pH, electrical conductivity, total suspended solids, total dissolved solids, total solids, chemical oxygen demand, total hardness, alkalinity, dissolved oxygen, biological oxygen demand, chlorides, sulphates, phosphates, nitrate, lead, chromium, zinc and oil and grease were analyzed in the supernatant (APHA, 1998).

Formulation of Anaerobically Digested Distillery Effluent (ADDE) medium for *Spirulina platensis*

The standardized ADEE medium contained 1000 ml of distillery effluent medium (60% conc.) were supplemented with carbon (16.8 mg L^{-1} $NaHCO_3$), phosphorus (0.5 mg L^{-1} K_2HPO_4), $FeSO_4$ 0.01 g L^{-1} and EDTA 0.08 g L^{-1}, pH adjusted to 9.0.

Table 1: The mass cultivation of *S. platensis* in digested distillery effluent medium during summer season

Cultivation system	**Cement tank**
Nutrients	Anaerobically digested distillery effluent medium (60% con.) supplemented with $NaHCO_3$ (16.8m g L^{-1}), K_2HPO_4 (0.5g L^{-1}), $NaNO_3$ (2.5 g L^{-1}), $FeSO_4$ (0.01 g L^{-1}) and EDTA (0.08 g L^{-1})
Light	Sunlight
pH	9.0 to 9.5
Temperature	40°C
Agitation	Manual stirring by plastic stick (30 min./day)
Culture depth	15 cm to 20 cm
Seeded culture	*Spirulina platensis*- CAS10, inoculum dose 0.25 g L^{-1}
Culture period	60 days
Harvesting	Filtration through muslin cloth
Drying period	Sun drying on plastic sheets
Season	April-May, 2018
Location	Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai nagar, Chidambaram, Tamilnadu

During cultivation period the growth parameters such as dry biomass, protein content, chlorophyll content, lipid content, total carbohydrate and phycocyanin were estimated at 10 days interval from 0 to 60 days by various methods which are given in Table 2.

Table 2: Methodology adapted for estimation of biomass, chlorophyll, protein, lipid, total carbohydrates and phycocyanin content of *S. platensis*.

S. No.	**Parameters estimated**	**Methodology adapted**
1.	Biomass	Pandey *et al.* (2010) [8]
2.	Chlorophyll content	MC Kinney (1941) [7]
3.	Protein content	Lowry *et al.* (1951)
4.	Lipid content	Foich and Lees (1957)
5.	Total carbohydrates	Hedge and Hofreiter (1962)
6.	Phycocyanin content	Horvath *et al*., (2013) [5]

Harvesting, Drying and storage

After growing of the culture, the culture was filtered by using fine nylon. Filtered algal samples were washed in distilled water to remove impurities. The washed samples were collected in a tray and is covered with sterile plastic cover. The tray is then kept in bright sunlight for 5-6 days to remove the moisture present in the algal sample. The dried culture was then collected in bags and kept for further use.

Results and Discussion

Growth of *S. platensis* CAS 10 strain under outdoor condition

The culture was grown in outdoor condition for a period of 60 days. It was observed that the growth parameters like protein, lipid, carbohydrate, phycocyanin content and dry biomass were increased from 0^{th} day till 60^{th} day in Fig. -1.

The culture was then transferred to a 1000 litre cement pond for further investigation. An average temperature of 42 ^{0}C was there during the cultivation which encourages the biomass yield of 12.15g DWm^{-2} day $^{-1}$ which supports the view expressed by Jimenez *et al.* (2003).

Bioremediation of anaerobically digested distillery effluent using *Spirulina platensis.*

The study revealed that anaerobically digested distillery effluent showed maximum reduction in the effluent characteristics *viz.*, colour, pH (7.5), electrical conductivity (1.13 5Sm-1), chemical oxygen demand (450 mgL^{-1}), Biological oxygen demand (280mgL^{-1}), Calcium (54.2 mgL^{-1}), sulphates (210 mgL^{-1}), nitrate (18 mgL^{-1}), lead (0.06mgL^{-1}), potassium (56.23 mgL^{-1}), manganese (1.05 mgL^{-1}) zinc (0.5mgL^{-1}) of distillery effluent after bioremediation using *S. platensis* (Table-3). The decrease of distillery parameter is likely due to that *S. platensis* absorbs the nutrient from the effluent. Higher pH was reported by Sivakumar *et al.* (2011) when they analyzed the tannery effluent and the reduction in the manganese content is in the conformity of their absorption during the phycoremediation experiments (Dwivedi, 2012) [2].

Conclusion

After the treatment of distillery effluent with the use *Spirulina platensis* it was observed that the physico-chemical characteristics of the distillery effluent were changed. This change indicates that the Spirulina can be used as good source for Bioremediation. This experiment also indicates that the micro algae samples can be used to reduce the chemical nature of the anaerobically digested distillery effluent.

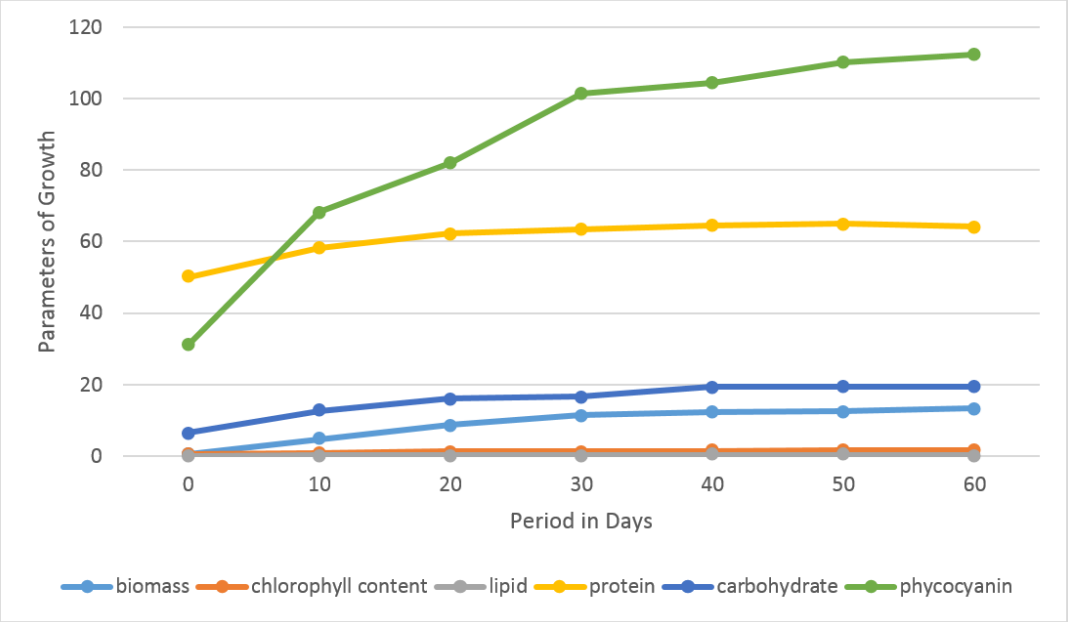

Fig 1: Growth of *S. platensis* CAS 10- strains in outdoor condition

Table 3: Phycoremediation of anaerobically digested distillery effluent using *S. platensis* CAS 10

S. No.	Parameters	Physicochemical characteristics of anaerobically digested distillery effluent		BIS Standards for irrigation
		Before Phycoremediation	After Phycoremediation	
1	Colour	Light brown	colourless	colourless
2	pH	8.3	7.5	5.5 -9.2
3	BOD (mg/L)	2250	280	200
4	COD (mg/L)	28880	450	250
5	Oil and Grease(mg/L)	14	8.3	10
6	Temperature(^{0}C)	33	30	28 -35
7	Electrical Conductivity(dsm^{-1})	8.50	1.13	1.00
8	Total dissolved solids (mg/L)	950	400	2100
9	Calcium(mg/L)	332	54.2	50
10	Iron (mg/L)	2.0	1.65	5.0
11	Sulphate (mg/L)	2210	210	
12	Nitrogen(mg/L)	1682.8	16.0	100
13	Lead(mg/L)	0.065	0.04	0.1
14	Zinc(mg/L)	0.56	0.20	5.0
15	Manganese(mg/L)	2.035	1.05	2.0
16	Potassium(mg/L)	3108	56.23	

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