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03

IMPACT OF INDUSTRIAL DYES ON ALGAL GROWTH

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Abstract: *The deterioration of water quality because of industrial dye discharges is becoming an emerging environmental problem throughout the world. Dyes are directly discharged into nearby water bodies which in turn affect the aquatic ecosystem and human health. Dyes were degraded by means of biological methods. This study involves the application of two algae (Chlorella vulgaris and Spirulina) and oxidation pond in the decolourisation of dyes. The UV spectromax M3 spectrophotometer was used in reading absorbance values. Methylene blue (MB) (20 mg/l, 50 mg/l and 100mg/l), Eriochrome Black T (EBT) (20mg/l, 50mg/l and 100mg/l) and Melanoidin (100mg/l, 250mg/l and 500mg/l) at different concentrations were used for this study. Decolourisation was recorded in a 10-day experiment. In Spirulina, maximum dye removal was 92.37 percent (20mg/l) in methylene blue dye, 54.24 percent (20 mg/l) in EBT and 45.89percent (100mg/l) in melanoidin while in oxidation pond, the maximum dye removal was 91.84 percent (20mg/l) in methylene blue dye, 93.22percent (20mg/l) in EBT and 46.1 percent (100mg/l) in melanoidin and in Chlorella vulgaris, the maximum dye removal was 91.3 percent (20mg/l) in methylene blue dye, 79.25 percent (20mg/l) in EBT and 57 percent (100mg/l) in melanoidin. The dye removal was dependent on algal concentration and dye concentration. Gel puncture method was used to determine the toxicity of individual dyes on algae (Mixed sample, Scenedesmus and Lake Sample) at varying concentration of dyes.*

Key words: Dyes, Algae (Spirulina, Chlorella vulgaris), Dye Removal Efficiency, Gel Puncture Method.

Introduction

Dyes may be defined as substances that, when applied to a substrate provide color by a process that alters, at least temporarily, any crystal structure of the colored substances (Bafana *et al* 2011). Dyes are classified according to their application and chemical structure, and are composed of a group of atoms known as chromophores, responsible for the dye color. These chromophore-containing centers are based on diverse functional groups, such as azo, anthraquinone, methine, nitro, arilmethane, carbonyl and others. In addition, electrons withdrawing or donating substituents so as to generate or intensify the color of the chromophores are denominated as auxochromes. The most common auxochromes are amine, carboxyl, sulfonate and hydroxyl (Arun Prasad 2010).

Disposal of industrial effluents into natural water bodies has been of much debate in the recent decades. The collective impact of industrial pollutants on the biota of natural water bodies has been studied extensively, but there are not many studies on the impact of individual pollutants on the biota. Present day consumer-oriented industries like textile, leather, printing, food, paper and pulp, etc. use various dyes to improve the perceptual quality of their products. During the manufacturing process, nearly 10-15 percent of dyes are discharged in the effluent. The concentration of dyes in industrial waste water is around 10-500 mg/L (Doble et al. 2005; Mirbolooki et al. 2017).In recent times more than 10,000 synthetic dyes and pigments are commercially available. Moreover, the dye manufacturing industries worldwide produce over 7×10^5 tons of dyes annually (Crini 2006).

Stringent government regulations push the industries to treat the effluent to a high standard before discharging into the natural water bodies (Satapathy et al. 2015). Currently, the effluents are treated by means of physical and chemical methods. Though they are effective, they are either costly, or involve toxic sludge generation which should be treated again. Biological methods are considered for waste water treatment due to their economic and non-polluting nature. Biological methods have the tendency to oxidize harmful organic pollutant present in the waste water into simple compounds like water and carbon dioxide. Some microbes can build up inorganic pollutants, thereby removing them from waste water. The separation of potent species and thereby, degrading the pollutant is one of the biological aspects of waste water treatment. In recent years, many studies are focused on microorganisms (bacteria, fungi) that have the capacity to biodegrade and /or biosorb the dyes present in wastewater.

Micro algae are known to remove the dye by two processes viz. biosorption and bio degradation. The term biosorption includes number of independent metabolisms taking place in the cell wall. Micro algae degrade dye for their nitrogen source, by removing nitrogen, phosphorous and carbon from the waste water. They are unique as they sequester carbon dioxide, one of the major contributors to greenhouse effect. Micro algae such as *Chlorella* and *Spirulina* grown in high-rate algae pond have been shown to be used in rubber effluent, textile waste water and sago starch factory waste water (Phang et al. 2000). The micro algae *Spirulina* is grown in larger amounts all over the world and its annual production is about 2000 tons (Celekli et al. 2009, 2011). The biomass of *Spirulina* can be used to mediate the pollutant binding because it has carboxyl, hydroxyl, sulphate, phosphate and other charged functional groups. It has been used successfully to remove heavy metal, organics and dye (Shahwan et al. 2008; Dotta et al. 2012; Celekli et al. 2018).

Therefore, the aim of this work is to use different microalgae (*Chlorella vulgaris*, oxidation pond and *Spirulina*) in bioremediation of different type of dyes. The dyes chosen were Methylene blue (MB), Eriochrome Black-T (EBT) and Melanoidin. Methylene blue and EBT are the major

constituents of textile industry. Melanoidin is not a constituent of a dyeing industry, but it is a major constituent of agro-based industrial waste waters and is an organic compound which is found to impart colour to the waste water.

Table 01: Bioremediation of Textile Waste water by using Microalgae

#	Micro algae species	Target	Biosorption capacity (mg/g)	Decolourization (percent)	References
1	*Chlorella pyrenoidosa*	Methylene blue	21.3	>90	(Vinayak et al. 2015)
2	*Chlorella sp.*	Methylene blue	-	99.9	(Hwan et al. 2015)
3	*Desmodesmus sp.*	Methylene blue	-	98	(Al-Fawwaz et al. 2016)
4	*Spirulinaplantensis*	RR -120	483.2	99	(Cardoso et al. 2012)
5	*Chlorella vulgaris*	Tectilonyellow 2G	-	63-69	(Acuner et al. 2004)
6	*Chlorella pyrenoidosa*	Textile waste water	20.8	80	(Vinayak et al. 2015)
7	*Chlorella vulgaris*	Textile waste water	-	77	(El-Kassas et al. 2014)
8	*Chlorococcumvitiosum*	Textile waste water	-	13	(Jaya Chitra et al. 2013)
9	*Oscillatoria*	Textile waste water	-	76	(Brahmbhatt et al. 2016)
10	*Chroococcusminutus*	FF sky blue	-	90	(Parikh et al .2005)

Materials and Methods

Algal Culture Maintenance:

Three mixed microalgal consortia were isolated from lakes in IIT Madras.The cultures were maintained in Bold's basal media at room temperature. Enrichment was done in 250 mL Erlenmeyer flasks with 0.1M Sodium bicarbonate as the inorganic carbon source. The light source for the growth of algae was provided by using 40W LED lamps (1000-3000 lux).

Table 02: Constituents of Bold's Basal Media (BBM)

#	Ingredients	Concentration
1	K_2HPO_4	0.075(g/l)
2	KH_2PO_4	0.175(g/l)
3	NaCl	0.025(g/l)
4	$NaNO_3$	0.25(g/l)
5	$CaCl_2 \cdot 2H_2O$	0.025(g/l)
6	$MgSO_4 \cdot 7H_2O$	0.075(g/l)
7	$ZnSO_4 \cdot 7H_2O$	0.00882(g/l)
8	$MnCl_2 \cdot 7H_2O$	0.00144(g/l)
9	MoO_4	0.00071(g/l)
10	$CuSO_4 \cdot 5H_2O$	0.00157(g/l)
11	$CO(NO_3)_26H_2O$	0.00049(g/l)
12	H_3BO_3	0.0115(g/l)
13	EDTA	0.0637(g/l)
14	KOH	0.031(g/l)
15	$FeSO_4 \cdot 7H_2O$	0.005(g/l)
16	H_2SO_4	1(ml/l)

Preparation of Dye*:*

The stock solutions of methylene blue,Eriochrome black T were prepared by dissolving 5mg dye in 5ml of phosphate buffer(pH=7.5).For the whole experiment, the pH was maintained at 7.5 by using phosphate buffer. For further experimentations, dilution was also done byphosphate buffer.

The stock solutions were stored in a conical flask at 4°C. Molecular weight of methylene and EBT are 319.85g/mole and 461.38g/mole.

Figure 01: Molecular Structure of methylene blue

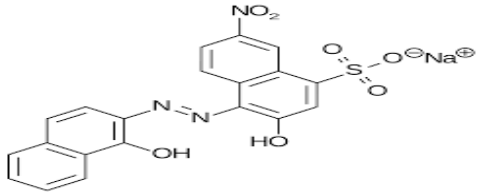
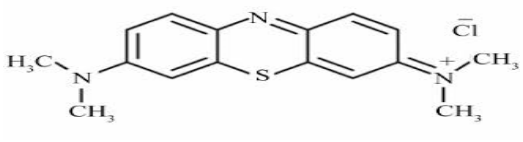
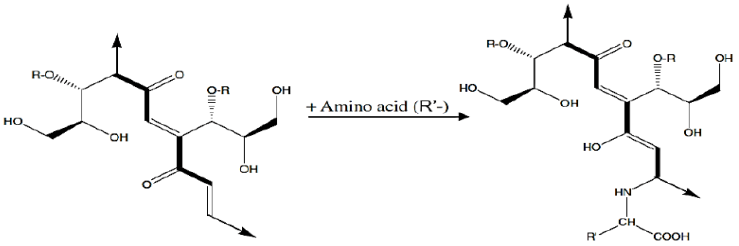


Figure 02: Molecular Structure of EBT



The stock solution of melanoidin was prepared by dissolving 1.0 M of glucose, 1.0 M of glycine and 0.5 M of sodium carbonate in 100ml of phosphate buffer. The solution was kept in the oven for 7 hours at a temperature of 95°C. The stock solution was then stored at 4°C. 1mL of the stock solution was treated with 5mL of acetone to induce phase separation. The aqueous phase was discarded and the concentrate was dried and weighed to obtain the yield. The stock solution was then diluted to get 100mg/l, 250mg/l and 500mg/l solution.

Figure 03: Carbohydrates based Melanoidin Molecular Structure

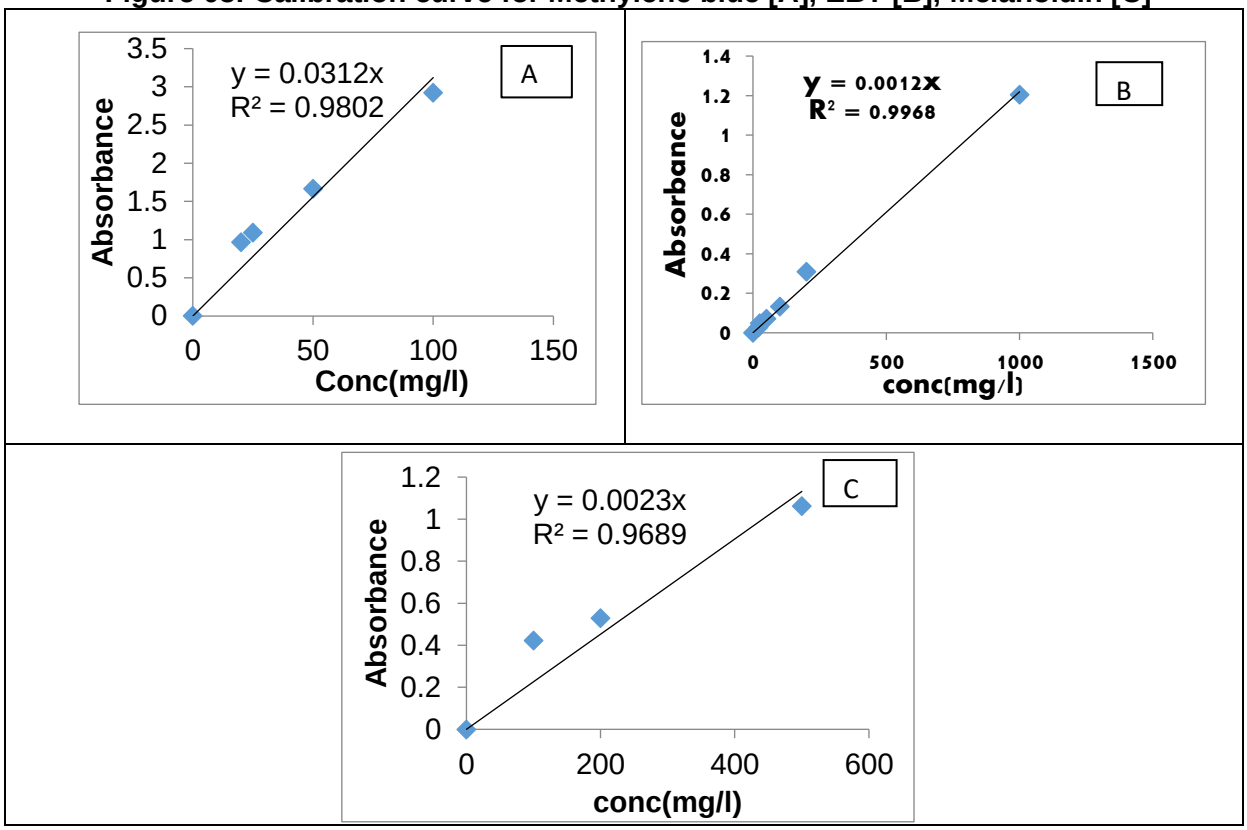


Methylene blue: Color index; C.I (52015), Maximum wavelength; λmax 668 nm, Molecular weight, MW, 319.9 g.mol-1 and Molecular formula ($C_{16}H_{18}N_3SCl$). EBT: Color index; C.I (14645), Maximum wavelength; λmax 600 nm, Molecular weight, MW, 461.38 g.mol-1 and Molecular formula ($C_{20}H_{12}N_3O_7SNa$). Melanoidin: Maximum wavelength; λmax 800nm, Molecular weight, MW, 5 - 40 kDa and Molecular formula ($C_{17\text{-}18}H_{26\text{-}27}O_{10}N$).

Adequate number of samples were collected and analysed in UV spectromax M3 spectrophotometer. The peak wavelength was found out for each dye and it was found to be 600nm, 668nm and 800nm for EBT, methylene blue and melanoidin respectively. Standards of methylene blue, EBT, melanoidin ranging from 0 to 500 mg/l were calibrated and the same was used for the series of the experiment.

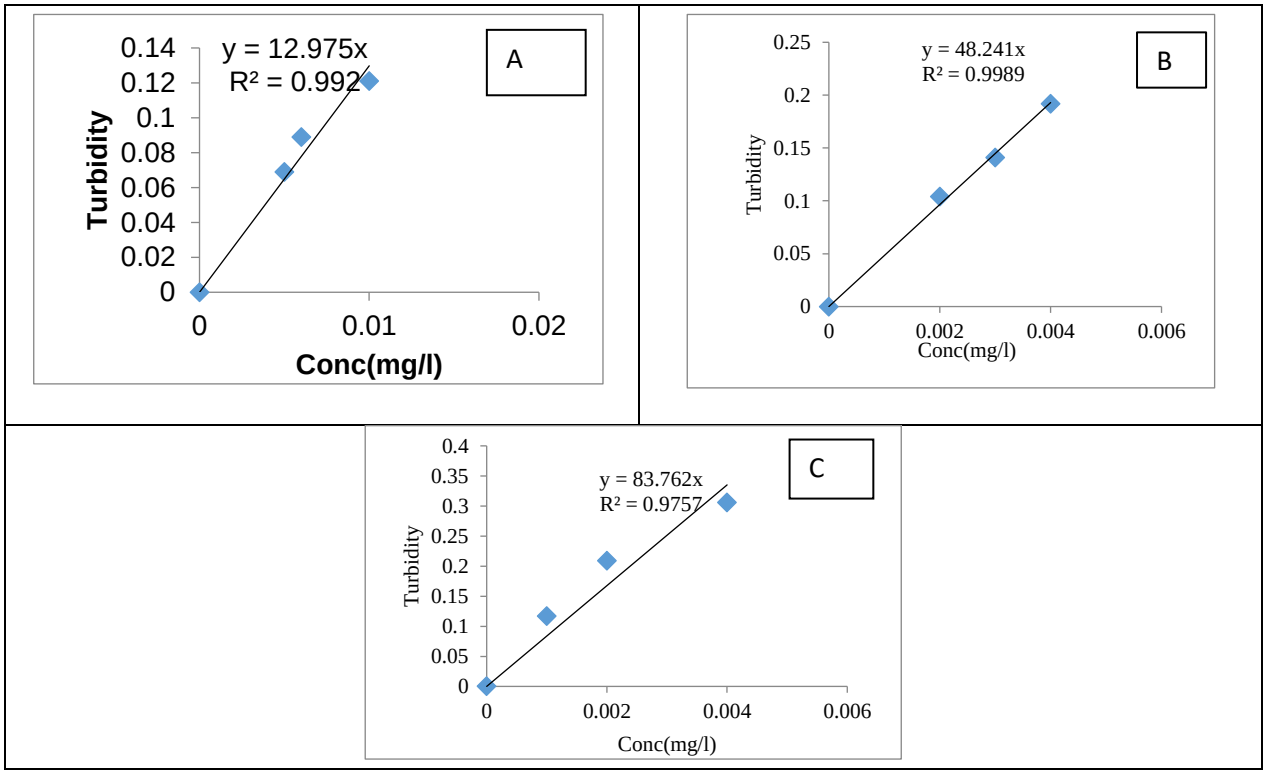
Figure 04: Spectrophotometer

Figure 05: Calibration curve for Methylene blue [A], EBT [B], Melanoidin [C]



The calibration curve for methylene blue, EBT, melanoidin are shown in Figure 05 [A], [B] and [C].Calibration curve for algae *Spirulina*, oxidation pond, *Chlorella vulgaris* are shown in Figure 06(A-C) at 680nm.

Figure 06: Calibration curve for *Spirulina* [A], Oxidation pond [B], *Chlorella vulgaris* [C]



Spread Plating for Algae

Agar acts as a medium for the attachment of algae. 3percent of 100 mL agar solution and 100 mL of 2 X BBM solutions were prepared and autoclaved at 121 °C for 15 minutes at 1 atm. Both the solutions were mixed together when the temperature was just above 60 °C. Then, 8g of $NaHCO_3$ (sterilised by UV or a 0.22 µm filter) and 0.04ml of vitamin solution (filter sterilized) were added. The agar solution was poured on the petri plate when it is hot and left for few minutes until it undergoes solidification. 0.1 ml of algae inoculum was transferred onto the center of the agar plate. An L-rod (flame sterilized by ethanol) was used to spread the algal inoculum over the agar evenly. Spread plate technique was the method used for isolation of microorganisms from the mixed culture. It can also be used as a qualitative way of assessing the toxicity of compounds through gel-puncture method. A well was made into the agar with the back portion of a 1mL micropipette tip after spreading the microbial culture. The sample for which toxicity has to be tested was then loaded into the well. In order to find out inhibition of dye (methylene blue, EBT and melanoidin) on algae, small wells were created on the agar plate .To that wells, 0.3 ml of dye at different concentration were added. The plates were incubated for 15 days at room temperature under 16 h/8 h light/dark period at 1500 lux using LED lamps

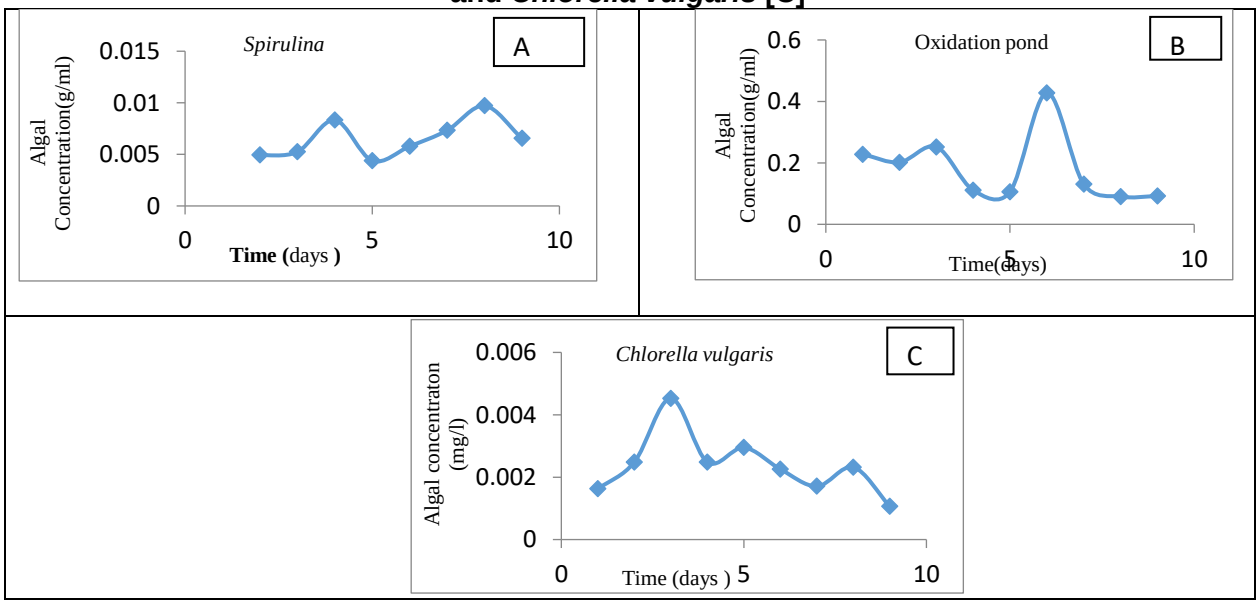
Figure 07 : Toxicity Analysis by Spread Plate Gel Puncture Method

Results and Discussion

Effect of Time on Algal Concentration:

The growth of algae increased gradually and reached the peak on day 3 . This is because the algae has grown by consuming the nutrients present in the media . After the day 3 , the growth of algae started decreasing due to the depletion of nutrients in the media .

Figure 08: Control for determination of algal growth: *Spirulina* [A], Oxidation pond [B] and *Chlorella vulgaris* [C]



Removal of Dyes by Algae:

Growth of microalgae in the media with methylene blue resulted in removal of methylene blue from the media. The removal might have occurred either by biodegradation or by biosorption or both. Removal was high with decreasing concentration of methylene blue. Initially, the removal rate was high, which can be attributed to biosorption. Then, a stable phase is seen after which there is again an increase in the rate of colour reduction which could be attributed to biodegradation.

Methylene Blue removal by Algae

The percentage of dye removed was calculated using the following equation:

Removal (percent) = $(C_i - C_f / C_i)*100$

where

C_i is the initial concentration of dye (mg/l) and

C_f is the concentration of dye after a period of time (mg/l).

Decolorization was determined based on an absorbance calibration curve of known standard solutions.

The methylene blue dye removal percentage ranged between 92.37 and 55.6 percent , between 91.84 and 47.87 percent and between 91.3 and 47.8 percent for *Spirulina*, Oxidation pond and *Chlorellavulgaris* respectively.

Figure 09: Methylene blue removal by algae: *Spirulina* [A], Oxidation pond [B], *Chlorella vulgaris* [C]

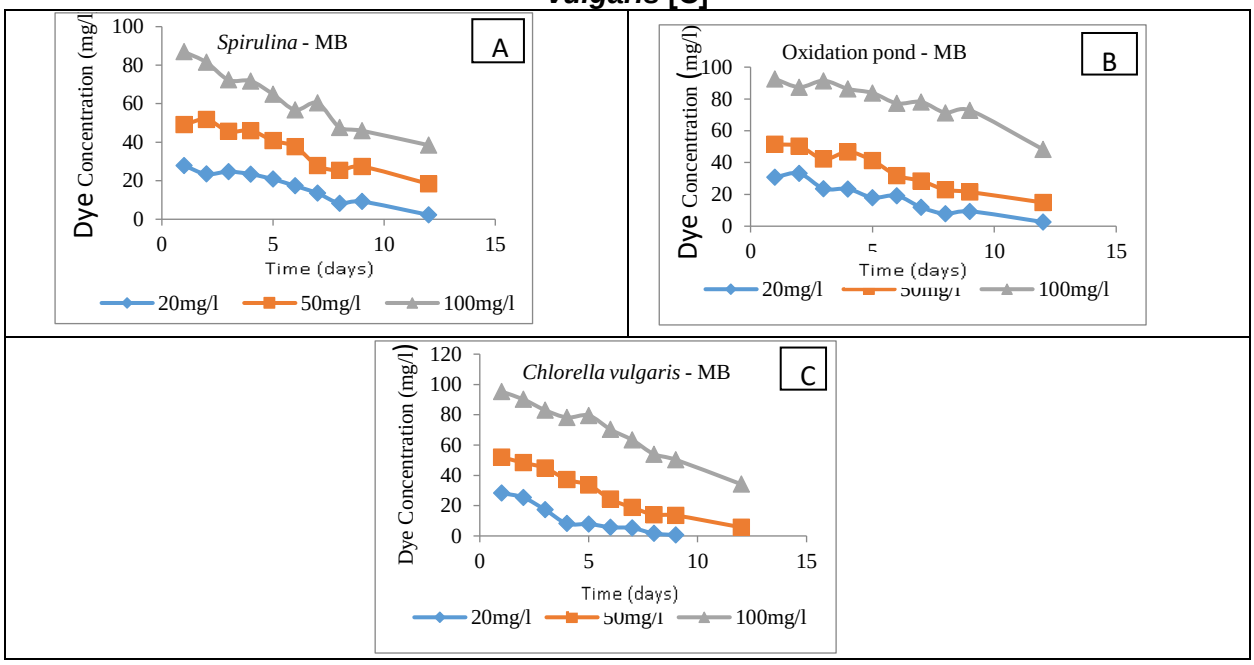


Table 03: Methylene Blue Removal Efficiency

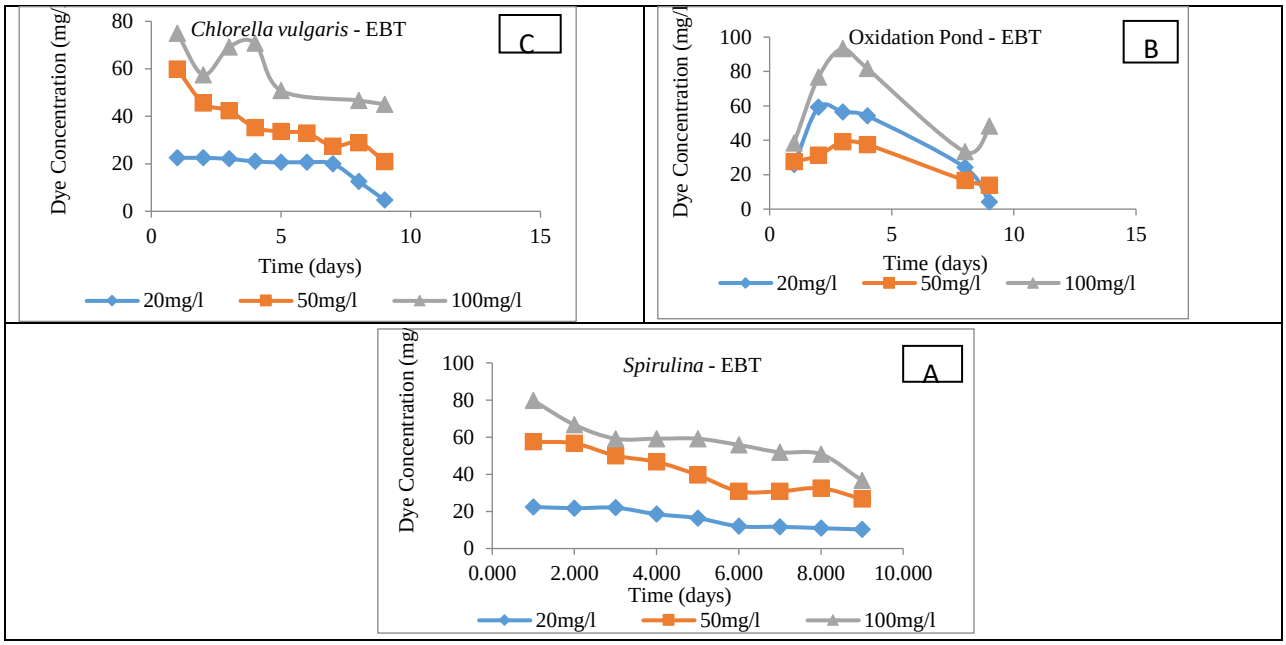
#	Algal species	Dye Removal Efficiency (Percent)		
		20mg/l	50mg/l	100mg/l
1	*Spirulina*	92.37	62	55.6
2	Oxidation pond	91.84	62.66	47.87
3	*Chlorella vulgaris*	91.3	71.11	47.8

EBT removal by Algae:

In case of EBT, initially the removal was high which may be due to biosorption .The removal was stable afterwards because the algae growth was restricted by the dye concentration and also due to the lack of media. Afterwards, the algae started adapting to the dye environment and started

growing thereby increasing the dye removal. The removal was very high at lower concentration (20mg/l) compared to higher concentrations (50mg/l, 100mg/l).

Figure 10: EBT removal by algae: *Spirulina* [A], Oxidation pond [B], *Chlorella vulgaris* [C]



The EBT blue dye removal percentage ranged between 54.24 and 36.7 percent, between 93.22 and 52.6 percent and between 79.25 and 40 percent for *Spirulina*, Oxidation pond and *Chlorella vulgaris* respectively.

Table 04: EBT dye Removal Efficiency

#	Algal species	Dye removal efficiency (Percent)		
		20mg/l	50mg/l	100mg/l
1	*Spirulina*	54.24	53.21	36.7
2	Oxidation pond	93.22	58.97	52.6
3	*Chlorella vulgaris*	79.25	64.4	40

Melanoidin Removal by Algae:

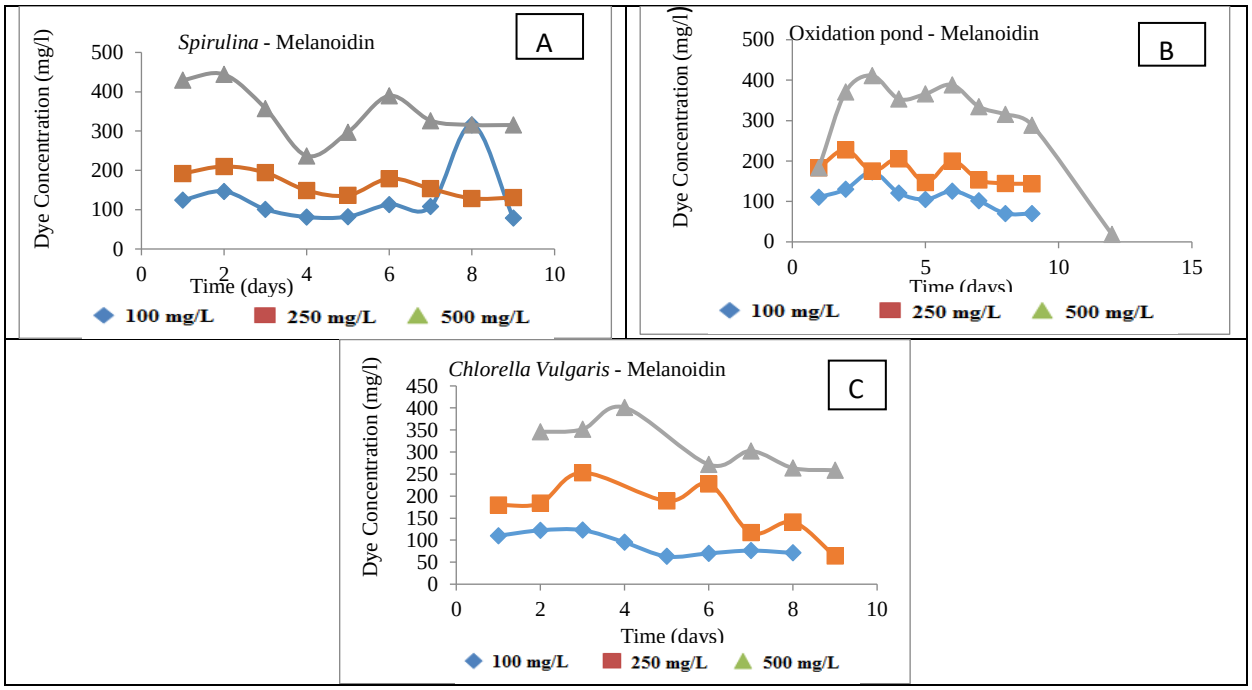
In melanoidin, the removal efficiency was very low even for the lower concentration of the dye. 100mg/l, 250mg/l and 500mg/l concentrations of dye affect (decrease) the growth of algae, there by bio sorption will not be significant. In this case, only less removal will take place. The dye removal efficiency for 100mg/l of melanoidin is around 40-50percent.

The melanoidin dye removal percentage ranged between 45.89 and 26.7 percent, between 46.1 and 22.1 percent and between 57 and 25.4 percent for *Spirulina*, Oxidation pond and *Chlorella vulgaris* respectively.

Table 05: Melanoidin Dye Removal Efficiency

#	Algal species	Dye removal efficiency (Percent)		
		100mg/l	250mg/l	500mg/l
1	*Spirulina*	45.89	32.06	26.7
2	Oxidation pond	46.1	21.6	22.1
3	*Chlorella vulgaris*	57	35	25.4

Figure 11: Melanoidin removal by algae: *Spirulina* [A], Oxidation pond [B], *Chlorella vulgaris* [C]

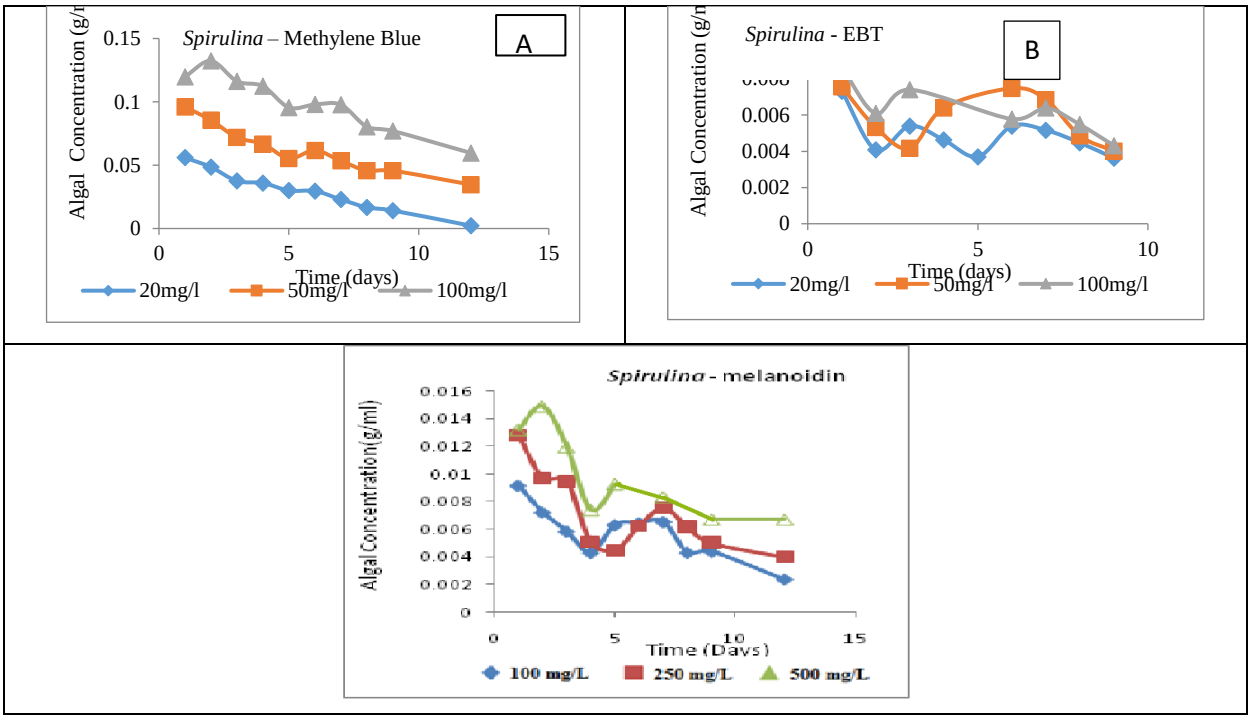


Effect of Dyes on Algal Growth:

Initially, there was a decline in biomass till day 4. This might be due to the toxic effect of dye that has hampered the growth of algae. Then from day 5, the growth stabilized with a slight increase in slope. It can be inferred that the algae might have got acclimatized to the conditions by entering into lag phase of growth in the substrate containing the dye. But, after day 7, since no extra media nutrient components were added, there was a decrease in algal growth due to nutrient limitation.

Effect of dye on *Spirulina*

Figure 12: Effect of dye on Spirulina: Methylene blue [A], EBT [B], Melanoidin [C]



Effect of dye on growth of Oxidation pond:

Figure 13: Effect of dye on growth of Oxidation Pond: Methylene Blue [A], EBT [B], Melanoidin [C]

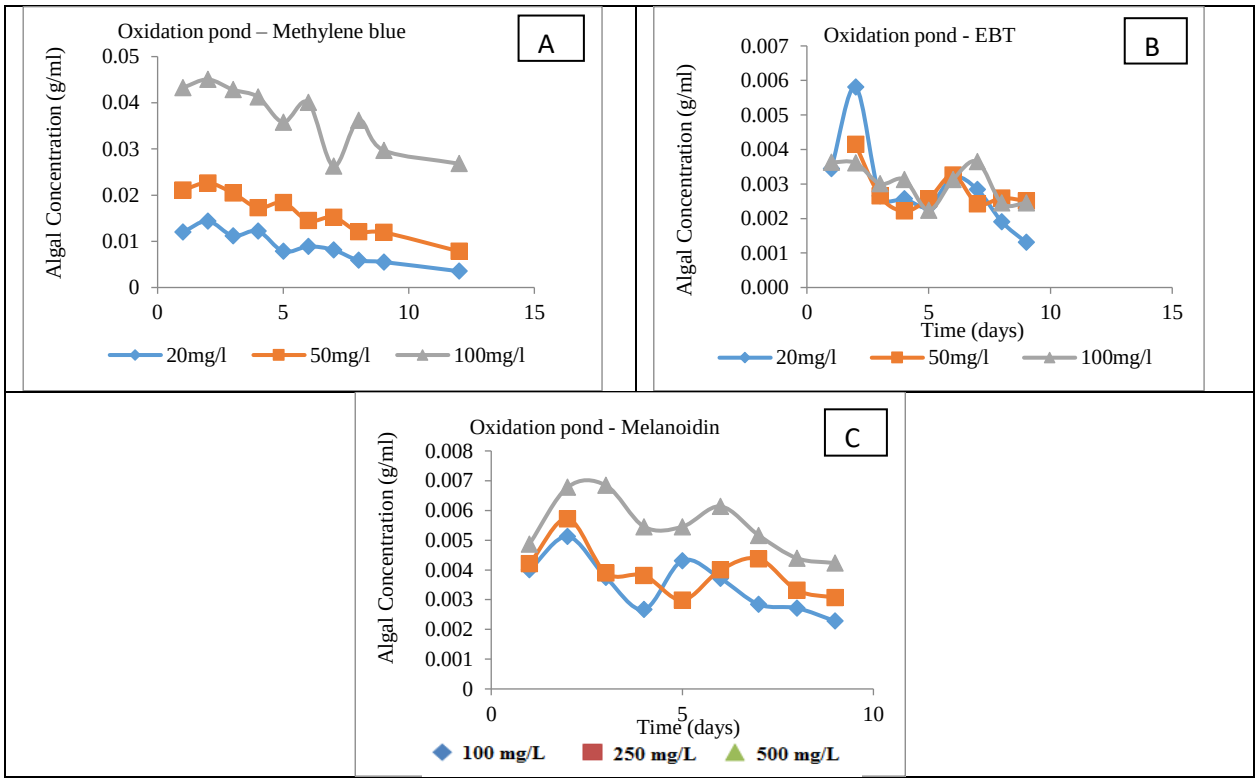
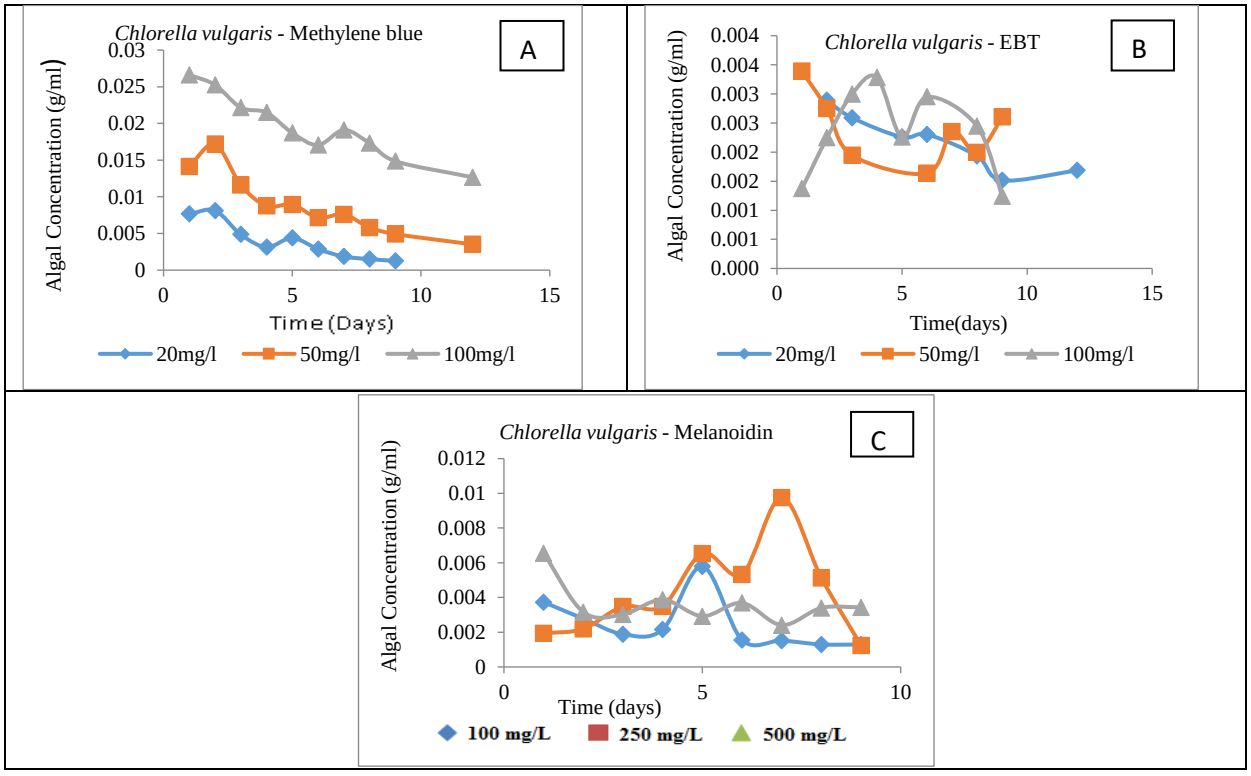


Figure 14: Effect of dye on *Chlorella vulgaris*: Methylene Blue [A], EBT [B], Melanoidin [C]



Effect of dye on Chlorella Vulgaris:

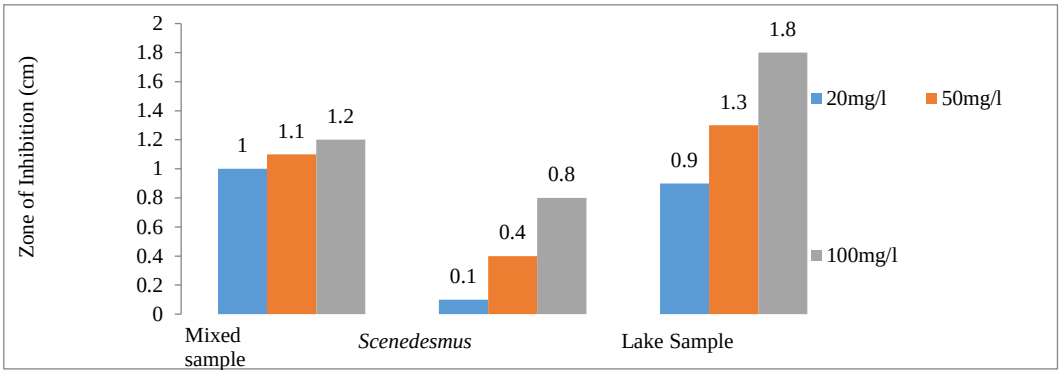
Spread plating – Zone of inhibition

The Zone of inhibition method helps in finding the effect of dye inhibition on attached growth. The Zone of inhibition of Methylene blue and melanoidin are shown in Figures 15 and 16 respectively.

1. Mixed sample
2. Scenedesmus
3. Lake sample

Methylene blue Zone of inhibition

Figure 15: Methylene blue Zone of inhibition

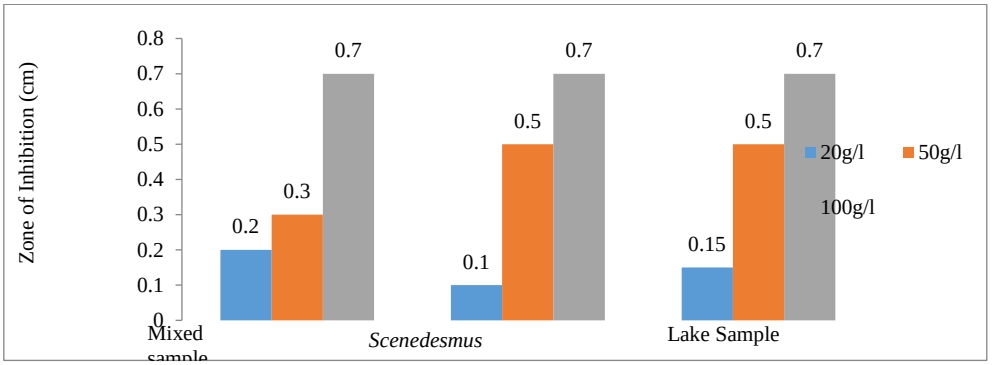


EBT Zone of inhibition

Eriochrome black T has no Zone of inhibition in three samples

Melanoidin Zone of inhibition

Figure 16: Melanoidin Zone of inhibition



From Spread plating , it was found that Eriochrome black T has no Zone of inhibition i.e., it has very low toxicity or no toxicity towards micro algae in attached growth .In case of methylene blue and melanoidin , the toxicity effect is more in 100mg/l and 100g/l respectively . The toxicity effect decreased with decrease in concentration of dye.

Conclusion

A series of experiments were conducted to study the effect of dye on algae and it was inferred that dye removal depends on the algal concentration and dye concentration. The dye removal efficiency is higher at the lower concentration of dye. Increase in concentration of dye decreases the dye removal efficiency. Compared with Methylene blue and Melanoidin, EBT has very low toxicity towards micro algae. Initially, there was a decline in biomass till day 4. This might be due to the toxic effect of dye that has affected (decreased) the growth of algae. Then from day 5, the growth remained stable with a slight increase in slope. It can be inferred that the algae might have got adapted to the conditions by entering into lag phase of growth in the substrate containing the

dye. But, after day 7, since no extra media nutrient components were added, there was a decrease in algal growth due to nutrient limit. The dye effluent from industries at a higher concentration is toxic to microalgae. The concentration of dye effluent from the industries should not be greater than 1mg/l.

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