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### RESEARCH ARTICLE

#### EVALUATION OF POTENTIAL OF *Ulva lactuca* IN BIODECOLORIZATION OF REACTIVE AZO DYE.

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#### Abstract

The present study investigated the potential of *Ulva lactuca* in dye decolorization and degradation. The optimum tolerance concentration of biomass was observed under lower concentrations of azo dye. The degradation potential of biomass was studied by FTIR (Fourier-transform infrared spectroscopy) analysis of azo dye for pre and post treatment. The biochemical response of the biomass by analysis of chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid, total soluble sugar and protein was observed decreased with increasing days and dye concentration. The surface characteristic of the biomass was studied by SEM (Scanning electron microscopy). The experimental results indicate the order of decolorization as reactive red-195 > reactive yellow -145 > reactive black -5. Thus, *Ulva lactuca* shows significant decolorization and degradation.

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#### Introduction:-

Many different groups of living organisms ranging from single celled to multicellular which are made up of eukaryotic cells are called algae. They possess nuclei, organelles, plastids, chlorophyll, protein, carbohydrate and fatty acid [1]. Algae capture carbon dioxide from the atmosphere, sunlight through photosynthesis and minerals from the environment which transforms inorganic substances into its simpler forms. Various methods are applied for the waste water treatment such as coagulation, flocculation, advanced oxidation process, ozonation, photocatalytic treatments etc [2]. These methods are costly and require heavy inputs of high energy [3]. On the other side biodegradation by biological methods such as bacterial, fungal and algal treatment proved to be best solution. The bacterial and fungal treatment methods are also required maintenance of the culture and environment conditions for the microorganism's survival. Algal treatments are helpful to remove phosphate, nitrogen, total dissolved solids and other contaminants and required low maintenance; It grows on nutrient rich waste water and generates no secondary waste [4][5].

Industrial waste water are generated from pulp and paper, poultry, dairy, metal finishing, pharmaceutical, textile, dyes and pigments, pesticides, chemical and drug formulation industries etc; but the dye industrial waste water concerned mainly for environmental pollution because the color imparts the transparency of water and affects the

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photosynthesis of aquatic phytoplanktons and aquatic plants. It also causes various skin disease and carcinogenic effect on humans if consumed without prior treatment. Various reports are available on dye waste water treatment such as azo dye effluent and congo red dye by various species of microalgae [6][7][8], methylene blue by *Caulerpa racemosa* and *Ulva lactuca* [9][10][11], Organic dye removal by *Sargassum horneri* [12]. Various scientists have worked on accumulation of heavy metal such as chromium, lead, copper, cadmium, phosphate by algae from waste water [13][14][15][16]. The present paper attempts to evaluate decolorization and degradation of reactive azo dye prepared in aqueous solution. Furthermore, this accumulated alga after treatment was studied for its biochemical response and surface characterization.

## Material and Methodology:-

### Seaweed collection:-

The algae *Ulva lactuca* was collected from Bet-dwarka near Okha coast of Gujarat, India shown in Image-1 (Longitude - 68°20' E to 70°40' E Latitude - 22°15' N to 23°40' N). It was washed there with seawater to remove sand particles and unwanted debris, particle adhered to it. Then kept in icebox and transferred to laboratory to wash three to four times with distilled water to remove surface particles and salts. Then it was kept on room drying at normal room temperature for 2-3 days, and used in the present study. The Image-2 indicates wet and dry algal biomass.



Image-1:- Map of Okha



Image-2:- Wet and Dry biomass of *Ulva lactuca*

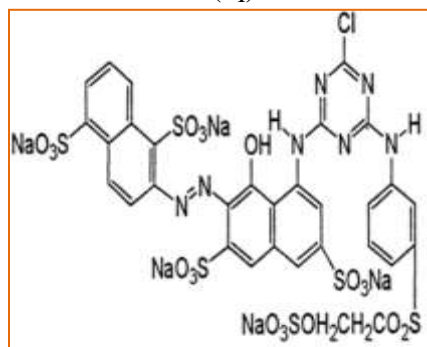
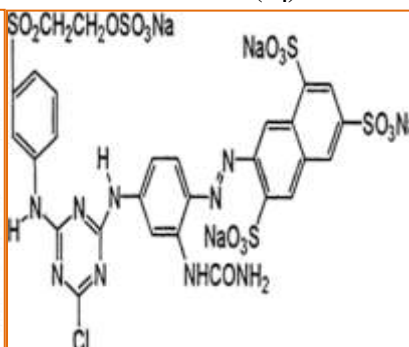
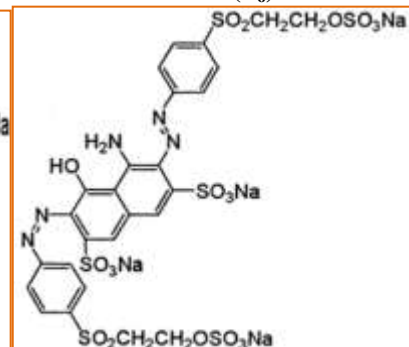
Reactive Red-195 (P<sub>1</sub>)Reactive Yellow-145 (P<sub>4</sub>)Reactive Black-5 (P<sub>6</sub>)

Figure-1:- Structure of Dyes

**Biodecolorization Treatment: -**

The powder sample of reactive red-195, reactive yellow-145, and reactive black-5 (Fig-1) was collected from dye manufacturing industries entitled as P<sub>1</sub>, P<sub>4</sub> and P<sub>6</sub>. They were prepared in aqueous form under 0.4 % W/V of concentration. Under the static ambient conditions the algae was inoculated in the solution and percentage decolorization was studied by UV-Visible spectrophotometer (Make- Shimadzu, Model- 1800) with given formula below:

$$(\%) \text{Decolorization} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

**Spectrophotometer Analysis:-**

The dye solutions were studied for maximum absorbance ( $\lambda_{\text{max}}$ ) wavelength by UV-vis spectrophotometer (Make- Shimadzu, Model- 1800) under 400 to 700 nm range. The maximum absorbance ( $\lambda_{\text{max}}$ ) was observed at 542 nm, 418 nm & 595.5 nm in P<sub>1</sub>, P<sub>4</sub> & P<sub>6</sub> respectively and optical density of individual dye was measured (Fig-2 and Fig-3).

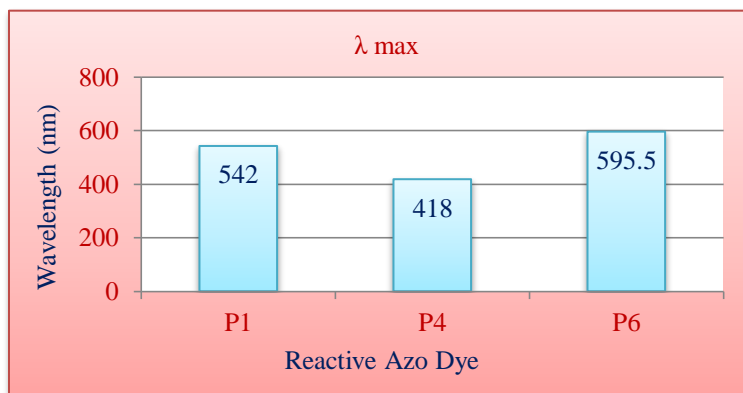
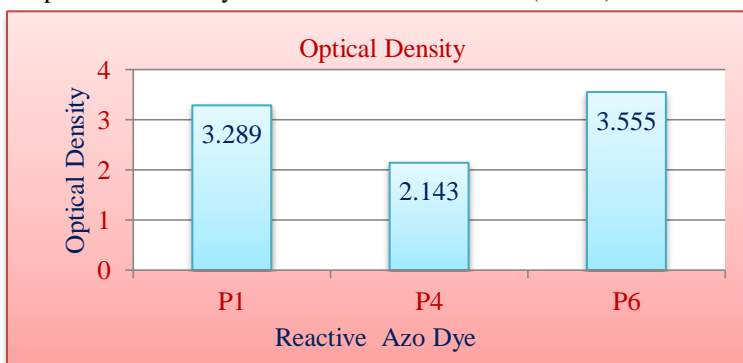
Figure-2:- Spectrometer analysis for the determination of ( $\lambda_{\text{max}}$ ) maximum absorbance

Figure-3:- Spectrometer analysis for determination of Optical Density

**Fourier transform infrared analysis (FTIR):-**

The FTIR analysis of pre and post treatment of azo dyes was obtained by FTIR spectrophotometer (Make: Perkin Elmer, U.S.A., Model: Spectrum GX) by K-Br disk (spectroscopic grade) method. The solutions were kept in oven in petri plates at 200°C temperature for 3-4 hours until the dry powder of the solution obtain. 2 mg of powder sample taken in 200 mg of K-Br with additional crushing and by hydraulic press prepared pallets and scanned under 4000 to 400 cm<sup>-1</sup> range in ambient conditions and spectra was obtained.

**Optimization of Tolerance concentration of dye on biomass: -**

The optimization of tolerance concentration of dye on biomass was studied. All the experiments were conducted in triplicates and standard error mean mentioned in the results and conducted for 8-16-24 day interval. The effluent solutions were prepared in 0.2 % V/V, 0.4 % V/V, 0.6 % V/V, 0.8 % V/V & 1 % V/V. The percentage decolorization was estimated by below formula:

$$(\%) \text{Decolorization} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

**Biochemical response of accumulated alga: -**

The accumulated alga was collected from post treatment of dye. The biochemical changes after dye treatment was studied by biochemical analysis such as chlorophyll-a, chlorophyll-b, total chlorophyll, carotenoid, protein and sugar with the help of standard methods of biochemical analysis by S. R. Thimmaiah [17].

**Scanning electron microscopy (SEM):-**

The surface characteristic of the alga for pre and post treatment with dye was studied by Field Emission Gun-Scanning Electron Microscopy (Make: FEI Ltd., Model: Nova NanoSEM 450)

**Result and Discussion:-**

The decolorization study indicates 90.66 %, 88% & 85 % reduction of color from the dye solutions of P<sub>1</sub>, P<sub>4</sub> and P<sub>6</sub> respectively (Fig-4) which shows that the *Ulva lactuca* has potential in the color removal from dye aqueous solution thus it can be used as decolorizing agent in waste water treatment system. The (λ max) maximum absorbance & Optical density is mentioned in Fig-2 & Fig-3.

Tolerance concentration of dye on alga was estimated under different concentration of dye in aqueous solution by determining % decolorization shown in Fig-5 This study indicates the maximum decolorization was observed in 0.2 % V/V of dye concentration and lower in 1 % V/V. it can be shown as 0.2 > 0.4 > 0.6 > 0.8 > 1 % V/V. Thus, it describes that with increasing dye concentration the % decolorization was observed decreased. The experiment also reveals that with increasing days the decolorization rate become steady from 16-24 day. This is occurred due to the availability of vacant space in the tissues of the biomass initially and with increasing time and dye concentration it gets more accumulated by dye components which lowers the adsorption and results the decreasing rate of decolorization. After this treatment, the accumulated alga was studied further for its biochemical response by biochemical analysis of chlorophyll-a, chlorophyll-b, total chlorophyll, carotenoid, protein and total soluble sugar,

In the present study values of chlorophyll-a, chlorophyll-b, total chlorophyll was observed maximum in 8 day under 0.2 % V/V concentration of dye and minimum value was observed in higher concentration. Similar result was observed in carotenoid, protein, total soluble sugar content. The results are shown in Fig-6 to Fig-9 Thus the experimental results indicates that with increasing dye concentration and time the biochemical content of the biomass was observed decreased.

The scanning electron microscopy (SEM) for pre and post treatment of dye by *Ulva lactuca* indicated in Image-1 which shows in pre treatment the algal surface was observed smooth and even but after the post treatment with dye it was observed damaged, uneven surface and swollen cells. This is occurred because of accumulation of dye functional groups inside the cells.

The Fourier transform infrared analysis (FT-IR) gives the information concerning functional groups variations for pre and post treatment of dye (Fig-10 to Fig-15). The FT-IR spectra indicate the presence of various functional group such as O-H alcohol, S=O sulfate, S=O sulfonic acid, S=O sulfonyl chloride, S=O sulfone, C-O aliphatic ether, C-O vinyl ether, O-H carboxylic acid, C-O alkyl aryl ether, C=C alkane disubstituted (cis), Methylene group,

C-N amine, C-O secondary alcohol, C-O tertiary alcohol were found in dye P1, P4 & P6. These bands are resulted because of structure of dye. In post treatment spectra indicates decrease in peak intensities and band shifts which is reported in Table-1. Post treatment spectra shows the potential removal of various functional groups such as S=O sulfone, S=O sulphonic acid, S=O sulphonyl chloride, S=O sulphate, O-H bonded alcohol, O-H carboxylic acid, N-H amine, C-H alkane, C=C cyclic alkene, C=C conjugated alkene, Methylene strong, C-O tertiary alcohol, C-O aliphatic ether, C-O secondary alcohol, C-N amine clearly represents the dye degradation. The minor changes in peak frequencies resulted due to adsorption of dye onto alga. The active site inside the alga binds with dye functional groups causes break down of the dye structures which resulted the biotransformation or biodegradation.

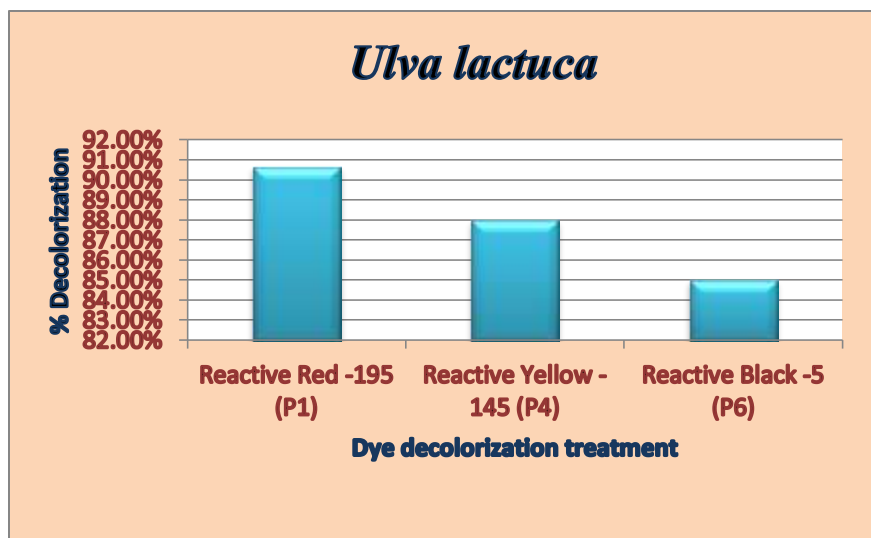


Figure-4:- Percentage color removal of dye from aqueous solution (Biodecolorization)

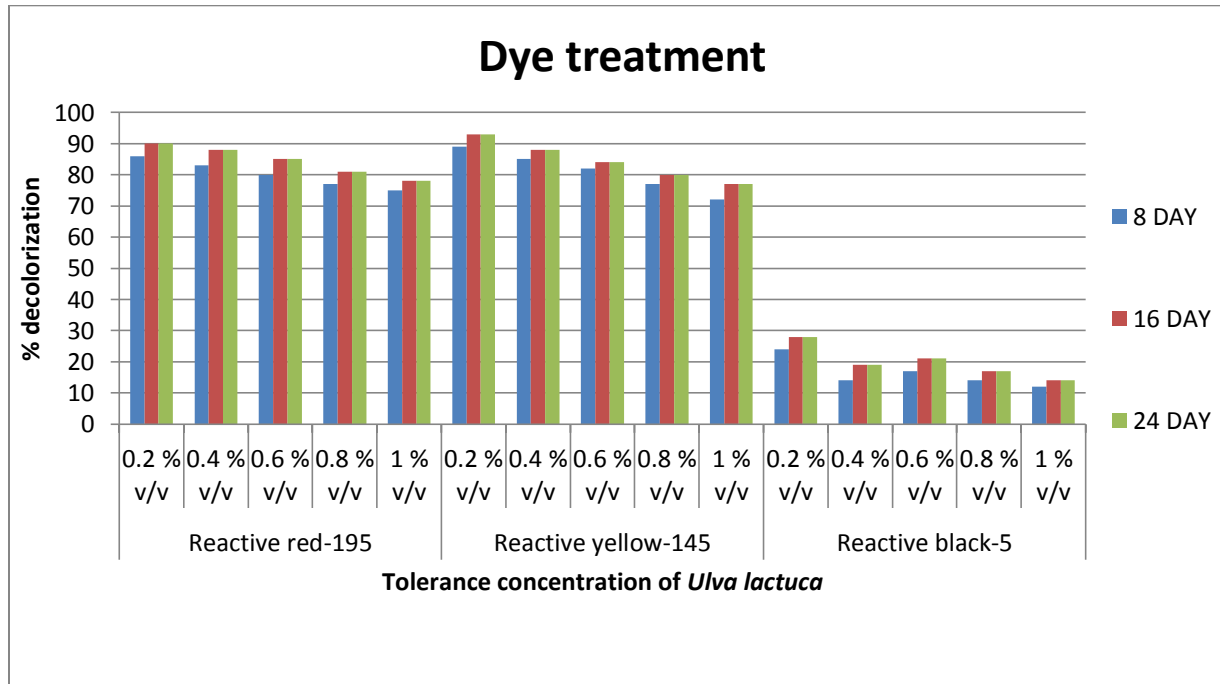
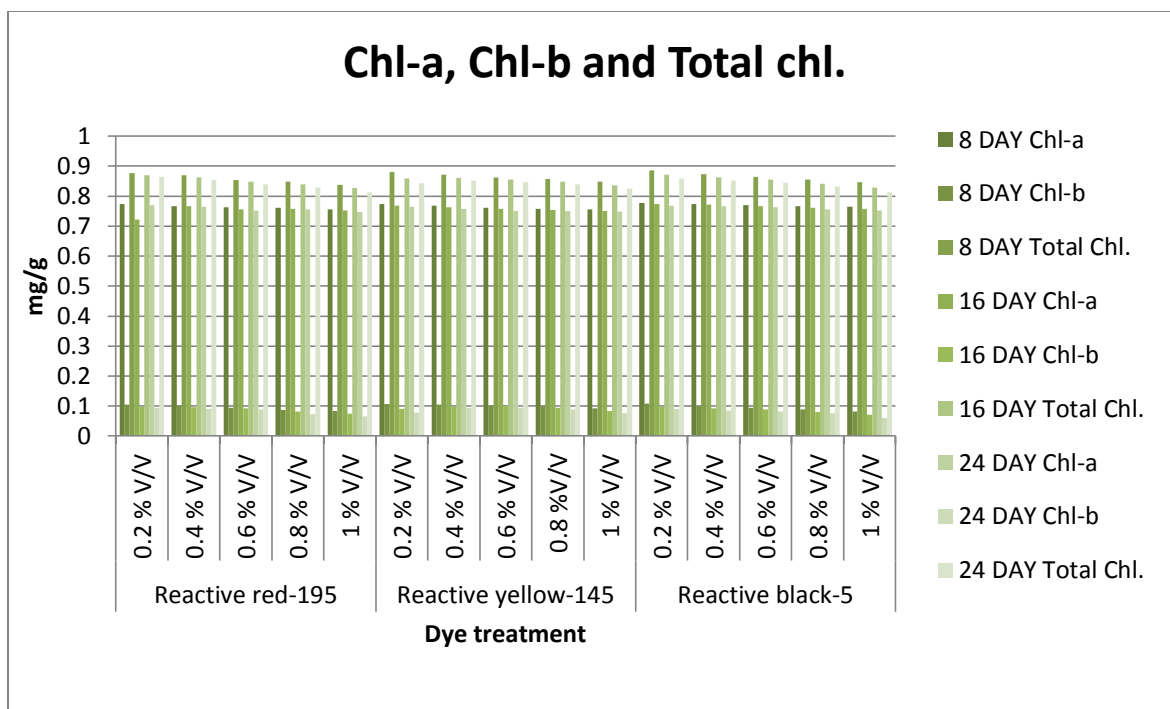
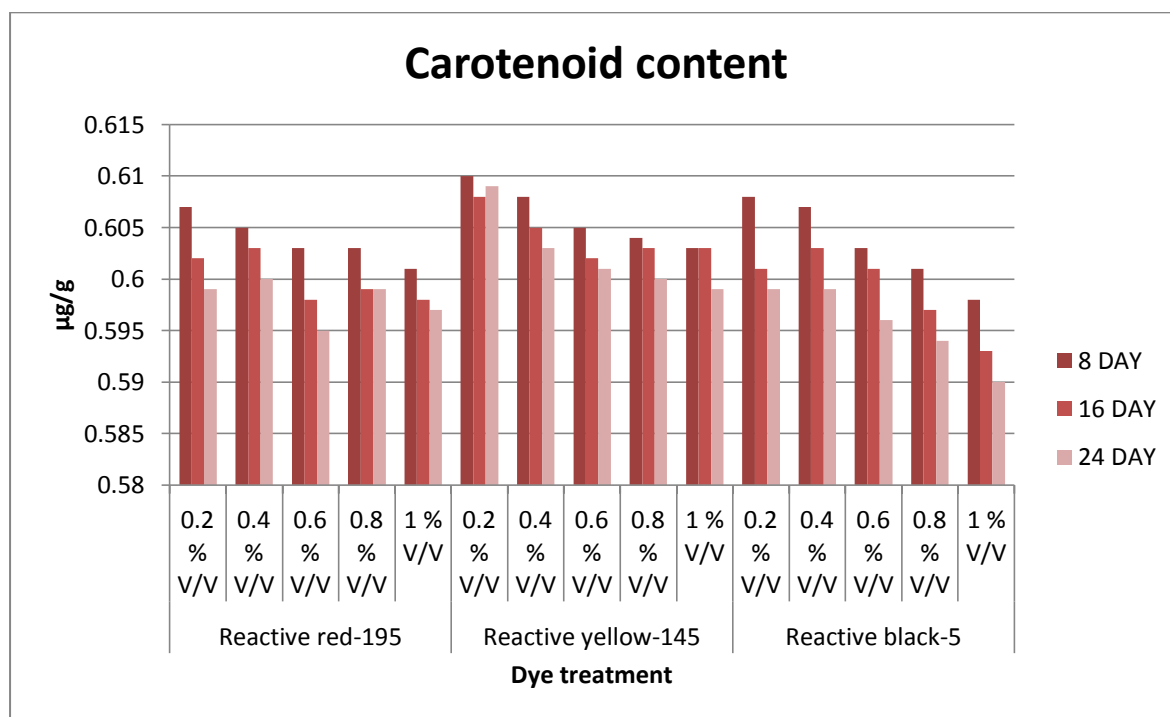


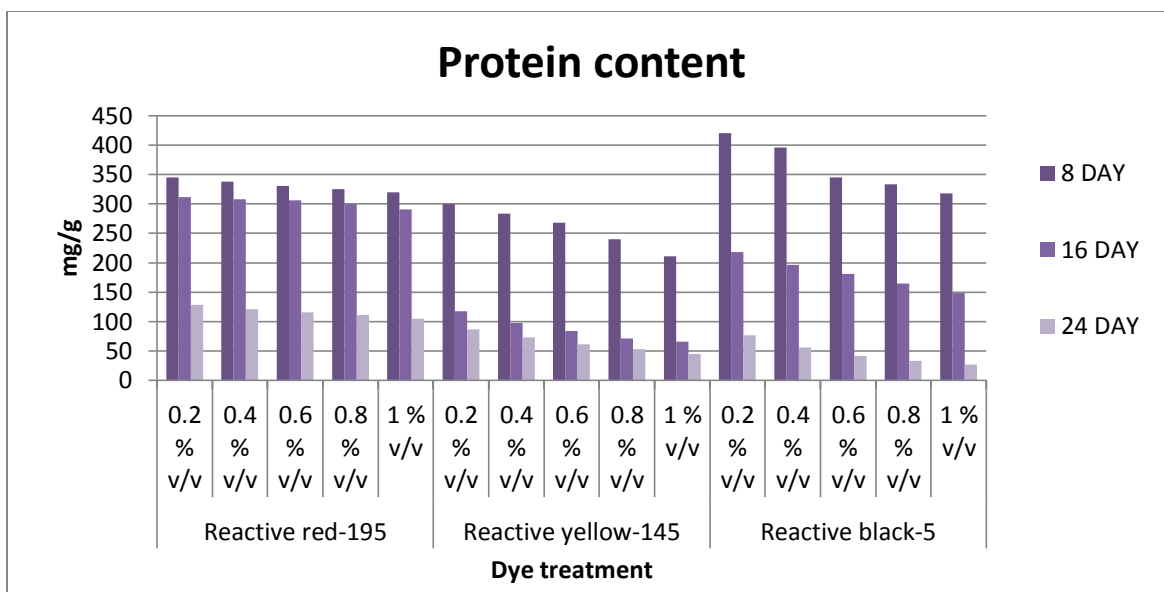
Figure-5:- Tolerance concentration of *Ulva lactuca*



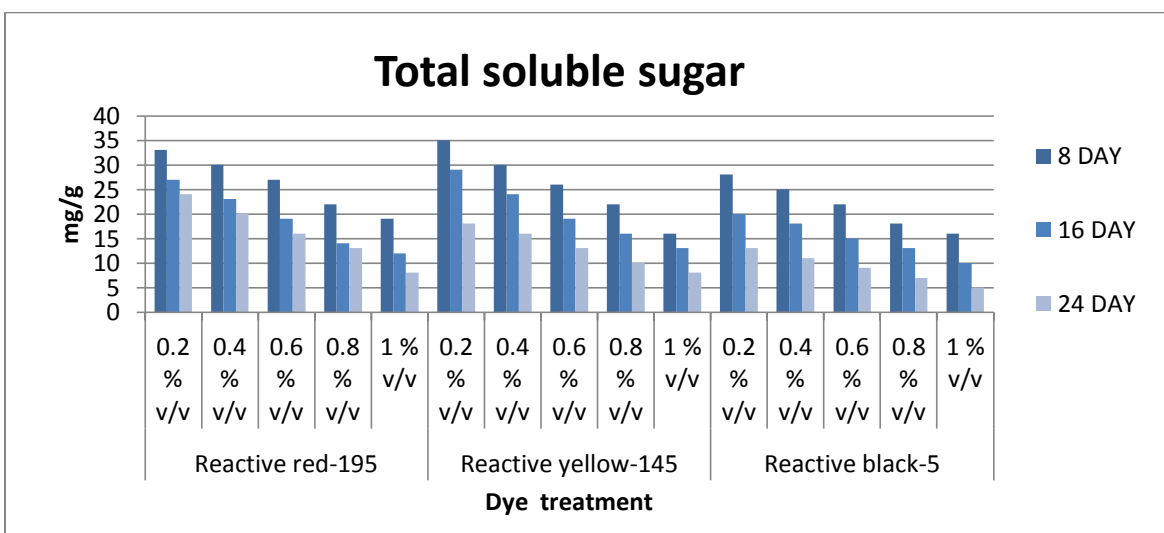
**Figure 6:-** Effect of post treatment on Chlorophyll-a,b and Total Chlorophyll content



**Figure 7:-** Effect of post treatment on Carotenoid content

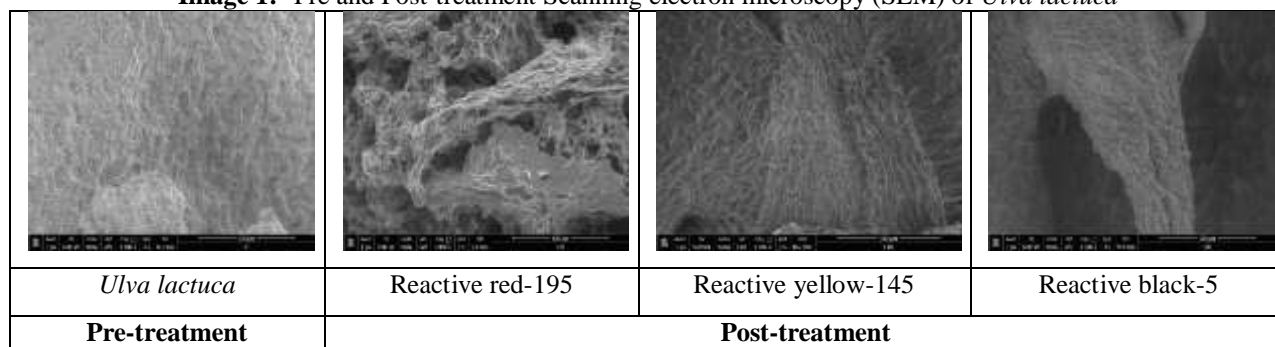


**Figure 8** Effect of post treatment on Protein content



**Figure 9:-** Effect of post treatment on Total soluble sugar content

**Image 1:-** Pre and Post-treatment Scanning electron microscopy (SEM) of *Ulva lactuca*





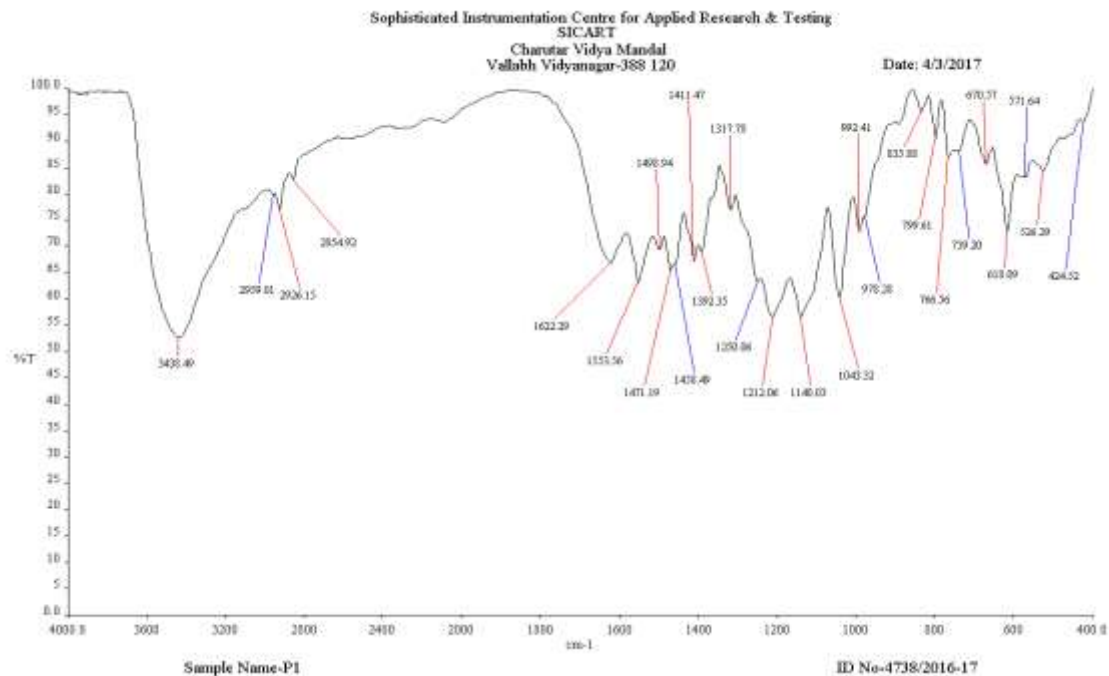
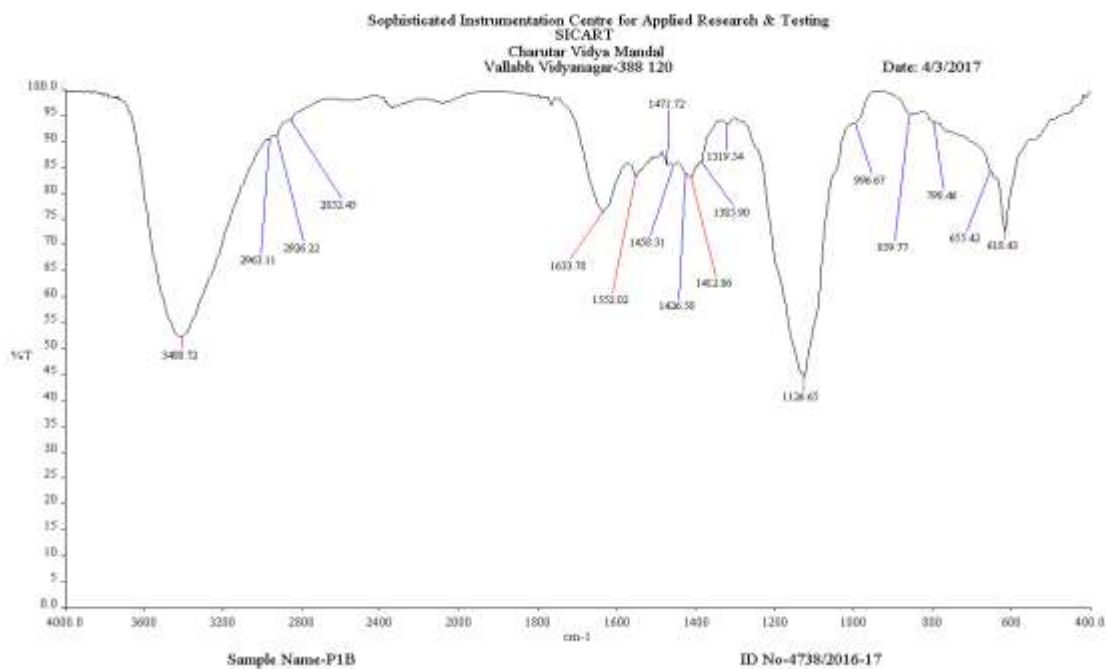
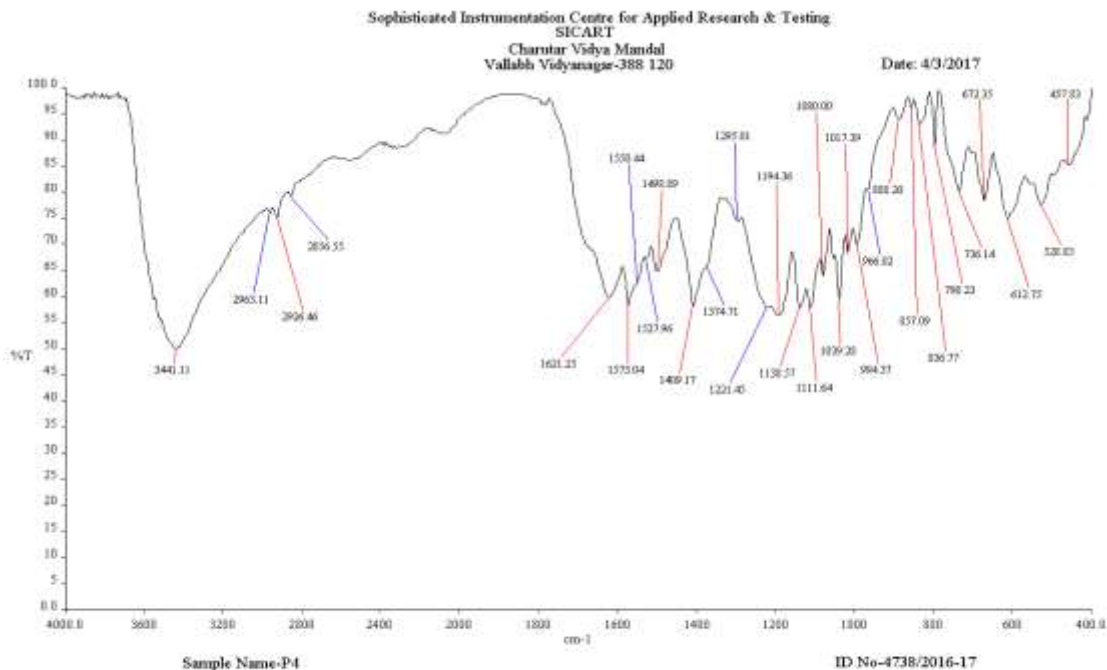


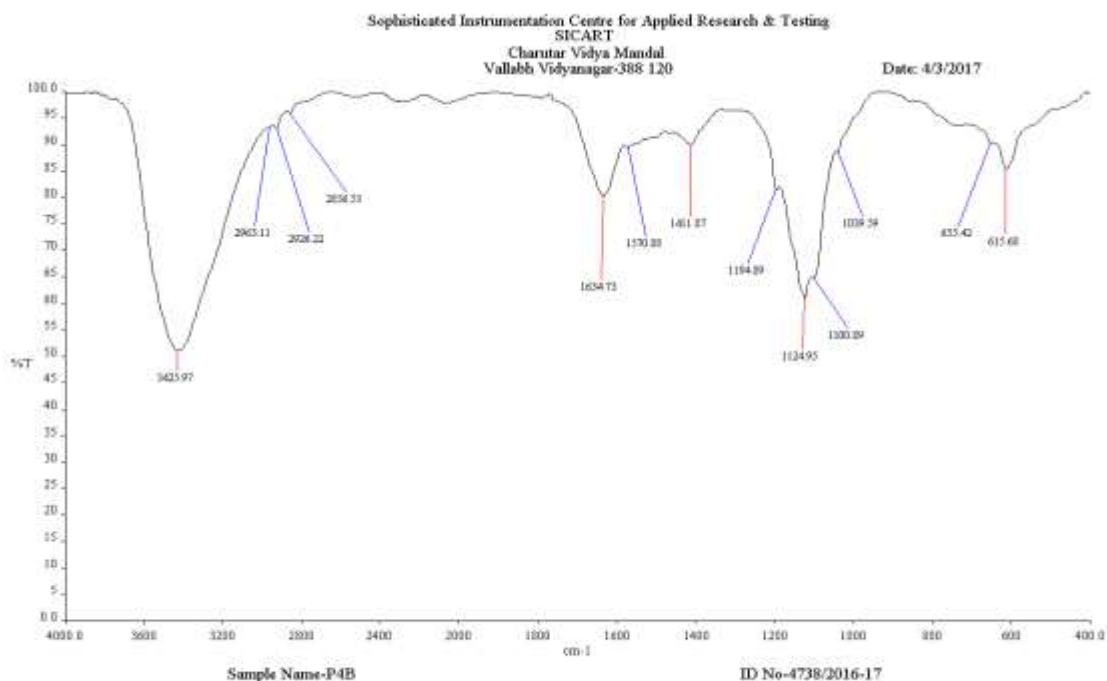
Figure-10:- Spectra of Reactive red-195

Figure-11:- Spectra of treatment of Reactive red-195 with *Ulva lactuca*

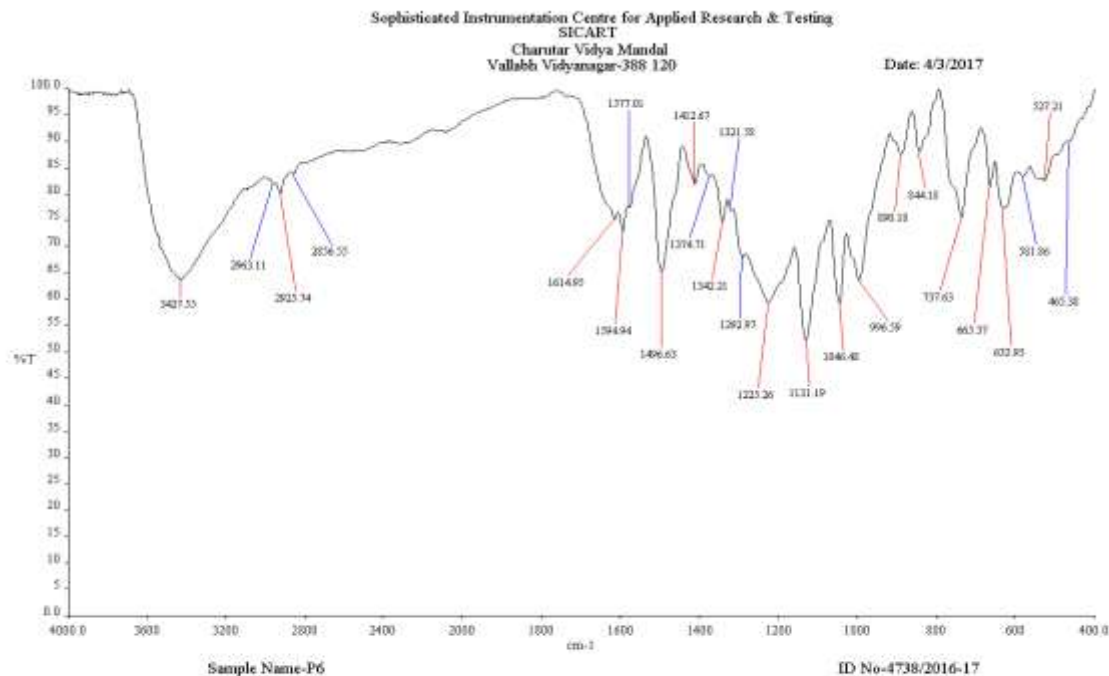




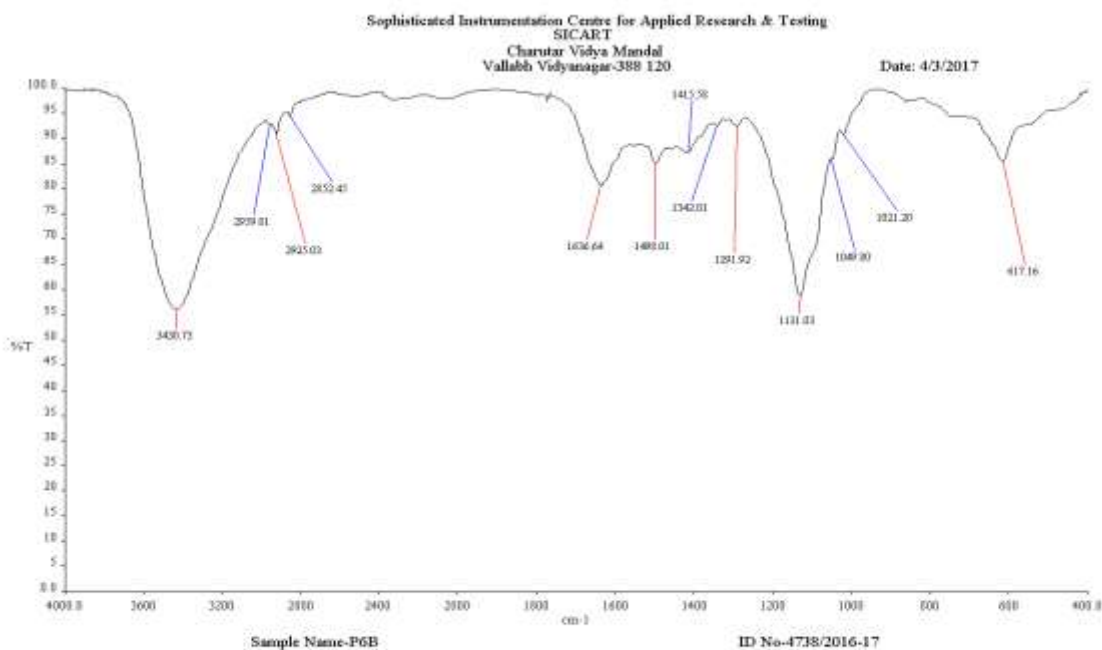
**Figure-12:-** Spectra of Reactive yellow-145



**Figure-13:-** Spectra of treatment of Reactive yellow-145 with *Ulva lactuca*



**Figure-14:-** Spectra of Reactive black-5



**Figure-15:-** Spectra of treatment of Reactive black-5 with *Ulva lactuca*

**Table-1:-** Biodegradation of dyes by *Ulva lactuca*

<i>Ulva lactuca</i>	Adsorption bands (cm <sup>-1</sup> )			Assignment
	Initial	Final	Difference	
P <sub>1</sub> -Reactive Red 195	3438.49	3408.72	29.77	N-H primary amines, O-H alcohol
	1622.29	1633.78	-11.49	N-H Primary amines
	1553.56	1552.02	1.5	C=O Carboxylate salts, amino acid zwitter ions
	1471.47	1471.72	-0.25	Methylene strong, O-H alcohol bending, S=O stretching sulphate,
	1411.47	1412.86	-1.39	S=O Sulfate
	1317.78	1319.54	-1.76	O-H Phenol
	1140.03	1126.65	13.38	C-O Primary alcohol, C-X Fluroalkanes
P <sub>4</sub> -Reactive Yellow 145	3441.11	3425.97	15.14	N-H primary amines, O-H alcohol
	2926.46	2926.22	0.24	C-H alkane
	1621.25	1634.73	-13.48	N-H Primary amines
	1573.04	1570.88	2.16	N-H primary amines
	1409.17	1411.87	-2.7	O-H alcohol bending, S=O stretching sulfate
	1138.57	1124.95	13.62	C-X Fluroalkanes
P <sub>6</sub> -Reactive Black 5	3427.55	3430.75	-3.2	N-H primary amines
	2925.75	2925.03	0.72	C-H alkane
	1614.95	1636.64	-21.69	C=N stretching imine/oxime
	1412.67	1415.58	-2.91	O-H alcohol bending, S=O stretching sulfate
	1342.21	1342.01	0.2	
	1225.26	1291.92	-66.66	S=O stretching sulfone, C-O stretching tertiary alcohol
	1131.19	1131.03	0.16	C-N amine, C-O aliphatic ether, C-O stretching secondary alcohol

**Conclusion: -**

All the above discussion suggests that *Ulva lactuca* has potential ability in decolorization of dye. The FT-IR spectra show removal of dye components after treatment which represents the degradation of dye. It shows potential ability of decolorization under lower concentration of dye. Optimum tolerance concentration of biomass was observed under lower concentration of azo dye. Biochemical response of the alga after treatment shows with increasing dye amount the biochemical content inside the biomass was found decreased. The scanning electron microscopy analysis reveals after exposure the surface of the biomass was found uneven, swollen and broken due to accumulation of dye component inside the cells of the alga. Thus, the present study indicates the *Ulva lactuca* has potential capability for dye degradation and decolorization.

**Acknowledgement: -**

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