JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

A STUDY ON THE BIOREMEDIATION USING OSCILLATORIA TENUIS ON DOMESTIC SEWAGE WATER.

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Abstract : Algae has been using as a source of phycoremediation in many areas like industrial, textile, pharmacological, nuclear and cosmetics industry. Being autotrophic and efficient in growing the adverse conditions, algae can be reared in multiple scale for the pollution treatment. In addition to this a lot of value added products can also be segregated from these algae. In this study, a waste-water isolated alga has been used for the study. It has been treated with domestic sewage collected from the natural water bodies dumbed in the highly populated area. The study had been carried out for 30 days and the water quality variations studied at restricted laboratory conditions. In this study we can found out that the algae can reduce the pollution and BOD, COD of the sewage water and the treated water can be reused for other purposes like irrigation.

IndexTerms **- Phycoremediation, Oscillatoria tenuis, sewage water, BOD, COD, UV-Spectrophotometry.**

I. INTRODUCTION

Pollution is an avoidable malice generated as a result of modernization and industrialization. The intrusions to the natural energy flow due to anthropological activities generate a wide range of pollutions (Bhutiani *et al.*, 2016). Algae being the major producers of the earth with primitive life mechanisms have the ability to grow in any adverse conditions (Deepa *et al.*, 2019). Phycoremediation is the process of employing macro or microalgae for wastewater treatment. It has many advantages over the conventional methods, which are very costly, energy consuming and generating high amount of sludge hence it is accepted throughout the world. This method involves the use of macro or microalgae for the removal or biotransformation of pollutants, including nutrients and xenobiotics from wastewater (Ahmad *et al.*, 2013). Over the last few decades studies were undertaken to diverse microalgae such as *Chlorella, Spirulina, Nostoc* and *Oscillatoria* for bioremediation (Sharma and Khan, 2013). The fundamental assumption is that the microalgae are versatile to convert the contaminants into non-hazardous resources, enabling the treated water to be recycled or reused or safely discharged. This technology is low in cost and it is an effective approach to remove excess nutrients, contaminants in wastewater and generating possibly useful biomass. (Deepa. K. P, 2019). Besides emerging trend of employing microalgae in phyco-remediation of toxic heavy metals due to its high binding affinity, abundance of binding sites and large surface area (Cameron *et al.*, 2018). These algae can be used for the bioremediation and they can also be utilized for getting the value-added hydrocarbons, algal chemicals and bio-pigments. The Sewage disposal is a major problem in most of the populated cities in India. Algal species can improve the quality of mine water by reduction in pH, Temperature, Nitrate, Iron, Chloride, Fluoride, Total Hardness, Sulphate, Calcium and Manganese (Gupta and Nikhil, 2014). Microalgae can act on complex eco-toxins to simpler hydrocarbons and thereby made to ecofriendly and value-added byproducts (Deepa *et al.*, 2020). These algae itself can also be act as cleansing agent on various ecotoxins.

II. MATERIALS AND METHODS

The domestic sewage water had been collected from the Korai river, located near Dheeran nagar, Tiruchirappalli, a densely populated area. The collected river water had been tested for various analysis test like, BOD, COD, TDS, DO, Calcium, Magnesium, Sodium, Potassium and its pH. The algae Oscillatoria tenuis had been isolated from the waste waters from the hydrocarbon spills in Tiruchirappalli district and isolated, purified and maintained at a laboratory condition of 28+℃, 3000 − 4000 lux light intensity.

The study was conducted in 250 ml Conical flask (Borosil Ind. Pvt, Ltd), with varying concentration of sample (sewage water) ranging from 50, 100, 150, 200, 250 ml and 250 ml distilled water as negative control and 250 ml BG11+ (algal medium) as positive control.

About 5 ml of pure culture of algae has been treated with various concentration of polluted water and its biodegradation had been studied for 30 days. UV- Spectrophotometer study at the wavelength of 450-630 nm (540 nm) and Turbidity percentage at the prescribed wavelength had been noted for these 30 days. All these studies were done in sterile conditions, triplicates and the average of values has been taken as result. At the final, the biomass of the algae grown has also been calculated. The physicochemical parameters used were detailed below:

2.1 BIO-CHEMICAL OXYGEN DEMAND (BOD)

A desired volume of distilled water with 1 ml per litre of phosphate buffer, $MgSO_4$, CaCl2 and FeCl3 were mixed and aerated for 30 minutes. This is known as dilution water. 2 sets of BOD bottles were filled with the respective solutions of dilution range. One bottle was incubated with label at 20 degree Celsius in a BOD incubator for 5 days. Initial DO level was estimated in the other set of bottle and noted down. After 5 days the final DO was estimated and the BOD was calculated using the following formula

BOD mg/1 = (Initial DO-Final DO) × Dilution factor.

2.2 CHEMICAL OXYGEN DEMAND (BOD)

20 ml of the sample was taken in a round bottomed flask and a pinch of mercuric sulphate was added. Anti-bumping granules were added. 5ml of Sulphuric acid-silver sulphate mixture was added to the solution in the flask and mixed well to facilitate the dissolution of mercuric sulphate. 10ml of potassium dichromate was added and 25ml of sulphuric acid-silver sulphate mixture was added. While adding the mixture, the round bottomed flash should be kept in ice water bath. This was to prevent the escape of fatty acids due to higher temperature (Hollender et al., 2019). Then the RB flask was connected to the reflux condenser and refluxed for 2 hours.

After 2 hours the flask was cooled and 80ml of distilled water was added and mixed well. This was titrated against FAS solution using ferrion indicator. The end point was the sharp colour change from blue green to wine red. Simultaneously, the blank was refluxed in the same manner using distilled water instead of sample with the same amount of chemicals..

2.3 TOTAL DISSOLVED SOLIDS (TDS)

A measured volume of well – mixed sample was transferred filtered through the filter to a pre- weighed dish and evaporated at approximately 2°C below the boiling point of water. After evaporation, the dish was dried for at least 1 hour at 180°C and cooled in a dessicator. After cooling, the dish was weighed..

2.4 DETERMINATION OF TOTAL HARDNESS

20ml of the sample was taken in a conical flask. To this, 2ml. Of buffer solution and a spatula of Eriochrome Black – T were added. It was titrated against EDTA solution taken in a burrete. The endpoint was the colour change from wine red and blue (Patel and Kanungo, 2012).

2.5 DETERMINATION OF CALCIUM HARDNESS

20ml of the sample was taken in a conical flask. To this,2ml of NaOH solutions and a spatula of Calcon indicator was added. It was titrated against EDTA solution. The endpoint was the colour change from pink to purple.

2.6 ESTIMATION OF MAGNESIUM

The amount of magnesium was determined by finding the difference between the total hardness and calcium hardness. The results were expressed in mg/L.

2.7 ESTIMATION OF SODIUM

Flame photometer was switched on and the flame was lit following the instructions given in the user's manual. The standard solution were aspirated into the flame and the instrument was calibrated. The sample was filtered through a filter paper to remove any suspended matter which otherwise clog the capillary of the instrument. The filtered sample was aspirated into the flame and the concentration of sodium present in the sample was determined.

2.8 ESTIMATION OF POTASSIUM

Flame photometer was switched on and the flame was lit following the instructions given in the user's manual. The standard solution were aspirated into the flame and the instrument was calibrated. The sample was filtered through a filter paper to remove any suspended matter which otherwise clog the capillary of the instrument. The filtered sample was aspirated into the flame and the concentration of potassium present in the sample was determined.

2.9 DETERMINATION OF pH

Samples and buffer solutions were brought to the room temperature. Temperature of both buffer solutions of sample was measured and temperature control was set. The probe of the electrode removed from the storage solution, rinsed and blotted dry with soft tissue. The probe was immersed into the first buffer solution and the selector switch was set to read pH. "Set buffer" control was adjusted until the meter reading agreed with the known pH of the buffer solution the probe was removed, rinsed in distilled water, blotted dry with tissue and placed in a beaker containing second buffer solution and the pH was read. (If the meter does not agree exactly with the known pH, calibration has to be repeated until correct readings are obtained). After calibration was over, the probe was rinsed with distilled water, blotted dry with tissue and immersed in sample solution and the pH reading was noted (Carstea et al., 2016).

III. RESULTS AND DISCUSSION

The biochemical analysis of the sewage water collected had been attached here with (Annexure- 1). The table for the algal biomass in terms of UV- Spectrophotometer study in the 540 nm wavelength has been given in the Table -1.

Table-1- Algal biomass- UV- Spectrophotometer study

	Ist day	10th day	20th day	30th day
50 ml	0	0.1	0.2	0.3
100 ml	0.2	0.6	0.7	0.5
150 ml	0.6	0.8	0.9	0.6
200 ml	0.12	1	1.1	1.12
250 ml	0.04	0.6	0.4	0.7

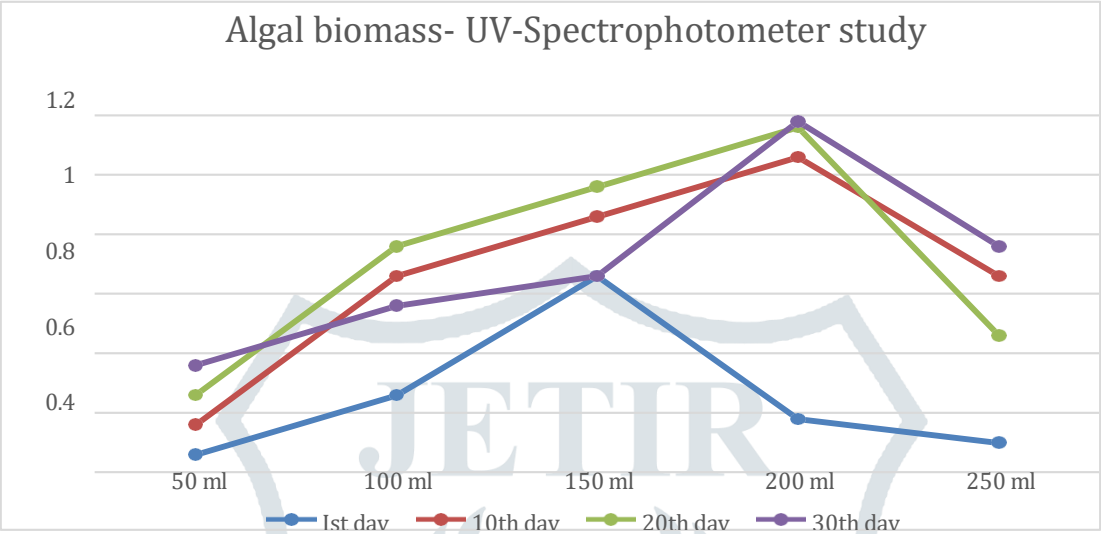
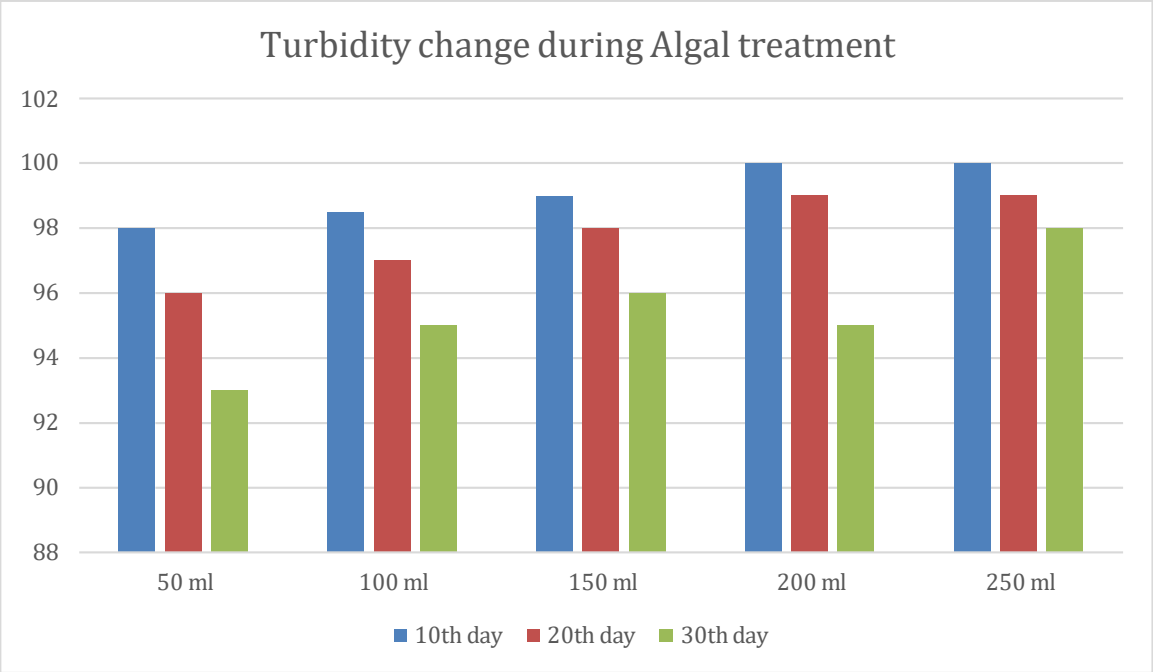


Table -2 Turbidity change during Algal growth

Turbidity	10th day	20th day	30th day
50 ml	98	96	93
100 ml	98.5	97	95
150 ml	99	98	96
200 ml	100	99	95
250 ml	100	99	98



The pictures showing the algal growth and the colour change in the sewage water can be observed in the below pictures.

First day

Tenth day

20th day

30th day

The study of biodegradation will be completed only after the successful amount of algal biomass cultured during the study. For this the sterile pre-weighed petri-plates were used and algal biomass was lyophilized, powdered and weighed under normal room temperature. The difference on the weight of petri-plates remain as the weight for the algal grown, Table-3.

Table-3 Algal biomass yield.

Sl no	Sample concentration	Weight in gms.
1	50 ml	0.01
2	100 ml	0.11
3	150 ml	1.12
4	200 ml	2.01
5	250 ml	1.53

IV. DISCUSSION

From the biochemical analysis, it is clear that the BOD and COD of the sewage water had been changed drastically from 3.24 to 11.35 and 136 to 108 respectively. The microalgae and macroalgae used earlier also shows major changes in the Also the TDS, total calcium and other ions in the sample had also been reduced by the algal treatment. Algae being adapted to live on adverse conditions can withstand the organic and inorganic wastes in the sample and utilizes the pollutant to the growth of algae and bio accumulate these particles as organic form inside system (Diamantini, et, al 2018). So, this is a type of natural biodegradation without causing any ecological side- effects (Leonga Y.K. and Chang, J.S. 2020). That's why algae have been widely utilizing in this sector for large scale industries and mixed farming industries (Bwapwa, et.al 2017)). Plant with medicinal property can also be used for natural remediation. The mass invitro propagation of plants yield products and also used for bioremediation. The vast usage of space and time remains the obstacle for relying in higher plants for bioremediation (Deepa. K.P 2019).

At the spectrophotometric study, algal biomass had been studied for 30 days and based on their turbidity the chart had been plotted. While looking on the chart, we can find out that the 200 ml of concentration of sample the biomass is showing high. A gradual

increase from the lower to higher concentration can be observed and after it in higher concentration the radial change in the biomass, turbidity, yield of the algal biomass had been observed.
A change in the biochemical parameters especially the BOD, COD of the water sample and the transparency of the algal medium shows the growth of algae using the degradable waste in the sample and the evaluation of the treated sample shows the capability of algae in biodegradation. This study proves this algae can also be used for sewage treatment like that of treating in textile effluent and hydrocarbon spills that had been carried out previously.

IV. CONCLUSION

The investigation from this study showed that Oscillatoria tenuis is a useful bioremediation tool in bioremediation of wastewater polluted sites. Employment of this method in large scale bioremediation of domestic wastewater (sewage) pollution is essential. The algae can also be taken for fatty acid profiling, while their biomass can also be treated for anti-microbial studies (Deepa and Thajuddin, 2023). The multiutility of algae are more to be utilized in all energy needs of humans (Neha et .al 2014).
In present study, domestic wastewater sample (sewage) was tested and treated with algae i.e., Oscillatoria tenuis. After treatment, a reduction was observed in all the physico-chemical parameters. The algal culture treated wastewater and showed a sharp reduction in BOD and COD and also the TDS, total calcium and other ions in the sample had also been reduced by the algal treatment. Algae utilizes organic pollutants for their steady and rapid growth. Through the results presented here it was shown that good, stable and effective wastewater treatment is achieved, then waste water reuse become increasingly safe. The study concludes that algae (Oscillatoria tenuis) was effective in the reduction of organic pollutants in domestic (sewage) wastewater treatment.

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