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Biodegradation of Different Concentration of dye (Congo red dye) by using Green and Blue Green Algae

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ABSTRACT: Releasing of textile dye effluents into general water bodies is a major environmental and health problem. Color removal, in particular, has recently become of major scientific interest, as indicated by the multitude of related research reports. During the past two decades, several physico-chemical decolorization techniques have been reported, few, however, have been accepted by the textile industries.

Their lack of implementation has been largely due to high cost, low efficiency and inapplicability to a wide variety of dyes. The ability of microorganisms to carry out dye decolorization has received much attention. Green algae and blue green algae are considered as an important source for decolorizing dye and textile effluent. The dye Congo red and textile dye effluent is chosen for this investigation and the green algae *Haematococcus sp., Chlorella sp., Chlorella vulgaris, Scenedesmus obliquuss, S. officinalis,* and *S. quadricauda* and blue green algae *Arthospira maxima* was used for the decolorization process. Chlorophyll, protein content of this organism was tested before and after the treatment. *Haematococcus sp* shown the maximum degradation among all the seven microalgae was found at 10ppm which was 98%, which decolorize the textile effluent efficiently in short period of time.

Key words: Congo Red Dye, Blue green algae, Green algae, Chlorophyll, Protein, Decolorization

INTRODUCTION

Today, more than 100,000 commercial dyes are available in market and nearly one million tons per annum are produced, whereas 10% of dyes are released in environment and natural resources as dyestuff waste (Jadhav *et al.,* 2010). This production increases day by day to meet the needs of growing population, also increases the release of dye effluent. The disposal of these colored substances poses one of industry's major problems in waste water treatment. This is because the discharge of colored wastes is not only damaging to the aesthetic nature of the receiving streams but also toxic to aquatic life and even carcinogenic or mutagenic in nature (Puvaneshware *et al.,* 2006). The treatment of textile effluent involves mainly physical and chemical methods, which are often very costly (Robinson *et al.,* 2001). It is difficult to treat dye wastewater by chemical and physical processes because of the complex molecular structures. Furthermore, the disposal of the concentrated sludge creates another problem. There has been increased interest in using biological methods for remediation of textile wastewater, especially in color removal. Most studies have concentrated on the use of fungi and bacteria to treat colored wastewater (Tastan *et al., 2010*; McMullan *et al.,* 2001). However, additional carbon sources are required for such systems. In recent years, the use of microalgae in bioremediation of colored wastewater has attracted great interest due to their central role in carbon dioxide fixation. In addition, the algal biomass generated has great potential as feedstock for biofuel production (Huang *et al.,* 2010).

Azo dyes are characterized by the presence of one or more azo group (-N = N-) bonds to aromatic rings (Bafana *et al.*, 2008). Azo dyes may be decolorized by cleavage of the azo bond, to which the color is associated (Wuhrmann *et al.,* 1980). The reduction of azo dyes results in the formation of aromatic amines that mostly cannot be metabolized, with the exception of a few examples bearing hydroxyl and carboxyl groups, which can be fully degraded (Razo-Flores *et al.,*1996) Hence, the majority of azo dyes are mutagenic and carcinogenic to humans as well as other animals removal of hazardous industrial effluents is one of the growing needs of the present time. Congo red is water soluble, yielding a

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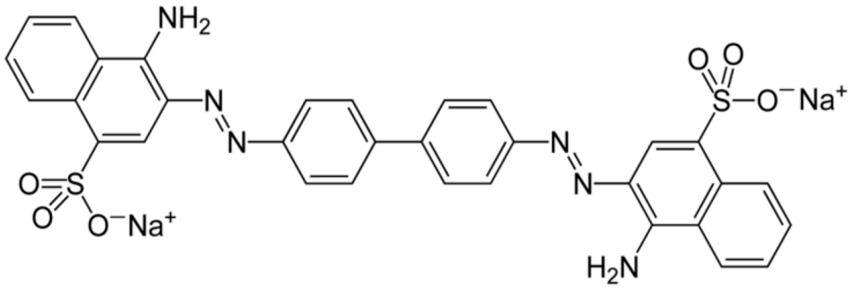

Fig. 1. Chemical structure of Congo red dye

red colloidal solution, its solubility is better in organic solvents such as ethanol. It has a strong, though apparently non-covalent affinity to cellulose fibers. However, the use of Congo red in the cellulose industries (cotton textile, wood pulp & paper) has long been abandoned, primarily because of its toxicity and tendency to run and change color when touched by sweaty finger (Zvezdelina *et al.*, 2012).

Recently the application of algae has been receiving increasing attention in the field of waste water decolorization. This is due to the versatile ability of the algae to degrade, partially or completely various dyes. Many researchers have studied the effect of algae and enzymes on decolorization characteristics since immobilization provide distinct stability over free cells. Algae have been studied in the field of decolourization of industrial effluents (Semple *et al*., 1999). Algae are ubiquitous naturally and serve as one of the biomaterials with high capacity for removing dye from contaminated waters as they are photosynthetic organisms distributed in nearly all parts of the world and in all kinds of habitats. Marungrueng and Pavasant reported that many functional groups (such as carboxyl, carbonyl, hydroxyl, phosphoryl and amide) making up the algae cell wall plays the important role in dye removal. Several other factors play important roles in dye bioremediation (Marungrueng *et al*., 2007). Among these factors, pH, dye concentrations, and amount of biomass of biomaterials are quite important. (Wafaa *et al*., 2008).

In the present study, experiments on the degradation of Congo red dye at different part per million's (viz., 2, 5, 10,15 and 20) was carried out on 5th, 10th and 15th day using these different algae (*Arthospira maxima, Haematococcus sp., Chlorella sp., Chlorella vulgaris, Scenedesmus obliquuss, S. officinalis*, and *S. quadricauda)* and we have evaluated the efficiency of each of this alga to degrade or decolorize the dye's intensity in the medium by estimating the pigment chlorophyll-a, b and protein content and also the decolourization percentage of the dye was estimated by scanning the medium from 250 to 700nm wavelength before and after the growth of the alga in the medium.

MATERIALS & METHODS

The algal strains were obtained from the culture collection of SDNB Vaishnav College for Women, Chrompet, Chennai-44. The algal strains were Arthospira maxima, Haematococcus sp., Chlorella sp., Chlorella vulgaris, Scenedesmus obliquuss, S. officinalis, and S. quadricauda. The green algal cultures were maintained in Bold Basal Medium and the blue green algae (cyanobacteria) was maintained in Zarrouks medium at 24±10C in a thermostatically controlled room, illuminated with fluorescent tubes (Philips 40W) providing 30µEm2/s and 12:12 h light/ dark cycle. The chemical structure of Congo red dye used for decolorization study is shown in Fig 1.

The growth study was carried out for a period of 15 days for all algal species under laboratory conditions. After every five day interval sample was withdrawn and the amounts of dry weight, pigments were estimated. After every five day interval 5ml of the sample was withdrawn and centrifuged at 5000rpm for 10minutes and the amounts of dry weight, pigments namely, Chlorophyll-a & b, (Mac Kinney 1945) and total protein (Bradford 1976) were estimated. The experiments were carried out in saline bottles containing algal cultures and azo dyes at different concentration (viz., 2, 5, 10, 12, and 15ppm). The cultures were incubated at 16-20°c for 15 days. The dye concentration was determined every after 5 days (e.g., 5th, 10th and 15th) by measuring the absorbance of cell-free supernatant of the sample at the maximal absorption wavelength (λ_{max} 250 to 700nm). (Hafez, 2008) The sterile cell-free medium was chosen as control. The changes in the dyes absorption spectra were recorded by using a UV-Vis spectrophotometer (U-2900). Decolourization percentage was calculated as follows:

$$\text{Decolourization (\%)} = \frac{\text{Initial absorbance - Observed absorbance} \times 100}{\text{Initial absorbance}}$$

RESULTS & DISCUSSION

Microalgae with its added advantages such as a long history of its food use, easy cultivation and high nutritional content make it a valuable source for immune modulating studies. Scientists are increasingly turning their attention to algae as ingredient factories, particularly the nutritional components. It is known that biomass adsorption is effective when conditions are not always favorable for the growth maintenance of the microbial population, because the use of biomass has its advantages, especially if the dye containing effluent is very toxic. Treatment of dye effluent presents several problems mainly due to the toxicity and recalcitrance of dyestuffs. Discharge of dye effluent into the natural streams may be toxic to the aquatic lives. Colour affects the nature of water and inhibits the sunlight penetration into the stream and reduces photosynthetic activity. In the present

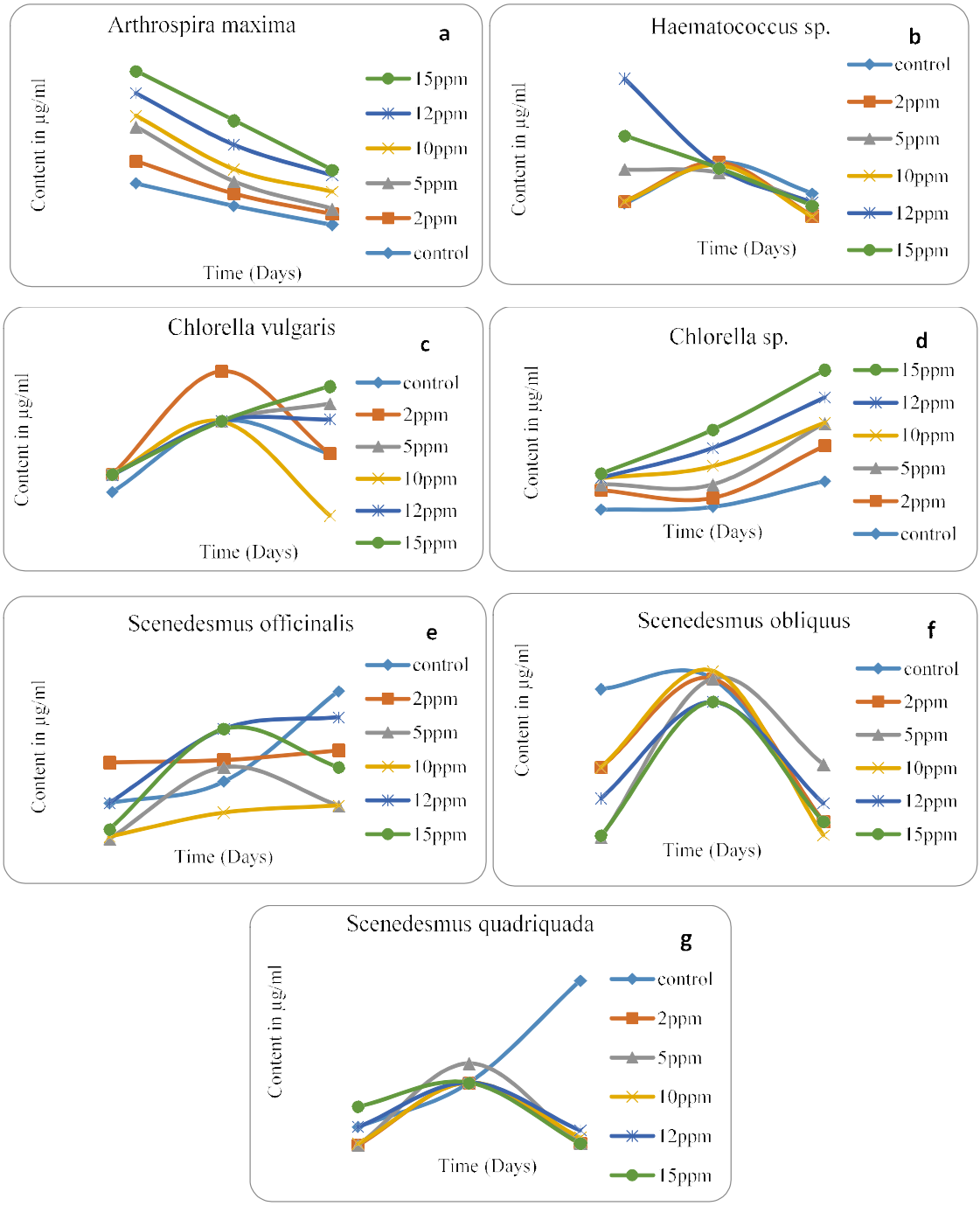

Fig. 2. Effect of Different Concentration Of dye on the Chlorophyll-a Content

investigation we have used different microalgae for the degradation of different concentration of dyes. The pigment analysis of different algae in our studies showed the presence of chlorophyll, protein in various amount when treated with dye in different concentration when compared to the control. The growth of organism is depicted by chlorophyll-a content deposition. *Arthospira maxima* treated with all the different concentration of Congo red dye showed the decrease in its dye concentration (Fig.2a). *Haematococcus* sp. when treated with different concentration the content of chlorophyll-a showed the highest accumulation on 5th day at 12ppm) and then the chlorophyll content started to decrease on 10th to 15th day (Fig.2b).

Chlorella showed a different trend when compared to *Arthospira maxima Haematococcus, Scenedesmus* . In *Chlorella sp.* the chlorophyll-a irrespective of the different dye concentration showed no changes and it seems to be that chlorophyll-a content accumulation does not show an affect of the dye when compared to control (Fig.2c). *Chlorella vulgaris* also showed the same trend to the *Chlorella sp* (Fig.2d). *Scenedesmus officinalis* when treated with different concentration of dye the chlorophyll-a showed the decrease in 10ppm then 15ppm, 12ppm and 5ppm the maximum concentration was seen in 10th day but on 15th day simultaneously decreased for all concentration while in control it showed an increasing trend from 5thto 15th day (Fig.2e). *Scenedesmus obliquus* and *Scenedesmus quadriquada* showed the same trend by having increase in 10th day followed by decrease in 15th day. The chlorophyll-a content showed a drastic decrease in all the concentrations of dye.

In *Haematococcus* the different dye concentration viz., 2, 5 and 10ppm (Fig.3c) showed increase in

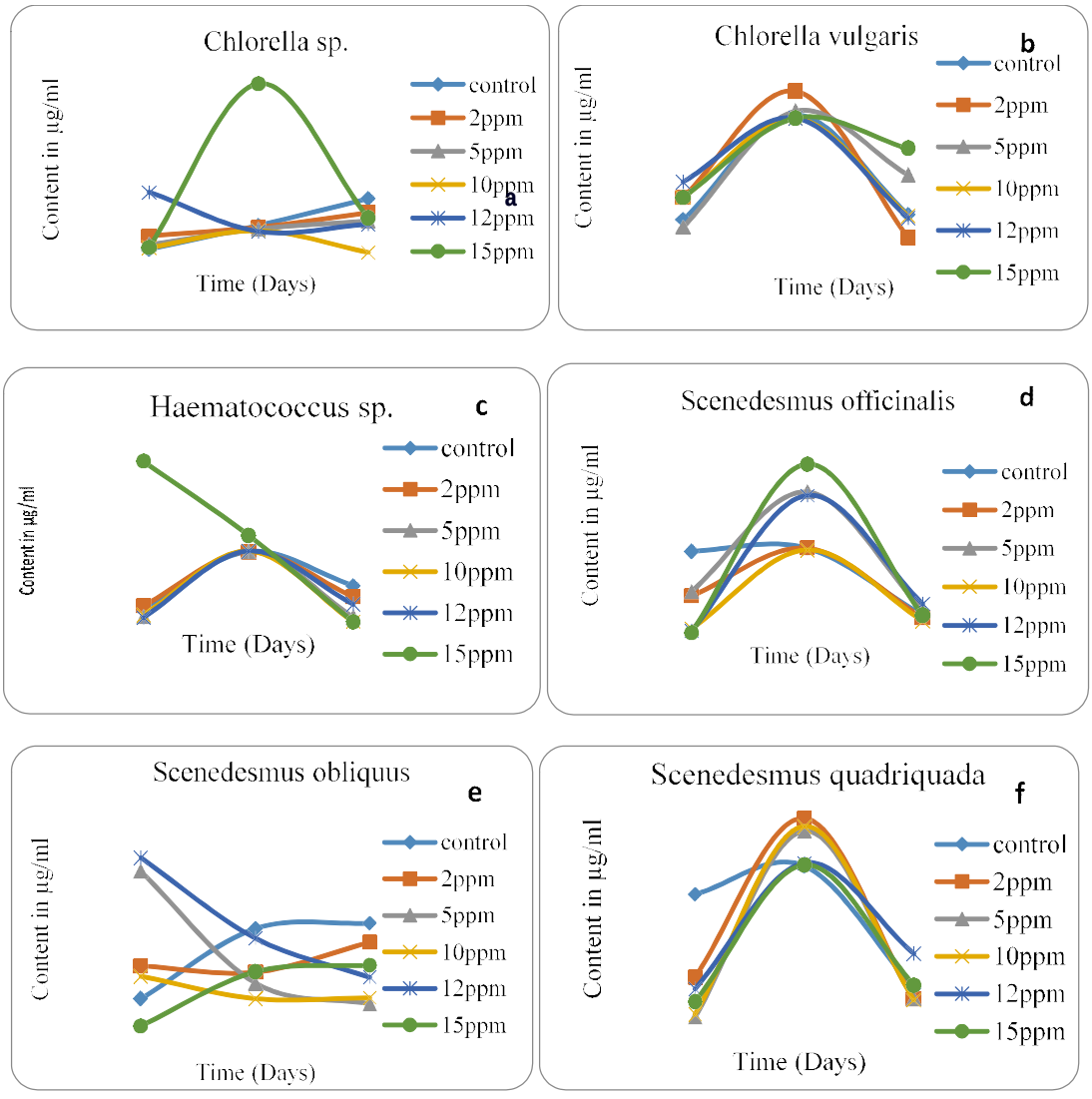

Fig. 3. The effect of Different Concentration Of dye on the Chlorophyll-b Content

chlorophyll-b content on 10th day followed by a decrease on 15th day. Among them the highest concentration of 15ppm showed a significant decrease in chlorophyll-b content, which kept constantly decreasing from 10th to 15th day. In the C*hlorella sp*. (Fig.3a) all the different concentration of dye did not show much affect on chlorophyll-b content accumulation. It showed increasing content of chlorophyll-b on 5th, 10th and 15th day, and the maximum content of chlorophyll-b was found to be on the 15th day. The highest concentration of the dye, 15ppm showed the different trend as the chlorophyll-b increased on 10th The chlorophyll-b content for *Chlorella vulgaris* seemed to increase

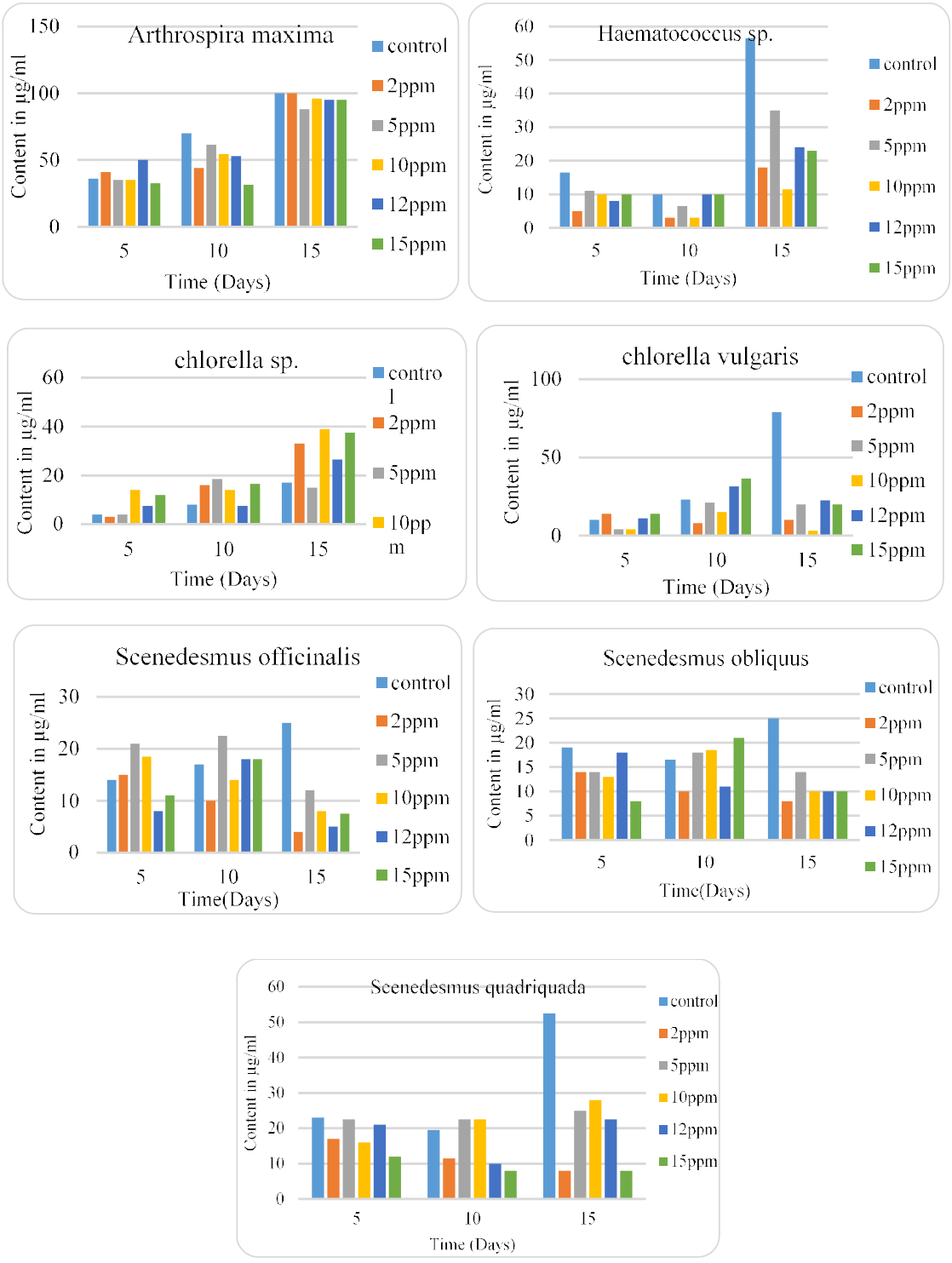

Fig. 4. The effect of Different Concentration Of dye on the Protein Content at 5 days interval

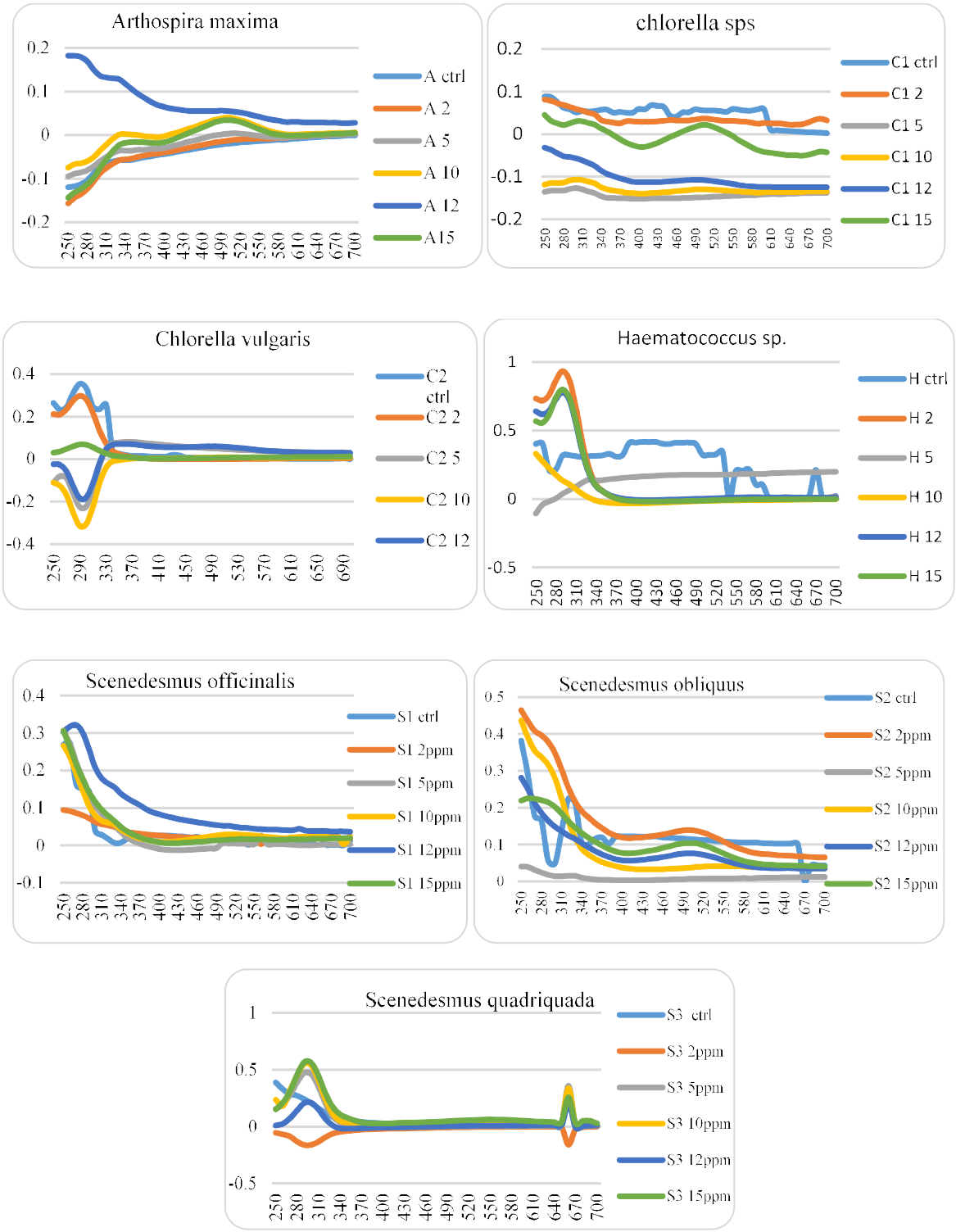

Fig.5. UV-Vis Spectrum of decolourization of different Concentration of Congo red dye using different algae on 5th day interval

from 5th day to 10th day and later it totally decreased (Fig.2b).Among the three *Scenedesmus* species *S. officinalis*, *S. quadriquada* showed the similar patterns of accumulation of chlorophyll-b and they significantly decreased on 15th day while in *S. obliquus* except concentration of 15ppm all the other ppms showed the decreasing content(Fig.3d, e & f). The protein content does not seems to be

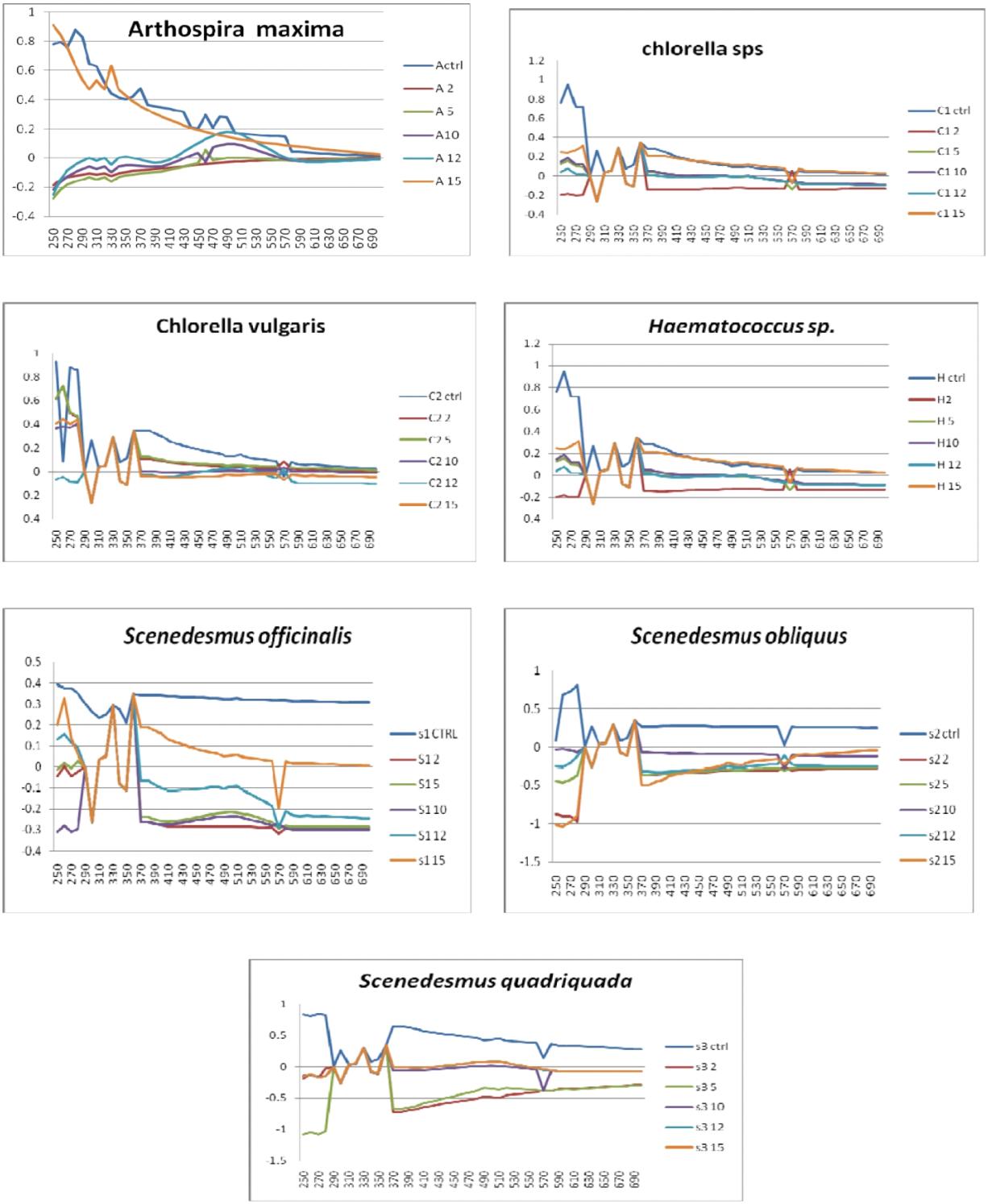

Fig. 6. UV-Vis Spectrum of decolourization of different Concentration of Congo red dye on 10th day interval.

much affected by the various dye concentration. The protein content in *Arthrospira*, *Chlorella sp.*, *Haematococcus* increased. Among all the algae used only *Scenedesmus* showed a deleterious effect of the dye and its seemed to have lowered the protein content of the alga (Fig.4a-g). The degree of decolurization for Congo red dye by some green algae and blue green algae were studied at 2, 5, 10, 12 and 15 ppm dye concentration for every 5 days interval. In *Arthorospira* the maximum decolorisation observed

Fig. 7. UV-Vis Spectrum of decolourization of different Concentration of Congo red dye on 15th day interval.

at 2ppm which was 46%. *Haematococcus sp.* had shown the maximum degradation among all the seven microalgae. In this the maximum decolorisation was found at 10ppm which was 98% followed by 2ppm(96%), 5ppm(94%), 12ppm(92%), and 15ppm(40%). Among the two chlorella species studied Chlorella vulgaris showed the highest decolourisation percentage which was maximum at 5ppm and 10ppm(99%) followed by

12ppm(81%) and 15ppm(55%). The Chlorella sp. used show the highest decolourisation percentage at 15ppm (77%). We are carried out studies on decolourisation in three species of *Scenedesmus (S.officinalis; S.obliquus; S.quadriquada)* among three *S.quadriquada* showed the maximum decolourisation when compared other two species. In *S.quadriquada* maximum decolourisation percentage was 97% which was seen for 5ppm followed by 12ppm. This was followed by *S.officinalis* in which the maximum decolourisation was 2ppm (89%) followed by 12ppm (79%) and 15ppm(78%).*S.obliquus* showed maximum decolourisation at 15ppm which was 52 %.The result obtained that the amount of color removal varies with varying dye concentration (Figs. 5, 6 & 7). The dye removal is highly concentration dependent and approximately attributed to bioconversion (Aydin and Baysal 2006). The decolourization was found to be due to both biological azo dye reduction and adsorption (Chen *et al.*, 2003) .The increase of decolourization of azo dyes seems to be related to the molecular structure of the dyes (Jinqi *et al.*, 1992). The rapid decolourization of congo red dye was observed after 5 days and then become slow with the time (Daneshvar *et al.*, 2007). This was caused by strong attractive force between the azo dyes molecules and the algae; fast diffusion onto the external surface was followed by fast diffusion into algae cells to attain rapid equilibrium.

CONCLUSIONS

The presence of dyes imparts an intense color to effluents, which lead to environmental problem. It may be concluded that algae undoubtedly have the potential to rapidly, efficiently and effectively remove dyes to low concentrations. Moreover, this bio sorption process could be adopted as a cost effective and efficient approach for decolorization of effluents ant it may be an alternative to more costly material. This prepares the theoretical foundation for the design, optimization and scale-up of the process.

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