



## SPIROGYRA GRACILIS - A POTENT ALGAE FOR THE REMEDIATION OF TEXTILE DYEING EFFLUENT

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The potential of *Spirogyra gracilis*, a fresh water green algae was investigated as a biosorbent for the remediation of textile dyeing effluent. The effects of operational parameters (biomass concentration, incubation time, pH and temperature) on the decolourization of the effluent were examined. The results obtained revealed that *S. gracilis* could decolorize the effluent up to 74% at pH 4, 30°C and 1 g/L (w/v) biomass concentration after 96 hrs of incubation. Analytical studies like UV-visible spectroscopy, FT-IR and GC-MS analyses were performed. The ultraviolet spectra of the effluent before and after decolourization suggested the occurrence of biodegradation in addition to biosorption of dyes. The metabolites were identified by GC-MS and the functional group analysis was performed by FT-IR spectroscopy. Adsorption of the dye on the cell surface was further confirmed by scanning electron micrograph of the biomass before and after treatment with *S. gracilis*. Phytotoxicity study using *Vigna radiata* was performed to evaluate the toxicity of metabolites produced after decolourisation of textile effluent.

**Keywords:** *Spirogyra gracilis*, Textile dyeing effluent, Decolourisation, Phytotoxicity

Increasing environmental pollution caused by toxic dyes is a matter of great concern to the mankind. Effluent released from dyeing units are highly objectionable when discharged into open water without any proper treatment. The presence of dyes in effluents is not only highly visible, but also cause environmental and health problems to human beings and aquatic animals (Eftekhari et al., 2010). Dyes undergo alteration in chemical structure and leads to the formation of new xenobiotic compounds which have a serious impact on the environment. The presence of colouring material in aquatic bodies also reduces the penetration of light and photosynthetic activity (Benguella and Benaissa, 2002 and Mittal et al., 2007). Exposure of human beings to untreated effluent may cause skin irritation, allergy, cancer, nausea, vomiting, paralysis, damages the liver, kidney, reproductive system, brain and central nervous system severely (Shen et al., 2009). The existence of dyes in the discharged effluents induces harmful effects on the liver, gills, kidneys, intestines and gonads of aquatic organisms (Daneshvar et al., 2007).

Increasing public awareness about the toxic nature of these dyes has encouraged the development of innovative treatment methods for converting these organic dyes to inoffensive compounds by many environmental organizations (Stylidi et al., 2003). Recently the government has also established environmental limitations with regard to the quality of colored effluents and also insisted to have compulsory dye houses to

decolorize the effluent before discharge (Shen et al., 2009). Therefore, the treatment of effluent containing dyes has been a challenging problem among environmental technologies and it has always been necessary to find efficient dye removal methods (Khataee and Zarei, 2011 and Soon and Hameed, 2011).

The removal of dyes from textile effluents has been practiced for several decades through conventional physico-chemical methods such as, ozonation, photooxidation, electro-coagulation, adsorption, activated carbon, froth flotation, reverse osmosis, ion exchange, flocculation, chemical precipitation, membrane separation, evaporation and ionic exchange resin which are usually expensive and sometimes not effective (Chowdhury and Viraraghavan, 2009, Forgacs et al., 2004, Rajkumar et al., 2007, He et al., 2008, Radha et al., 2005, Daneshvar et al., 2004a and b, Gupta et al., 2004a, Jain et al., 2003, Mittal et al., 2005 and Robinson et al., 2001). This physico-chemical treatment method generates large amounts of sludge, which leads to secondary pollution and formation of hazardous by-products (Khehra et al., 2005). Hence, biological methods using bacteria, fungi, algae and plant seems to be an alternative, low-cost, reliable, eco-friendly and cost-effective technique for effluent treatment in which the discharge of sludge will be minimised (Srinivasan and Viraraghavan, 2010, Zhao et al., 2006, Pandey et al., 2007, Kaushik and Malik, 2009, Khataee et al., 2010 and Vafaei et

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al., 2012).

In the present study, algae have been selected as the candidate biosorbent since it has high binding affinity to the textile dyes (Tsai and Chen, 2010). Numerous studies have been carried with *Spirogyra* sp. which can effectively remove the dyes from aqueous solutions (Bishnoi et al., 2007, Venkata Mohan et al., 2007, Lee and Chang, 2011, Rajfur et al., 2012 and Mohan et al., 2008).

The objective of the present study is to investigate the decolourisation of textile dyeing effluent using dried biomass of *Spirogyra gracilis*. Optimum biosorption conditions were determined as a function of biomass concentration, contact time, pH and temperature. Degradation studies were also carried out and the intermediates formed were extracted and analyzed using UV-visible, Gas chromatography - Mass spectrometry (GC-MS) and Fourier transform infrared (FT-IR) spectroscopy. Scanning electron microscopic (SEM) study was carried to understand the adsorption of dyes to the surface algal biomass during decolourisation process. Hence the toxicity of the metabolites formed upon biosorption of textile dyeing effluent by *Spirogyra gracilis* was assessed using *Vigna radiata*.

## MATERIAL AND METHODS

**Collection and identification of algae:** The algal species was acquired from the natural pond near the textile effluent discharging site. According to its morphology and microscopic observations, it was identified as *S. gracilis* belonging to the family Zygnemaceae. The species was authenticated by Botanical survey of India, Coimbatore, Tamilnadu, India (Authentication number -BSI/SRC/5/23/2014-1Tech./467). *S. gracilis* is a fresh water filamentous, non-branched green algae, found in the scum of ponds and slow-moving water. The algal biomass was washed with distilled water and dried at 80°C for 20h and ground in a mortar and pestle before use, to obtain larger surface area.

**Textile waste water:** The textile waste water used in this study was collected from a textile industry located in the Veppampalayam, Karur district, Tamilnadu, India, and the physico-chemical parameters were analysed (Alaguprathana and Poonkothai, 2015).

**Batch decolourisation operation:** Decolourization study was

performed using 1000 ml of the effluent sample (50%) inoculated with 1 g of algal biomass at room temperature (30±2°C) under batch contact conditions. To analyze the effect of different environmental factors on the efficiency of colour removal, the batch decolourization experiments were carried out at different biomass concentrations (1-5g/L), incubation periods (24-120h), pH values (3-10) and temperatures (25-45°C). Samples (5ml) were withdrawn after 96h of incubation and centrifuged at 10,000 rpm for 5 min and the supernatant was scanned at 585 nm, to study the decolourisation efficiency. Control was set without addition of *S. gracilis* and the percentage removal of colour from the effluent was calculated as follows:

Decolourisation (%) =  $(A-B)/A \times 100$ ; where A is the initial absorbance and where B is the final absorbance. Each experiment was conducted in triplicates.

At optimised conditions, the known amount of the algal biomass was inoculated and the percentage decolourisation was assessed.

**Determination of the degraded products of effluent:** To determine the intermediate products of textile dyeing effluent, the algal cells were resuspended in the effluent (50%) at a concentration of 1 g<sup>-1</sup> (w/v) and incubated at 30°C. After the incubation period, 50 ml of the suspension was collected, centrifuged at 10,000 rpm for 10 min to remove the cells and the supernatant was used for spectral analyses.

The supernatants of control and experimental samples were evaluated for decolourisation by UV-vis spectral analysis which was carried out using a Hitachi UV-vis spectrophotometer with the wavelength ranging from 200 to 800 nm (Khalaf, 2008). The ethyl acetate extract was evaporated using a rotary evaporator for control and experimental samples and then powdered. The powder was mixed with pure KBr at a ratio of 5:95, fixed in the sample holder, and FT-IR spectroscopy analysis (Perkin Elmer PE 1600) was carried out in the mid-IR region of 400-4000 cm<sup>-1</sup> at a scan speed of 16 cm/s (Karegoudar et al., 2009). The extracts extracted from control and textile dyeing effluent treated with *S. gracilis* were dissolved in methanol was used for GC-MS analysis (Mane et al., 2008). Analyses were performed using helium as a carrier gas with a flow rate of 1.1 ml/min. The temperature of the injector was maintained at 300°C and oven temperature was kept constant at 100°C for 2 min and was then increased to 250°C at a rate

of 10°C/min and to 280°C at 30°C/min. Analyses were carried out for about 20 min, and the compounds were analyzed using mass spectra in the National Institute of Standards and Technology (NIST) library (Kalpana et al., 2012).

**Scanning electron microscopic analysis:** Dried biomass of *S. gracilis* before and after biosorption of textile dyeing effluent were mounted on stubs coated with gold-palladium of 100-150 Å thickness and transferred to the sample chamber of SEM (Model JSM-6100) operated at 10 kV to confirm the surface adsorption of the dye onto the biomass (Neerven et al., 1990).

**Phytotoxicity studies:** The phytotoxicity studies were carried out at room temperature using *V. radiata* (10 seeds) followed by watering separately with 10ml of the effluent sample and 10 ml of the treated effluent daily. Control sets were also maintained by using tap water. On 7<sup>th</sup> day, the percentage germination, plumule and radical of *V. radiata* seeds were assessed.

## RESULTS AND DISCUSSION

**Effect of physicochemical conditions on decolourisation:** The effect of several physicochemical conditions (biomass concentration, incubation time, pH and temperature) on the decolourisation performance of textile dyeing effluent using *S. gracilis* was depicted in Fig.-1.

The biosorption efficiency of dyeing effluent at different biosorbent concentration (1-5g/L) revealed that increase in dose (Fig.-1a) gradually does not exhibit maximum colour removal. It was noticed that at initial concentration numerous unoccupied active sites are present in the biosorbent to which the dyes get early adsorbed. This, in turn, creates an aggregation of biosorbent which decreases its total surface area and limits the transportation of dyes present in the effluent to the active sites by increasing the diffusion path length (Rabia et al., 2011; Gode and Pahlivan 2005). Incubation time is a key parameter for decolourisation which reflects the kinetics of an adsorbate (Liu et al., 2013).

Fig.-1b illustrates the effect of incubation time on the decolourisation ability of the effluent. The decolourisation capacity increased linearly with increasing the time period of incubation from 24 - 96 h and a decrease in the adsorption capacity were noted at 120h (37%), specifying that the

decolourisation was significantly affected by the contact time. The rapid biosorption during the initial stage might be due to the effect of easy availability of dye molecules to bind with the active surface sites present in the biosorbent and when they are completely occupied by the dye molecules an equilibrium stage occurs and percentage decolourisation would be constant (Gupta and Suhas, 2009).

pH is an important parameter for biosorption which affects not only the removal capacity, but also the colour and solubility of dyeing effluent. The effect of different pH (3-11) on the sorption of textile dyeing effluent by *S. gracilis* showed pH 4 (79.7%) to be optimum for biosorption Fig.-1c. Extreme alkaline pH was found to reduce the decolourisation rate whereas acidic conditions favour decolourisation process. Textile effluents have complex organic compounds with different ionization potentials at various pH and their interaction with microbial biomass depends on the chemistry of a particular dye and the biosorbents (Waranusantigul et al., 2003). The decolourisation of effluent at optimum pH depends on the surface charge, degree of ionization and the dissociation of functional groups on the active sites of the biosorbent (Errais, 2011). Higher decolourisation obtained at lower pH values may be due to the electrostatic attractions between the negatively charged dye anions present in the effluent and positively charged cell surface. Hydrogen ion also acts as a bridging ligand between the algal cell wall and the dye molecules in effluent and the reduction in the adsorption capacity of dyes on algae with increasing pH can be attributed to change in surface characteristics and charge (Tien, 2002 and Tani et al., 2002). The results are also consistent with prior exploration in which the maximum decolourisation of multi component textile effluent was observed at pH 4 using *Spirogyra* species (Khalaf, 2008), *Enteromorpha prolifera* (Ozer et al., 2005) and *Sargassum cymosum* (Costa et al., 2010).

The capacity of *S. gracilis* for the biosorption of textile dyeing effluent was investigated at different temperatures (25-45°C). The results showed 75% decolourisation at optimum temperature (30°C) and a drastic decrease was observed at higher temperature Fig.-1d. The results mainly indicated that textile dyeing effluent was thermally stable under optimal temperature with *S. gracilis* and thermal deactivation occurred at higher temperatures which may reduce the decolourisation efficiency (Fan et al., 2013). Decreased decolourisation activity

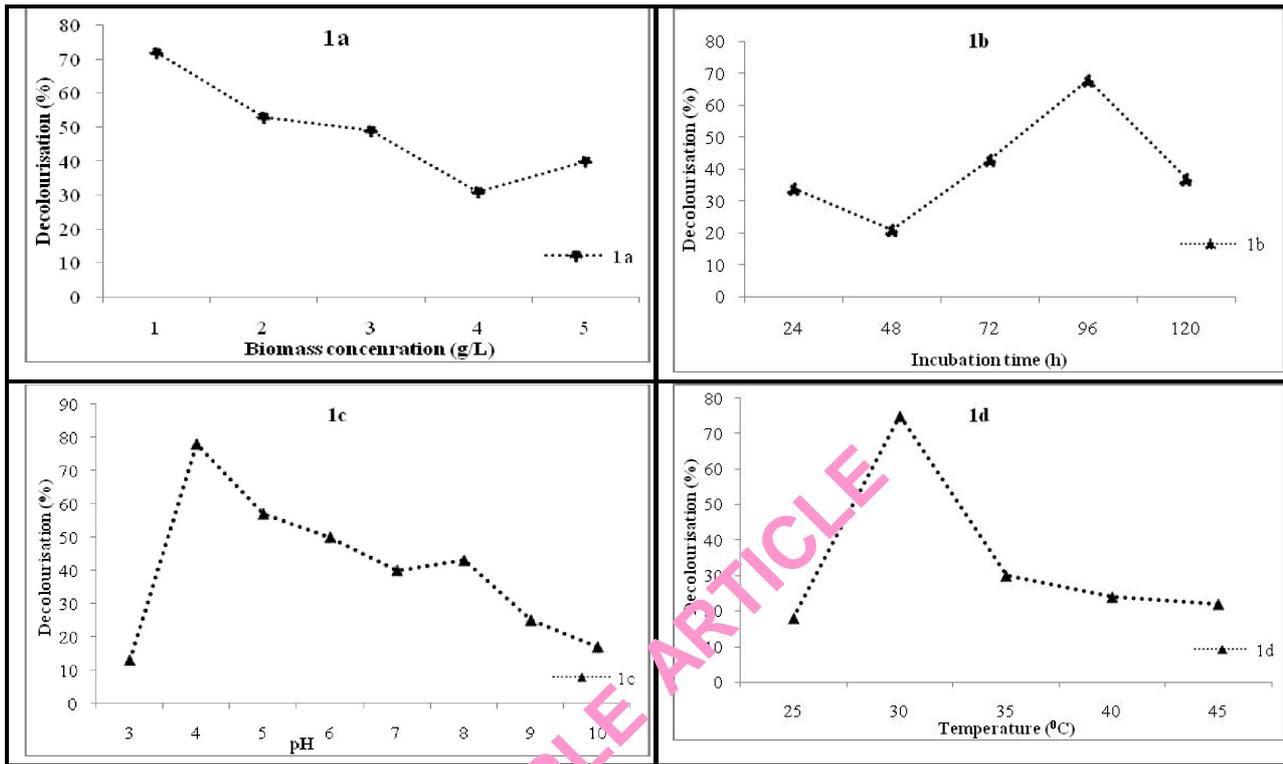


Fig:-1. The effect of several physicochemical conditions (biomass concentration (1a), incubation time (1b), pH (1c) and temperature (1d), on the decolourisation

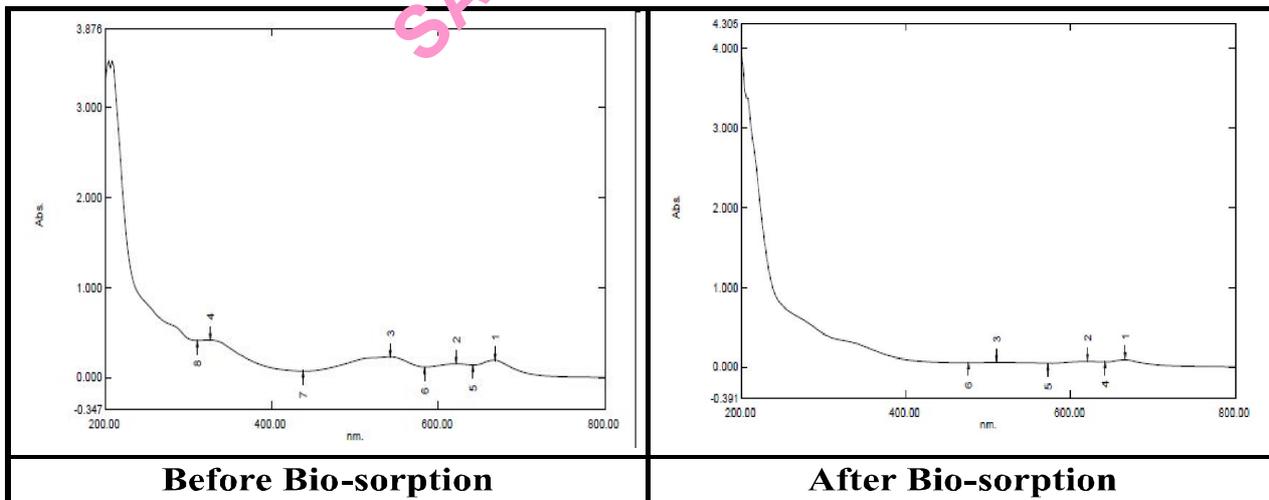


Fig:-2. UV-visible spectrum analysis of textile dyeing effluent before and after decolourization using *Spirogyra gracilis*.

beyond 30°C may alter the surface activity of biomass, cell viability and properties of the biosorbent may be denatured and the adsorptive forces between the dyes present in effluent and the active sites on cell surface may be reduced (Khalaf,

2008, Saratale et al., 2009, Chellababu et al., 2008 and Argun et al., 2008). Yu et al., (2001) reported that the temperature changes lead to a sudden alteration in the activation energy and change the adsorption capacity of the biosorbents.

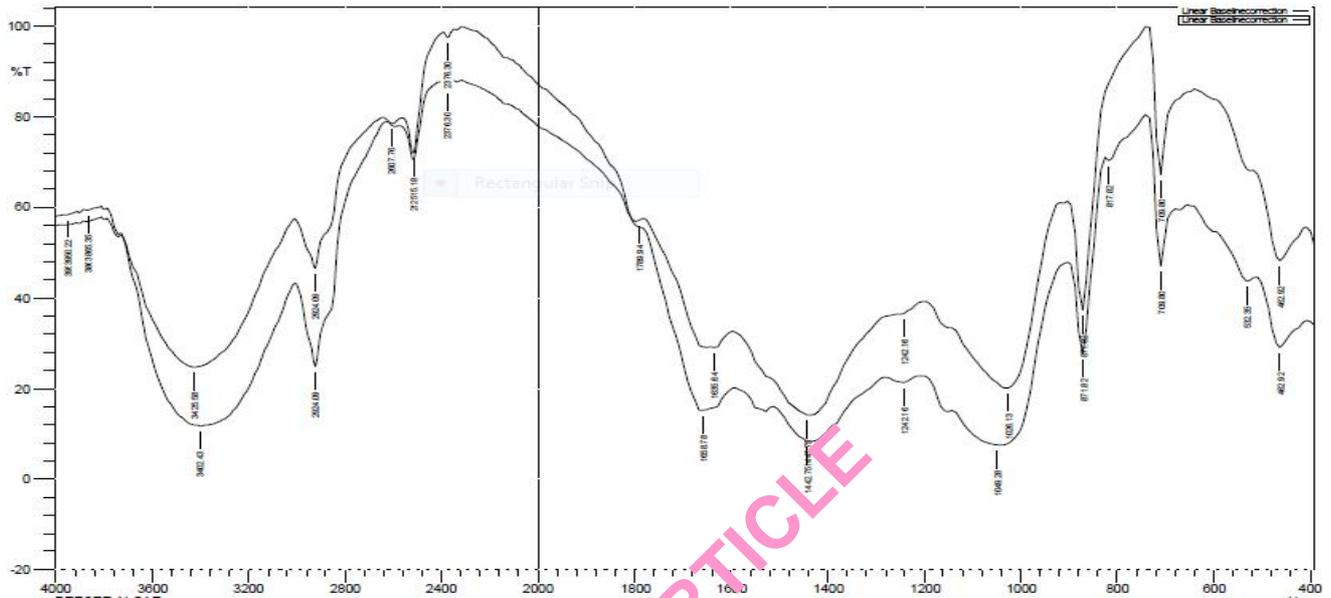


Fig:-3. FT-IR spectra of *Spirogyra gracilis*, before (a) and after (b) treated with effluent

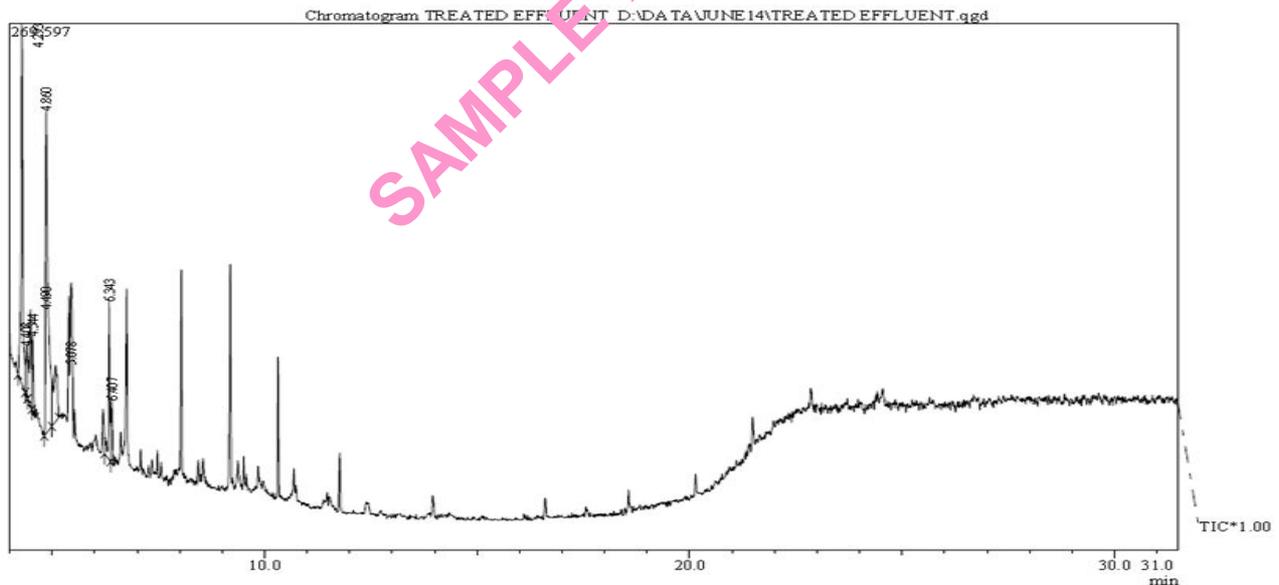


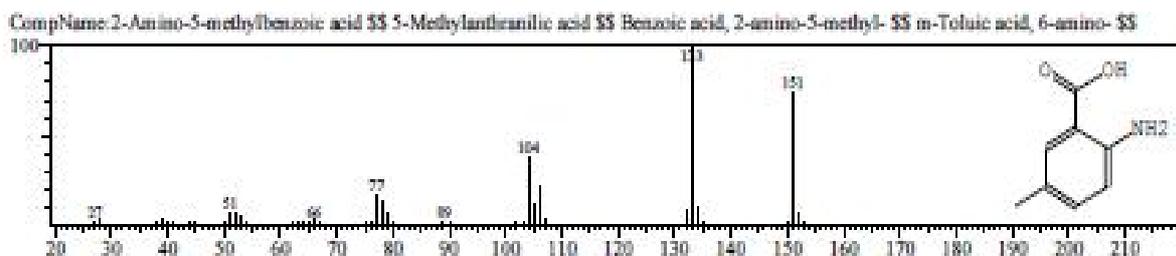
Fig:-4a. Gas chromatogram of degraded products of textile dyeing effluent treated with *Spirogyra gracilis*.

**Analysis of decolourised products:** UV-vis spectral analysis has been used to confirm the decolourisation process of effluent that was due to biodegradation or biosorption (Aksu, 2003). UV-visible spectral analysis of textile dyeing effluent and its degraded metabolites were performed in the absorbance of 200 nm to 800 nm Fig.-2. The treated effluent showed that the

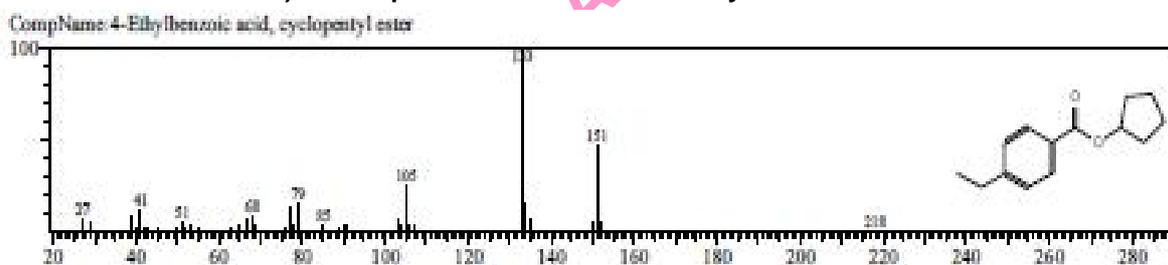
decreased in a peak at 666, 620 and 570 nm. This decreased of absorbance indicated that the removal of dyes from the effluent. The major visible light absorbance peak at 326 nm would completely disappear or the dyes may adsorb to surfaces by converting them into other products, which showed that the decolourisation of effluent was mediated by biodegradation.



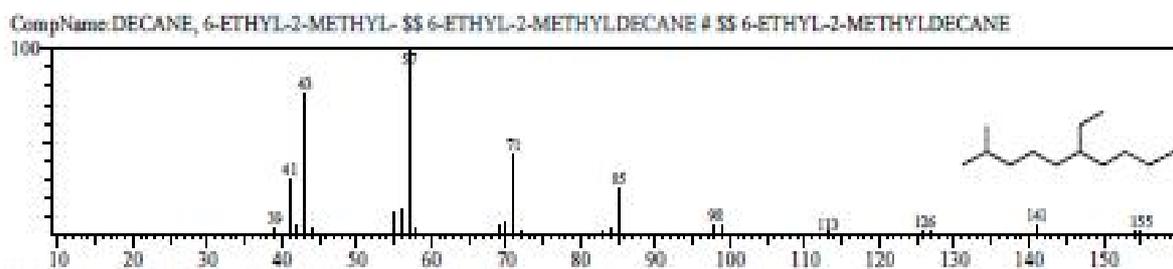
a) Mass spectrum of 4-hydroxy-4-methyl pentanone



b) Mass spectrum of 2-amino-5-methylbenzoic acid



c) Mass spectrum of 4-ethylbenzoic acid



d) Mass spectrum of 6-ethyl 2-methyldecane

**Fig.-4b. Mass spectrum of degraded products of textile dyeing effluent treated with *Spirogyra gracilis***

As a result, the presence of dyes in the effluent was degraded into another compound. Results specified that the dye removal from the effluent may be attributed to the sorption when. According to Chen et al., (2003) the degradation of dye can be observed either by the decrease in the major peak or formation

of new peaks. Hence decolourisation and biosorption of the textile effluent by *S. gracilis* occur simultaneously.

The significant changes between the FT-IR spectrum of textile dyeing effluent and its degraded metabolites postulate the effective biodegradation Fig.-3. The FT-IR spectra of both effluent

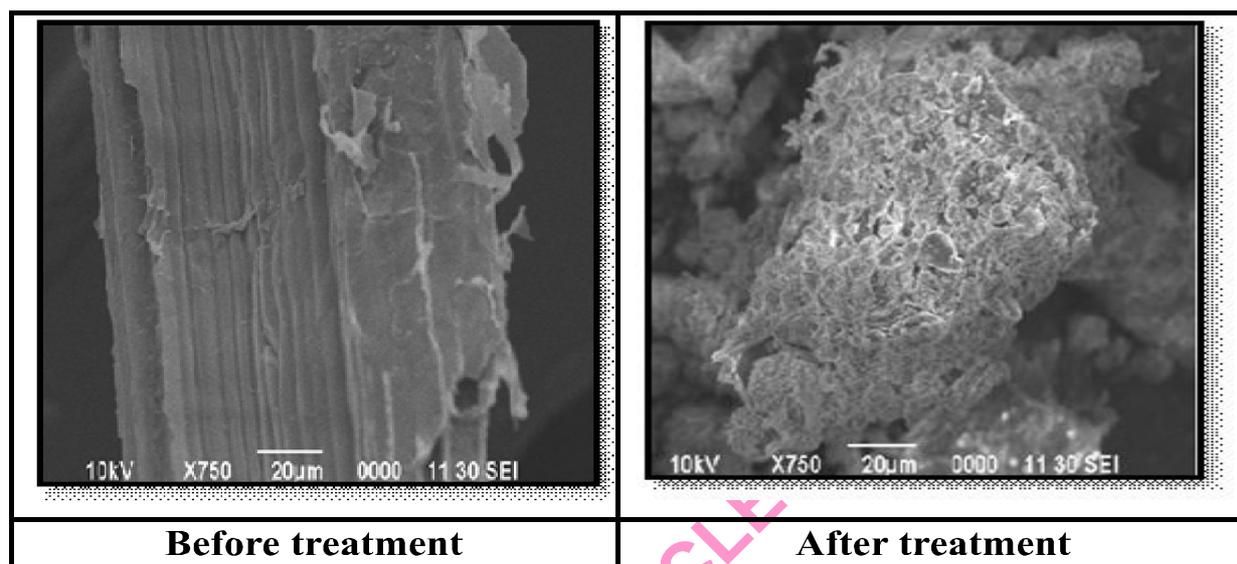


Fig:-5. Scanning electron micrograph of *Spirogyra gracilis* before and after treatment with textile dyeing effluent

Table-1. Assignment of products by GC-MS analysis of textile dyeing effluent

S.No	Retention Time (min)	Product assignment	Mol. wt	m/z values
1	4.29	4-hydroxy-4-methyl pentanone <chem>CC(O)(C)CC(=O)C</chem>	116	43 59 101
2	4.40	2-Amino-5-methylbenzoic acid <chem>CC1=CC=C(C(=O)O)N=C1</chem>	151	133 151 104 77
3	4.49	4-ethylbenzoic acid <chem>CC1=CC=C(C(=O)O)C=C1</chem>	151	133 151 105 77
4	6.34	6-ethyl 2-methyldecane <chem>CCCCC(C)CC(C)CC</chem>	184	43 57 71 85

and degraded compounds displaced three main peaks for functional groups; the strong bands at 3400 - 3430  $\text{cm}^{-1}$  for -OH and N-H stretching, the peak at 1658 - 1635  $\text{cm}^{-1}$  were attributed to the stretching of carboxylate group or C=C of

aromatics and the peak at 1049 - 1026  $\text{cm}^{-1}$  for -C=O- of alcohols, phenol, C-C-C of esters stretching. Although a few peaks at 871.82, 709.80, 532.35 and 462.92  $\text{cm}^{-1}$  involves the presence of S-O stretching. The FT-IR spectra of untreated

**Table-2. Toxicity study of textile dyeing effluent and its degradation products after 7<sup>th</sup> of the *Vigna radiata*.**

Parameters	<i>Vigna radiata</i>		
	Tap water	Textile dyeing effluent	Degradation products
Germination (%)	93	53	80
Plumule (cm)	19.3±3.48	2.31±3.20	17.82±4.22
Radical (cm)	5.87±3.76	1.61±1.39	4.92±2.65

and effluent treated with algal biomass were taken to obtain information on the nature of possible interactions between the functional groups of the biomass and the effluent. The stretching vibration of -OH, C-H, C=O and S-O group was shifted in treated effluent which might be due to the chemical interactions between the dye present in the effluent and the hydroxyl groups occurred on the biomass surface. The results of the present study also coincide with the findings of Yang et al., (2011) and Srinivasan and Viraraghavan (2010) who indicated that the presence of functional groups such as amine, hydroxyl and carboxyl could be responsible for dye biosorption for sequestration of contaminants from wastewater. The adsorption capacity depends upon the porosity as well as chemical reactivity of functional groups on the adsorbent surface (Kumar et al., 2010). The presence of metals in the effluent may change the IR spectral pattern such as band disappearance after saturation of active sites (Lin et al., 2005), band shifting (Pethkar et al., 2001) and elongation (Loukidou et al., 2004). Thus the analysis of the FT-IR spectra showed the presence of ionizable functional groups such as carboxyl, amino, amides and hydroxyl groups on the algal cell surface.

Gas chromatography is used for separating the complex mixtures into smaller compounds. The GC-MS analysis showed a major peak of degraded metabolites of textile dyeing effluent exposed several peaks at retention time of 4.29, 4.40, 4.49, and 6.34 min as shown in Fig.-4a. The mass spectrum revealed the formation of 4-hydroxy-4-methyl pentanone, 2-amino-5-methylbenzoic acid, 4-ethylbenzoic acid, 6-ethyl 2-methyldecane were illustrated in Fig.-4b. These compounds are identified using NIST mass database. The result of the product assignment was shown in Table-1. The GC-MS results indicated that the textile dyeing effluent was completely degraded into smaller units. The finding of the analytical studies collectively concludes that decolourisation was mediated by biosorption as well as biodegradation.

**Scanning electron microscopy:** Scanning electron micrographs of *S. gracilis* before and after treatment with textile dyeing effluent showed specified surface modifications in the biosorbent Fig.-5. The SEM analysis of algae before decolourisation showed a tangled mass of filament in net form, whereas the exposure of *S. gracilis* to effluent showed a rough, asymmetrical pores with a diameter of about 20µm or with a lot of macropores with uneven cell surface texture indicating the toxicity of dyes. The results of the present study corroborate with the findings of Phugare et al., (2010) who reported that the physical state of algal cells was disturbed when treated with effluent.

**Phytotoxicity study:** Degradation of effluent releases different intermediary products which must be nontoxic to the environment. Therefore, phytotoxicity tests were carried out using *V. radiata* to determine the toxicity of the degraded metabolites present in the effluent. The seed germination percentage of *V. radiata* was 53% in untreated effluent whereas it increased up to 80% in effluent treated with algae but lower than the seeds germinated in water (93%). The length of plumule and radical were reduced in plantlets grown in textile dyeing effluent when compared to those cultivated in water (control) Table-2. Presence of different dye molecules and their chemical compounds in the treated dyeing effluent inhibits the germination percentage and growth of green gram whereas metabolites produced after degradation of textile dyeing effluent by *S. gracilis* did not inhibit germination, the growth of a plant and found to be nontoxic.

## CONCLUSION

The present study demonstrates the ability of the *S. gracilis* to decolourise and degrade the dyes from the textile effluents. The alga was found to degrade the dyes from the effluent across a wide range of pH, temperature, biomass dosage and incubation time. UV-vis, FT-IR and GC-MS studies confirmed

the sorption of dye from the effluent by *S. gracilis* and the phytotoxicity study shows that the new metabolites formed after biodegradation were found to be nontoxic. Thus the results of a present study will form the basis for the development of cost-effective and robust indigenous technology for biosorption of dye-based effluents and will be a promising alternative to replace or supplement the physicochemical treatment process.

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