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Application of microalgae strains for potential use in phycoremediation of tannery effluents

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Abstract : Aquatic pollution is a man-made phenomenon, arising either by increasing the concentrations of naturally occurring substances or by adding non-natural synthetic compounds (Xenobiotics etc.) to the environment. Tannery effluents are marked as top prioritized pollutants among all the industrial wastes. Phycoremediation is an eco-friendly technique in which microalgae used as a bio-tool to treat the effluents without adding any chemical additives that enhanced the pH and DO and reduces the nutrients concentration. In the present study, the tannery effluent water samples were collected from tannery industry situated at Erode, Tamil Nadu, India to study the role of microalgae in tannery effluent water treatment. The tannery effluent water was treated with cultured strains of *Chlorella sp* (JM_{13}) and *Scenedesmus sp* (JM_{10}) for 15 days. Samples were periodically (every after 3rd day) analyzed for Physico-chemical parameters such as pH, phosphate, nitrate, BOD and COD using standard methods. The strain JM_{13} showed the best removal capacity of nitrate and COD while JM_{10} showed BOD and phosphate reduction up to 2 weeks. The combination of JM_{13} & JM_{10} reduces the COD and BOD at 88% and 89% respectively while nitrate and phosphate were reduced drastically at the rate of 90% and 97% respectively. The present investigation focuses on the phycoremediation of tannery effluent water using microalgae as safer, less expensive and more efficient approach to remove nutrients and metals. The output of this treatment may be filtered and converted as biofertilizer for agricultural practices.

***IndexTerms* - Microalgae, Tannery effluent, Phytoremediation, Chlorella, Wastewater.**

I. INTRODUCTION

Organic and inorganic substances which are released into the environment as a result of domestic, agricultural and industrial activities lead to organic and inorganic pollution (Abdel-Raouf *et al*., 2012). The relative severity of water pollution varies, according to a number of factors, including population, extent of industrialization, economic situation and institutional capacity. They increases tremendously and impose severe risks to availability and quality of water resources, in many areas worldwide. So, in terms of health, environment and economy, the fight against water pollution has become a major issue of the day. Industrial wastes are generated from different processes and the amount and toxicity of waste released varies with its own specific industrial processes. Tannery effluents are ranked as top among the worst pollutants among all industrial wastes (Das *et al*., 2017).

India alone about 2000–3000 tone of pollutants escapes into the environment annually from more than 3000 tannery industries, with high levels of toxic metals, chlorides, organic matter, organic and inorganic nitrogen compounds, aromatic and aliphatic ethoxylates hydrocarbon chains, fatty acids, dyes, arsenic, sulfides, BOD, COD, TDS and chromium concentrations in the aqueous effluent compared to the recommended permissible discharge limits (Dunn *et al*., 2013).

Secondary effluents from wastewater treatment plants may cause eutrophication in aquatic environments and upset the balance of the ecosystem. The major adverse ecological impacts caused by eutrophication are: increase in algae and aquatic plants, fish death, reduction of biodiversity and replacement of dominant species, increased water toxicity, and increased turbidity of the water and decreased lifespan of the lakes Leta *et al*., 2004).

Algae considered as green-cell factories that not only good scavengers of toxic chemicals but are also involved in oxygenation of the atmosphere and carbon dioxide sequestration, thereby making them a better candidate among bioremediation systems. Microalgae can be applied in the tertiary and quaternary purification steps because the growth of microalgae is usually limited by nitrogen or phosphorus and algae therefore remove these nutrients efficiently from the medium. Additions to those microalgae are reported for efficient removers of toxic organic compounds and heavy metals (Rai *et al*. 2005; Ajayan *et al*. 2015; Abdel-Raouf *et al*., 2012; Das *et al*. 2017). The wastewater treatment using microalgae has low energy demand because oxygen is supplied through photosynthesis rather than energy-intensive electromechanical blowers (Green *et al*., 1995). In addition to these advantages, the produced biomass can be used as animal feed, energy production, and fertilizers or to produce fine chemistry products such as pigments, polysaccharides, carotenes, sterols, vitamins, polly-unsaturated fatty acids (PUFA) and lipids (Lodi *et al*., 2003).

Keeping these all above points in consideration the present study has been designed to utilize the energy of wastewater for cultivation of microalgae that can be treated as the pollution removing technology approach. These micro unites of algae were cultured in tannery wastes to minimize the nitrogen and phosphate pollution in aquatic systems. This approach will be a tool for the resolution of pollution in water systems in the state resulted in environmental conservation for the welfare of the society.

II. Objectives of the Study

The objectives of the present study are

- To maintain the stock culture
- To analyse the water quality parameter of Raw tannery water
- To analyse the improvement in water quality of tannery effluent water treated with algal consortium

III. METHODOLOGY ADOPTED

The present study has divided into two major sections of study for which the methodology section outline plan and method that has applied are as follows;

3.1Sample collection, identification and incubation

Algal samples stocks were collected from the Algal Culture Laboratory, Zoology Department, Kongunadu Arts and Science College Coimbatore. The identified algae from *Chlorella sp* (JM_{13}) and *Scenedesmus sp* (JM_{10}) selected for the present study. The pre identified algal culture strain was kept separately and maintained in the same culture unit for further mass development and experiments.

The microalgae *Chlorella sp* (JM_{13}) and *Scenedesmus sp* (JM_{10}) used in this study, available in the experiment was conducted between September 2015 and February 2016.

3.1.1 Media used and cultivation conditions

For Stock culture: The identified algae were cultivated in the Erlenmeyer culture flasks of 500 ml initially by maintaining in modified Guillard medium. The stocks were maintained at 25°C, under constant aeration and continuous lighting of 3000 lux. All chemicals used in this study were of analytical reagent grade.

The growth until the exponential phase culture served as inoculums. The modified medium contained two different solutions. The methods of algal cultivation have been followed by Borowitzka (1998) and Dunn *et al* (2013).

For mass culture: Solutions for adsorption and nutrient utilization raw tannery effluent liquid were collected from a tannery located in Erode district, Tamilnadu, India. Raw tannery water was taken in a plastic tub of 10 L capacity and diluted up to 50% by mixing of distilled water. Mass culture was prepared by the appropriate dilution of the freshly prepared stock solution of algae (50 ml) in prepared raw effluent water.

3.2Experimental setup

Experiment – 1

The microalgae *Chlorella sp*. was evaluated for their ability to grow in Raw Tannery Water (RTW) and finalized for the selection of species for the present study. The stock culture was placed in 100 ml emerald flask and maintained in algae culture room in Zoology Department. Further the grown culture was transferred to the bigger flask i.e 500 ml and further 1 L respectively. Finally the mass culture of fully grown algal strains *Chlorella sp* (JM_{13}) and *Scenedesmus sp* (JM_{10}) was transferred to the effluent culture bucket.

At the same time, RTW was characterized by pH, DO, CO_2, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Nitrate, Phosphate, Chlorides, Sulphate using methods of APHA (2005).

Experiment – 2

The microalgae *Chlorella sp* (JM_{13}) and *Scenedesmus sp* (JM_{10}) was mixed with tannery effluent and cultured in 5 liters plastic buckets to evaluate for their ability to remove Nitrate and Phosphate from the tannery wastewater. To perform this experiment special set of buckets were used in three sets. The details of sets are as follows;

Set-1= *Chlorella sp* (JM_{13}) alone incubated with tannery effluent

Set-2= *Scenedesmus sp* (JM_{10}) alone incubated with tannery effluent

Set-3= *Chlorella sp* (JM_{13}) and *Scenedesmus sp* (JM_{10}) mixed 1:1 ratio and the consortium was incubated with tannery effluent

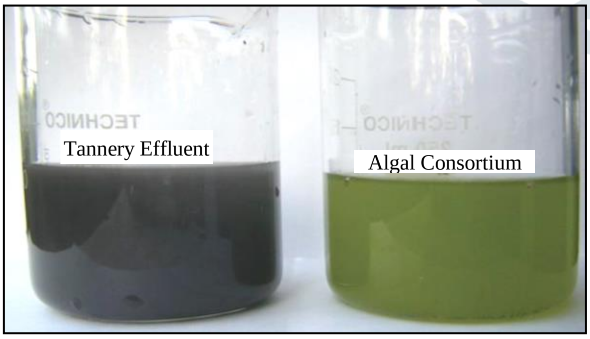

Figure 1: Image of tannery effluent and algal consortium prepared in the laboratory

Figure 2: Experimental setup of phycoremadiation of tannery effluent in algal consortium

The inoculums volume of *Chlorella sp* (JM_{13}) and *Scenedesmus sp* (JM_{10}) was standardized by giving the trial and error method up to 10 ml at 1 ml interval. For treatment, the prepared RTW was used in buckets without any further dilution.

Initially, the physico-chemical properties of the RTW were recorded prior to inoculation in experiment. The similar bucket without algal inoculums was maintained as control. The culture was grown in batch mode for 18 days at room temperature (25 °C) with a light intensity of 3000 lux with 24 h light condition. Treatment was aerated continuously by bubbling air from the bottom of the culture bucket with mechanical aeration system.

IV. RESULTS AND DISCUSSION

4.1 Results of Analysis of the biomass growth

The final cell density and biomass after RTW with algae exposure for a period of 18 days were compared to controls in RTW. Samples from each flask was taken and fixed with Lugol's iodine solution to measure the cell density using haemocytometer. The mean number of cells produced after exposing period was expressed and then the solution was characterized for reduction with respect to control.

Table 3: Physico-chemical change in experiments after algal treatments RTW cultures

Parameters	Initial Value	SET-1			SET-2			SET-3		
	Day 1	Day 7	Day 14	Reduction %	Day 7	Day 14	Reduction %	Day 7	Day 14	Reduction %
pH	7.9	7.6	7.5	-5.06	8.1	7.9	0	8.1	8.3	5.06
DO (mg/L)	4.2	5.4	5.8	38.1	5.1	5.4	28.57	5.9	6.2	47.61
BOD (mg/L)	260	180	55	-78.85	156	45	-82.69	156	27	-89.61
COD in (mg/L)	754	512	110	-85.41	542	131	-82.62	455	89	-88.19
Nitrate (mg/L)	4.3	0.9	0.8	-81.39	0.92	0.86	-80	0.83	0.43	-90
Phosphate (mg/L)	7.91	6.1	0.6	-92.41	5.8	0.5	-93.67	5.2	0.2	-97.47
Chloride (mg/L)	550	345	101	-81.63	448	112	-79.63	315	89	-83.81
Sulphate (mg/L)	82	61	37	-54.87	62	43	-47.56	58	27	-67.07
Calcium (mg/L)	170	122	64	-62.35	137	71	-58.23	102	46	-72.94
Magnesium (mg/L)	78	39	22	-71.79	41	34	-56.41	36	12	-84.61

In the present study, the results shows that the BOD, COD , phosphate, Nitrate showed that the microalgae cultures reduced the concentration of these parameter in about -78.85%, -85.41% and -92.41% , -81.39% respectively in the strain JM_{13} and the similar research of Rai *et al* (2005) show the ability of *Chlorella sp* to remove nutrients. Their report also showed that these algae can remove 86% inorganic nitrogen and 78% inorganic Phosphate from tannery water.

Various techniques are in place for exploiting faster growth rates and nutrient removal efficiencies of certain microalgae for the treatment of wastewaters from different sources. During the last four decades, phycoremediation has come in focus after evaluation work carried out by numerous investigators in similar ways. Among them, Dunn *et al*. (2013) examined the detoxification of tannery wastewater and nutrient removal using high-rate algal pond systems. Different microalgae such as species of Oscillatoria, Phormidium, Ulothrix, Chlamydomona, Scenedesmus have been evaluated for their ability to grow in tannery effluent and accumulate chromium (Rai *et al*. 2005; Ajayan *et al*. 2015). Notable reports on the use of Chlorella for bioremediation of tannery wastewater by Jaysudha and Sampathkumar (2014) in that *C. salina* or *C. marina* and *C. vulgaris* was used by Rai *et al*. (2005) for removal of nutrients from tannery wastewater. The results of the present investigation also well aligned with these reports.

Though the species Scenedesmus was taken in least consideration for treatment; despite of its high conversion efficiency of nutrients like nitrate and phosphates. For strain JM_{10} i.e. SET-2, maximum reduction of nutrients were recorded in case of Phosphate (93.67%), BOD (82.67%), COD (82.62%), and nitrate (80%). Similar results have been reported by Ajayan *et al*. (2015) and his team in different occasions.

The concept of application of consortium of algae in treatment of waste water was not new but it was adopted only in the field of biodiesel production or in the dairy waste water treatments (Hene *et al*., 2015). The present investigation also showed the promising results in reducing the nutrient levels present in the tannery waste water. Maximum reduction was recorded for Phosphate (97.47%) followed by Nitrate, BOD and COD at 90%, 89.61% and 88.19% respectively.

Similar results of Phosphate and nitrogen removal were obtained by Hene *et al*. (2015) and Ajayan *et al*. (2015) suggest that algae grow luxuriantly with great variety and abundance in waters rich in high amounts of nitrates, phosphates, Calcium and other oxidizable organic matter in the effluent also could be contributing to the growth of this microalgal strain (Murugesan and Sivasubramanian, 2005). In a study by Das *et al*. (2017) about tannery wastewater treatment by *Chlorella* sp it was found that Phosphate and nitrogen separation rate is almost 100% and 70% respectively during the first 5 days of the study. Ajayan *et al*. (2015) expressed that *Chlorella* sp can be used for the removal of phosphate and nitrate and also for algae production in tannery wastewater systems supports the present study.

Apart from the results obtained during present study, demonstrate higher reduction of nutrients (nitrates, phosphates and sulphates) by *C. vulgaris* from TW when compared with those reported by Ajayan *et al*. (2015), for Scenedesmus sp. and Adam *et al*. (2015), for Tetraselmis sp. Remarkably, the nutrient levels were reduced to well below the maximum permissible limits of BIS.

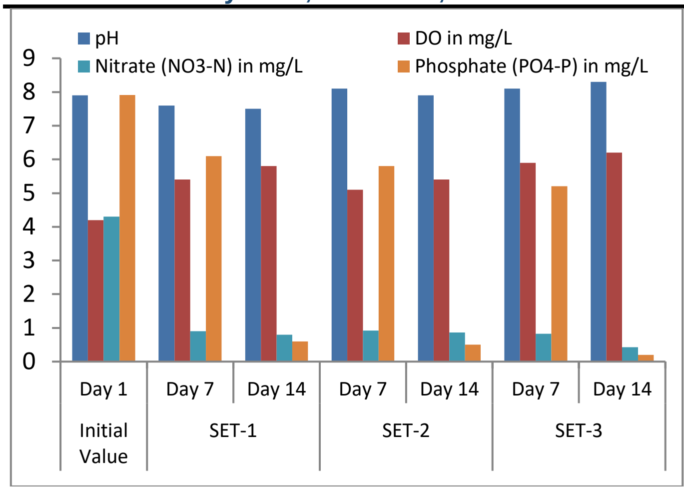

Figure 3: Effect of algal consortium on the concentration of pH, DO, NO3-N and PO4-P present in tannery effluent

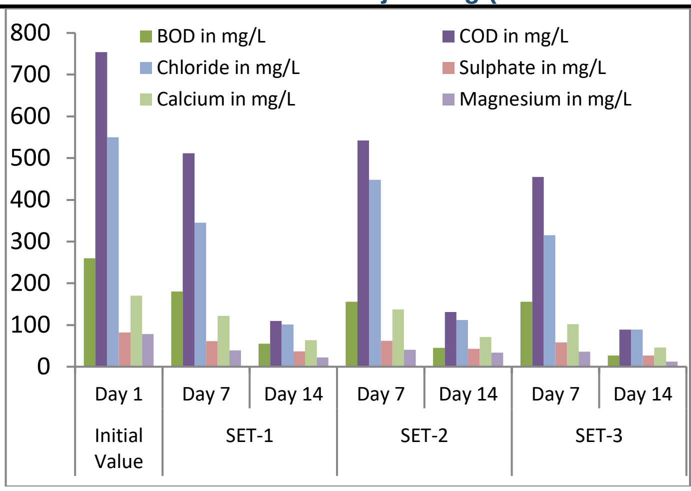

Figure 4: Effect of algal consortium on the concentration of BOD, COD, Cl, SO4, Ca and Mg present in tannery effluent

V. CONCLUSSION

Hence, the aim of this study is to compare the efficiency of microalgae *Chlorella* sp in removing pH, DO, CO_2, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Nitrate, Phosphate, Chlorides, Sulphate from raw tannery water and estimate the use of wastewater as a suitable medium for cultivation of these algae for biomass production.

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