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Biodecolorization and Biodegradation of reactive azo dyes by *Kappaphycus alvarezii* and optimization of biofertilizing potential

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Abstract

In recent years with increasing pollution and development there is a need of easily operatable, less costly and no secondary waste generation and environment friendly treatment methods required. It is an attempt to study as degradation and decolorization of reactive azo dyes by seaweed biomass of Kappaphycus alvarezii. This present paper discussed the color removal capabilities of dry seaweed biomass of Kappaphycus alvarezii (C) which gives 93.61%, 90.66% and 21.94% decolorization from P_1, P_4 and P_6 (reactive azo dyes) respectively. The FTIR study shows that the major dye functional groups were completely removed indicates the transformation or breakdown of dye molecules by the active sites of the seaweed biomass creates excellent result for the biodegradation and biodecolorization. After treatment the accumulated seaweed biomass was utilized for biocompost preparation as by-product and its applicability was studied by germination of Vigna radiata and Triticum aestivum. Pigment analysis chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoid was studied indicates the pigment concentration was found higher as compared to control (without compost) in both the plant species which represents the applicability of accumulated seaweed biocompost creates sustainable approach as by-product and no secondary waste generation can be used in waste water treatment systems.

Keywords: Decolorization, Degradation, FTIR Analysis, Dye, Seaweed, *Kappaphycus alvarezii.*

Introduction

Color is the main concern in industrial waste water treatment systems even after given the primary treatment some portion of the color remain in the water which can be removed by various physical and chemical treatment methods but they are too costly to operate and chemical treatment generates by product as part of treatment therefore waste water especially dyes containing waste water treatment by biological agent such as bacteria, fungi and algae or combined algal bacterial treatment is easy operating[1-5].

The bacterial and fungal treatment options are also required maintenance of microorganisms for its survivability; as compare to them the algae required no maintenance as it grows only on sunlight and carbon dioxide which helpful in the removal of nutrients from waste water and it does not generates secondary waste product, thus it becomes easy to operate, most cheaper and environment friendly method than other conventional treatment methods[6-10]. In the present study the first ever trial of reactive azo dyes treatment was taken under aqueous solution in laboratory condition at normal room temperature (25±5°C) by seaweed biomass *Kappaphycus alvarezii*. The used/accumulated alga is further studied for its biofertilizing potential thus it lowers the possibilities of by product/ secondary waste generation.

Materials and methods

Seaweed collection: The red seaweed biomass of *Kappaphycus alvarezii* was collected from Okha coast, Gujarat, India (Longitude - 68°20´ E to 70°40´ E Latitude - 22°15´ N to 23°40´ N) and immediately washed with seawater at source to remove unwanted debris, adhering sand particles and epiphytes then it was kept in icebox. Later on in the laboratory it was washed three to four times with distilled water to remove surface salt and impurities then it was kept on ambient conditions to make as room drying on normal room temperature for 4-5 days. This dried biomass of *Kappaphycus alvarezii* was used to study biodegradation and biodecolorization for further experiments.

Biodecolorization experiment: The dye powder samples were taken from the dyes industrial units of Padra, Ahmedabad and Khambhat. The three powder samples of reactive red-195, reactive yellow-145, and reactive black-5 (Figure-1) entitled as P_1, P_4 and P_6 was collected and prepared the aqueous solution under 0.4 % W/V of concentration of each dye powder. The red seaweed biomass of *Kappaphycus alvarezii* entitled as seaweed-C was inoculated under static ambient conditions. The Percentage biodecolorization was studied by UV-Visible spectrophotometer (Make - Shimadzu, Model - 1800). The decolorization efficiency was studied by formula given below.

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

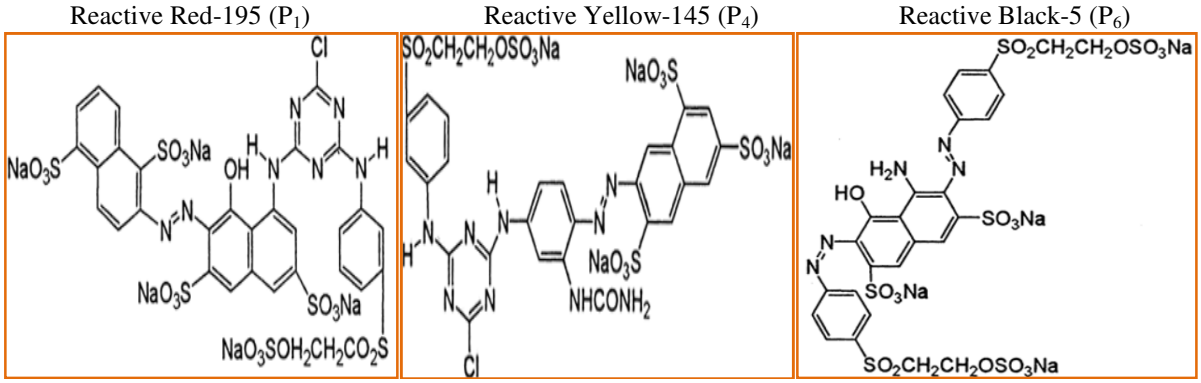


Figure-1: Structure of Dyes.

Spectrophotometer analysis: The Scanning was performed to study the maximum absorbance (λ max) wavelength of 0.4 % w/v of dye aqueous solution under 400 to 700 nm by UV-vis spectrophotometer (Make - Shimadzu, Model - 1800). These solutions were studied for decolorization (λ max) maximum absorbance was found at 542 nm, 418 nm and 595.5 nm for P_1, P_4 and P_6 respectively. The optical density was measured for 0.4 % dye solutions individually (Figure-2 and Figure-3).

Fourier transform infrared analysis (FTIR): The FTIR spectra of untreated and treated solutions of reactive azo dyes were taken with FTIR spectrophotometer (Make: Perkin Elmer, U.S.A., Model: Spectrum GX). It was obtained by KBr disk method. The samples were kept in oven for drying for 3 hours at 200°C temperature in petri plates and its dry powder was collected for analysis. The 2 mg of dried samples taken with 200 mg of KBr (spectroscopic grade) were carried out with additional crushing and prepared pallets by hydraulic pallet press and the spectra were scanned between 4000 to 400 cm^{-1} range under ambient conditions.

Biofertilizing potential: The accumulated algae can be utilized for biocompost therefore it is further studied for the biofertilizing potential. The mixture of dyes accumulated seaweed, cattle dung, dry and green leaves, kitchen waste were chopped in smaller sizes, prepare layer of soil, in next layer add moist cattle dung, add accumulated seaweed biomass, make 2-3 layers of wet and dry leafs, kitchen waste, waste fodder and sprinkle water to maintain moisture level upto 60-70 %, now cover the layer by cow dung slurry, kitchen waste, waste fodder repeat till the chamber is full cover the chamber by fodder waste and heap the soil till the shape gets convex and close the chamber for 6-8 weeks[11]. The biocompost is ready for the application in the field. The component such as organic carbon, nitrogen, phosphorus, potassium and carbon-nitrogen ratio were analyzed to study biofertilizing potential in percent by weight unit (%) and compared the data with Govt. guideline specifications of organic fertilizer [Schedule-4, {Clause 2(h) and (q)} Part-A] to determine its feasibility for application[12].

The prepared biocompost was studied for its potential effect on germination of *Vigna radiata* and *Triticum aestivum* seeds; the control was taken as without compost containing soil only and after the germination the pigment analysis such as chlorophyll a, chlorophyll b, total chlorophyll and carotenoid of control sp. and both the compost species was studied as qualitative analysis. Thus the experiment was carried out to determine the biofertilizing potential of accumulated seaweed biocompost for its applicability on the field as part of by product generated from waste water treatment system.

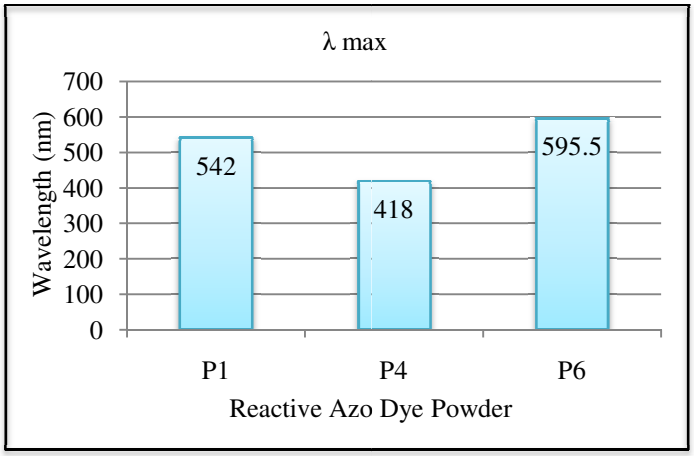


Figure-2: Spectrometer analysis for the determination of (λ max) maximum absorbance.

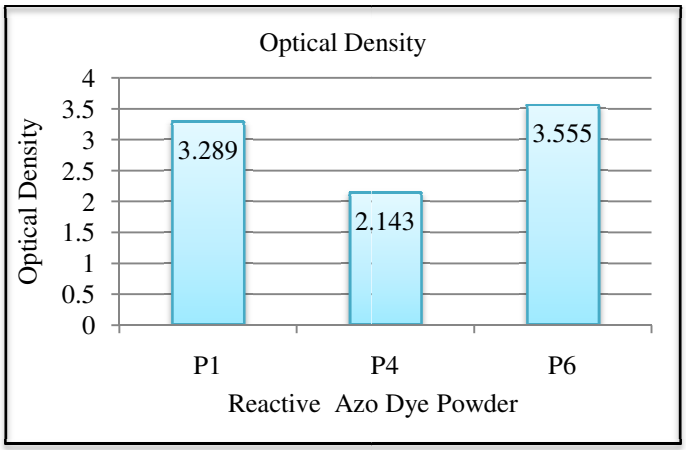


Figure-3: Spectrometer analysis for determination of Optical Density.

Results and discussion

Biodecolorization and Biodegradation study: Biodecolorization study shows 93.61 %, 90.66% and 21.94 % reduction of color from the dyes aqueous solution of P_1, P_4 and P_6 respectively (Figure-4) which indicates that the dry biomass of seaweed-C (*Kappaphycus alvarezii*) has potential capability for the color removal from aqueous solution therefore it can be used as decolorizing agent in the industrial waste water treatment system. The (λ max) maximum absorbance and Optical density is shown in Figure-2 and Figure-3. The decolorization of P_1, P_4 and P_6 shown in the Figure-5 represents the images of before and after treatment of dyes aqueous solution.

Fourier-transform infrared analysis (FTIR): The recorded FTIR spectrum gives information regarding functional groups variations for before treatment (control) and after treatment of dye solution (Figure-6 to Figure-11). The FTIR spectrum of dye solution indicates distinct and various adsorption peaks represents the existence of 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 and P6; These bands are resulted due to the structure of dye containing such functional groups. The comparison of the each spectrum with its original spectrum control indicates decrease in intensity of bands and band shifts which is reported in Table-1. The after dye treatment spectra clearly indicates potential removal of functional groups from the original dye (control) represents the degradation of dye. The complete removal of 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 were found absent after treatment of the dye as compared with control. The minor changes in the peak frequencies occurred because of adsorption of dyes onto seaweed biomass. The active sites in the cells of the biomass bind with the functional groups of dye and creats the bio transformation or degradation inside the cells causes break down of the dye structure resulted the decolorization and degradation[13,14].

Biofertilizing potential: The accumulated biomass were utilized for the biocompost preparation after the process the compost generated were analyzed for the total organic carbon, total nitrogen, total phosphates, total potash and C/N ratio as 20.45 %, 0.90 %, 0.30 %, 0.53 % and 22.72 respectively indicated in Table-2. The data were compared with the Indian Govt. standards of organic fertilizer which shows that the accumulated algal compost possess good potential as biofertilizer because the values are nearer to specifications indicates its applicability as biofertilizer.

The potential of accumulated algal compost were studied for its applicability as biocompost by experimenting germination of *Vigna radiata* and *Triticum aestivum* and its pigment analysis shown in Figure-12, Figure-13. The values indicates the good level of pigment present in the plants compare to control and germination was found better as compare to control as shown in images for both the species (Figure-14).

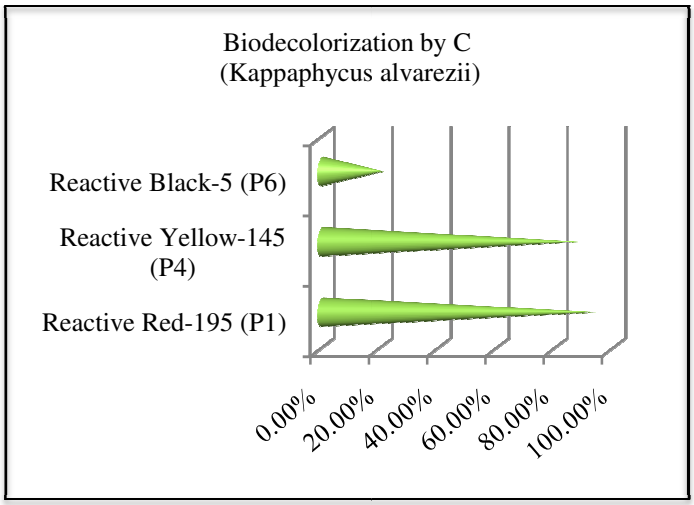
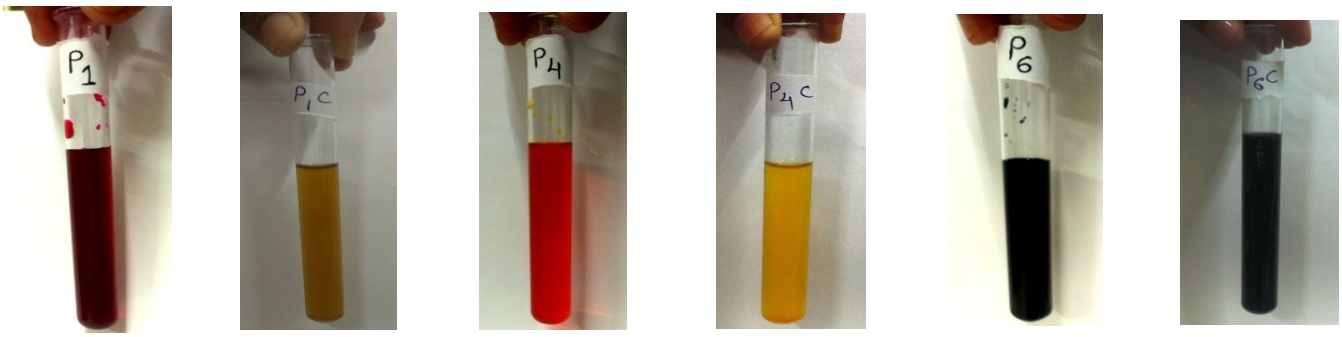


Figure-4: Percentage color removal of dyes from aqueous solution (Biodecolorization).



P_1 P_1C-after treatment P_4 P_4C-after treatment P_6 P_6C-after treatment

Figure-5: Image of control and after treatment of P_1, P_4 and P_6.

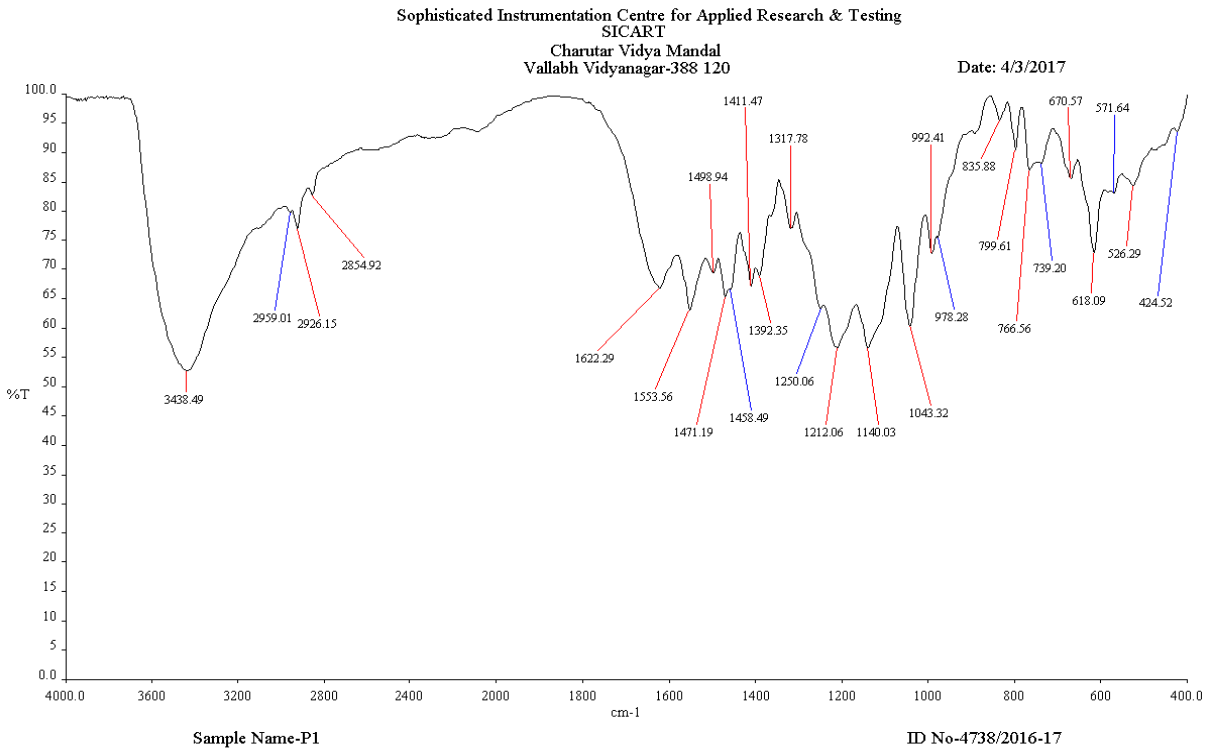


Figure-6: FTIR Analysis Chart of Conrol-P_1.

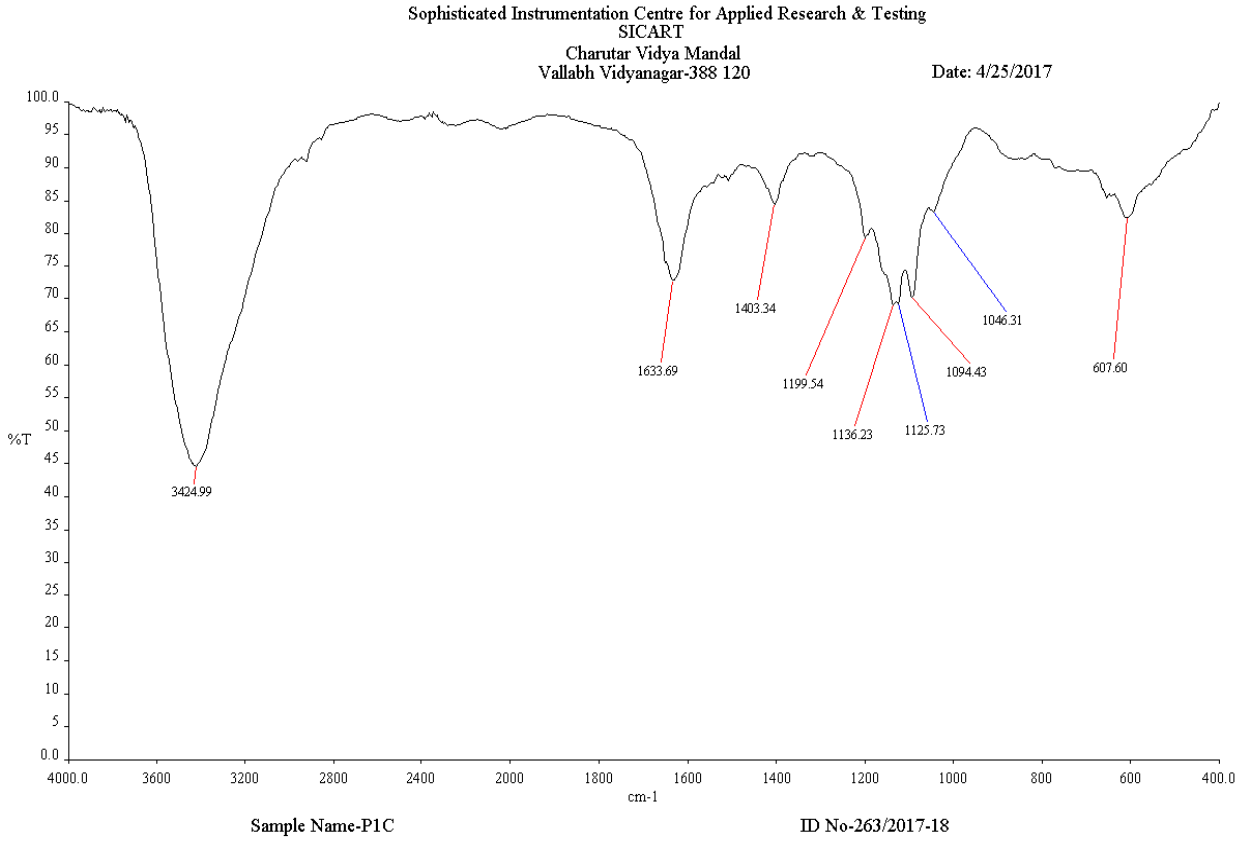


Figure-7: FTIR Analysis Chart of after treatment-P_1C.

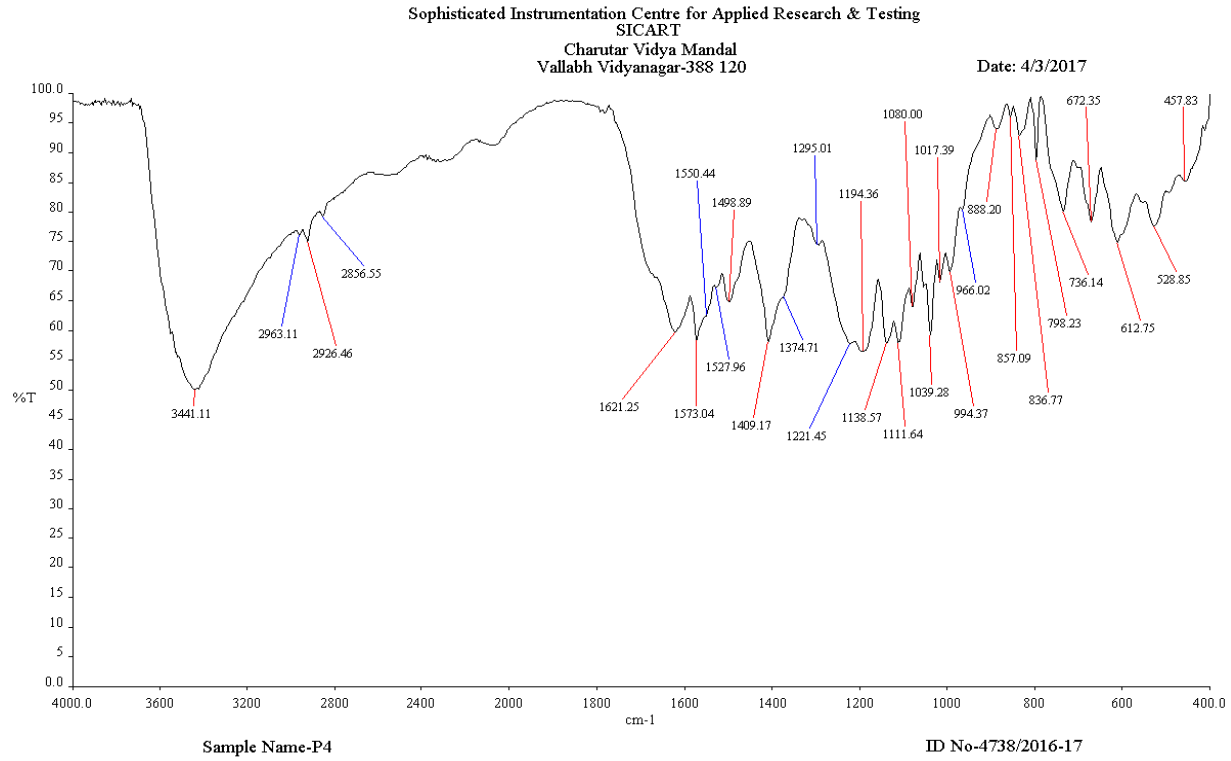


Figure-8: FTIR analysis Chart of Control- P_4.

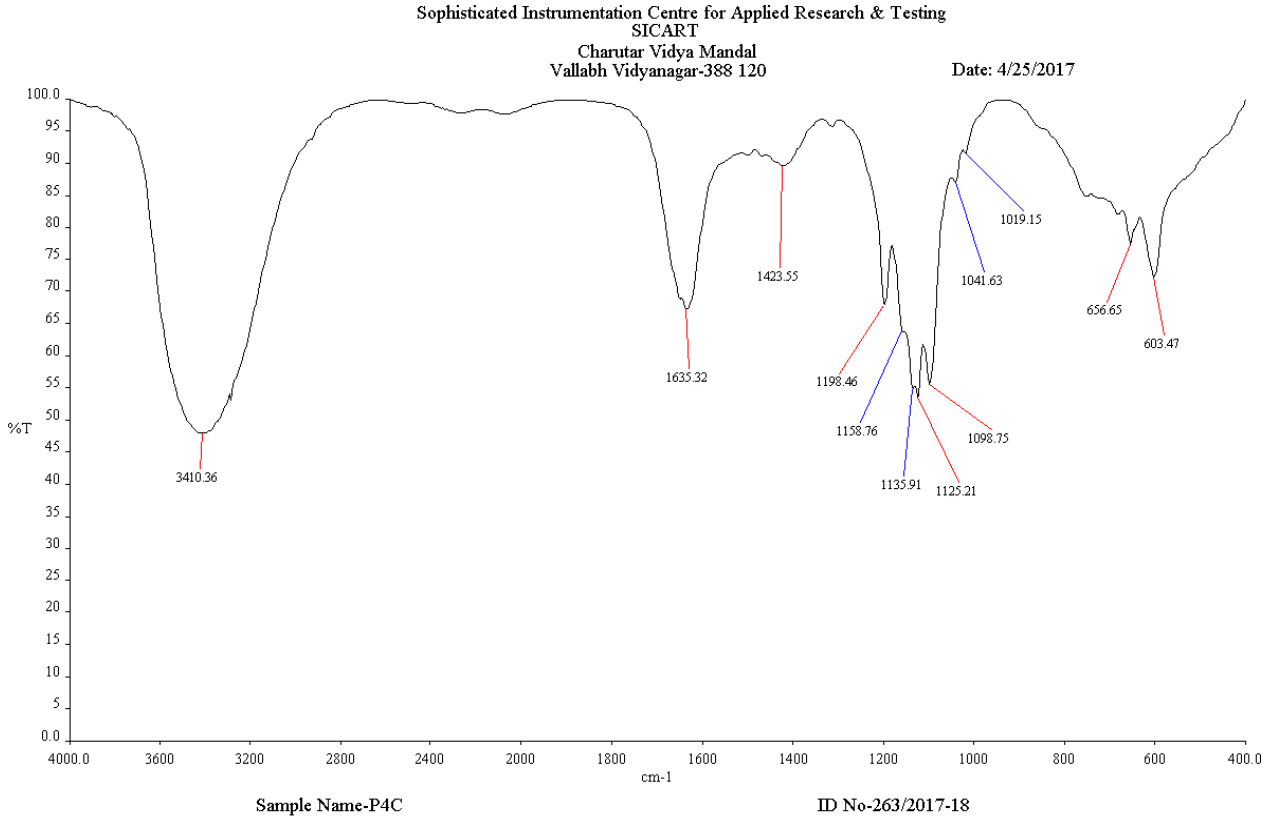


Figure-9: FTIR analysis Chart of Control- P_4C.

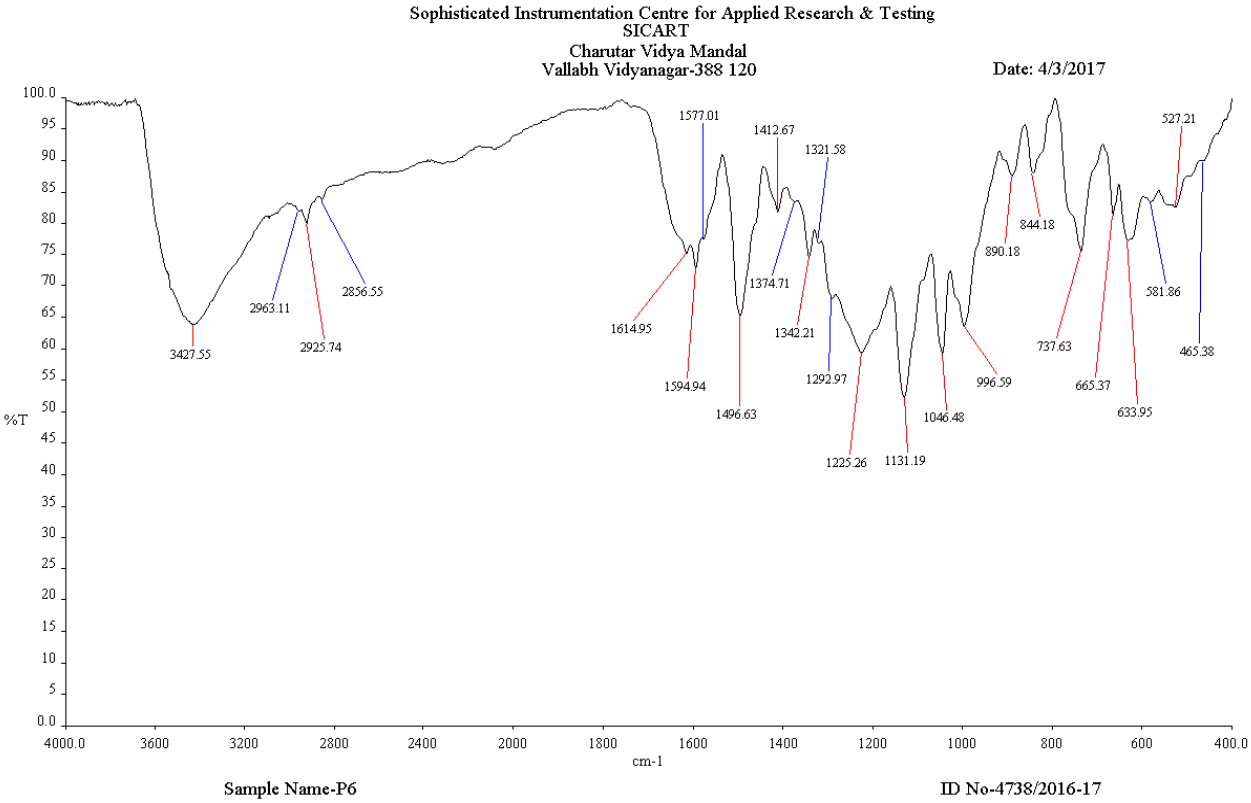


Figure-10: FTIR analysis Chart of Control- P_6.

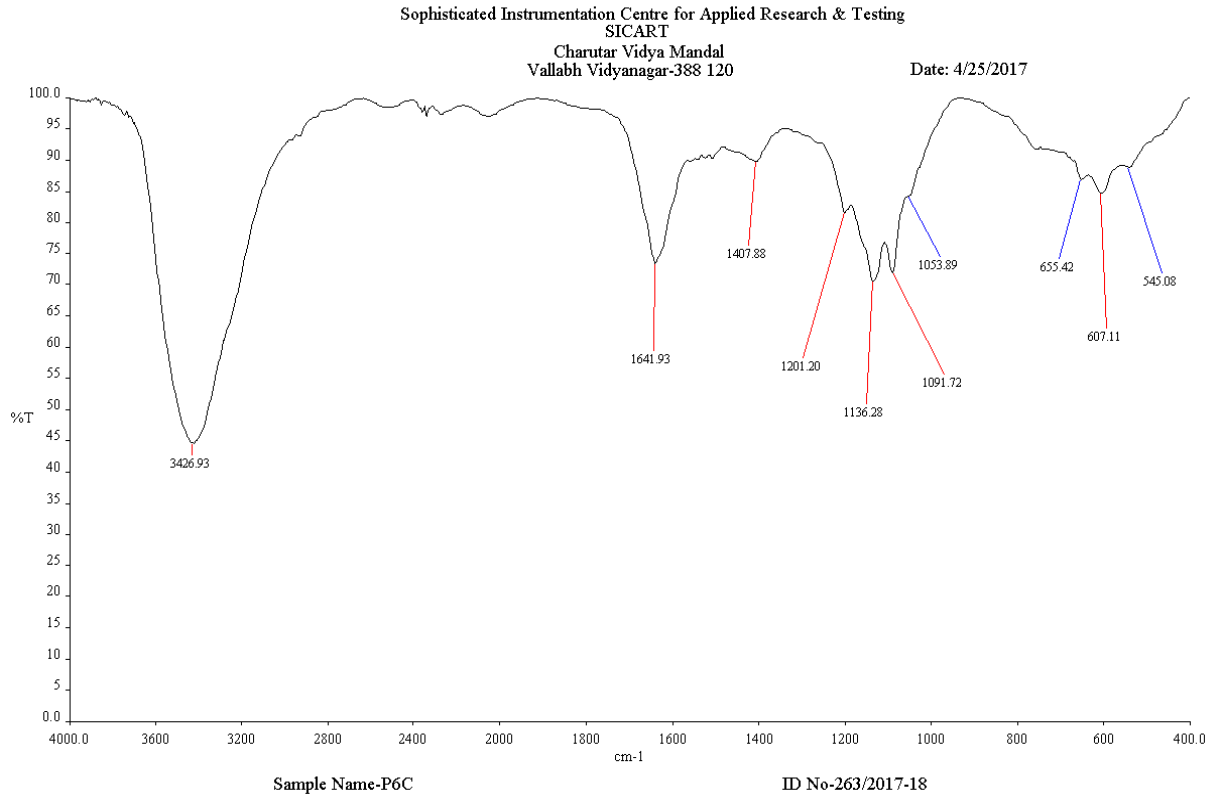


Figure-11: FTIR analysis Chart of Control- P_6C.

Table-1: Biodegradation of dyes by seaweed-C (*Kappaphycus alvarezii*).

Seaweed-C	Adsorption bands (cm^{-1})			Assignment
	Initial	Final	Difference	
P_1-Reactive Red 195	3438.49	3424.99	13.5	O-H stretching alcohol bonded
	1622.29	1633.69	-11.4	C=C conjugated alkene
	1471.19	1403.34	67.85	Methylene strong, O-H alcohol bending, S=O stretching sulphate,
	1212.06	1199.54	12.52	S=O stretching sulfate, S=O sulfonyl chloride
	1140.03	1136.23	3.8	S=O sulfone stretching, C-O tertiary alcohol stretching, C-O aliphatic ether stretching
	1043.32	1094.43	-51.11	C-O secondary alcohol stretching, C-N amine stretching
P_4-Reactive Yellow 145	3441.11	3410.36	30.75	O-H stretching alcohol intramolecular bonded
	1621.25	1635.32	-14.07	C=C alkane disubstituted (cis)
	1498.89	1423.55	75.34	O-H bending carboxylic acid
	1194.36	1198.46	-4.1	C-O stretching aromatic ester, C-O stretching tertiary alcohol
	1138.57	1125.21	13.36	C-O stretching tertiary alcohol, C-O stretching aliphatic ether
	1111.64	1098.75	12.89	C-N medium amine, C-O aliphatic ether, C-O stretching secondary alcohol
P_6-Reactive Black 5	3427.55	3426.93	0.63	O-H stretching alcohol bonded
	1614.95	1641.93	-26.98	C=N stretching imine/oxime
	1412.67	1407.88	4.79	O-H alcohol bending, S=O stretching sulfate
	1225.26	1201.20	24.06	S=O stretching sulfonyl chloride, C-O stretching alkyl aryl ether, C-N stretching amine, C-O vinyl ether
	1225.26	1136.38	88.98	S=O stretching sulfone, C-O stretching tertiary alcohol
	1131.19	1091.72	39.47	C-N amine, C-O aliphatic ether, C-O stretching secondary alcohol
	1046.48	1091.72	-45.24	C-O secondary alcohol stretching, C-N amine stretching

Table-2: Accumulated Algal Compost Analysis.

Biocompost Analysis	C (*Kappaphycus alvarezii)*	Specifications of Organic Fertilizer (Indian Govt. guidelines)
Total Organic Carbon	20.45%	12%
Total Nitrogen (as N)	0.90%	0.80%
Total Phosphates (as P_2O_5)	0.30%	0.40%
Total Potash (as K_2O)	0.53%	0.40%
C/N Ratio	22.72	< 20

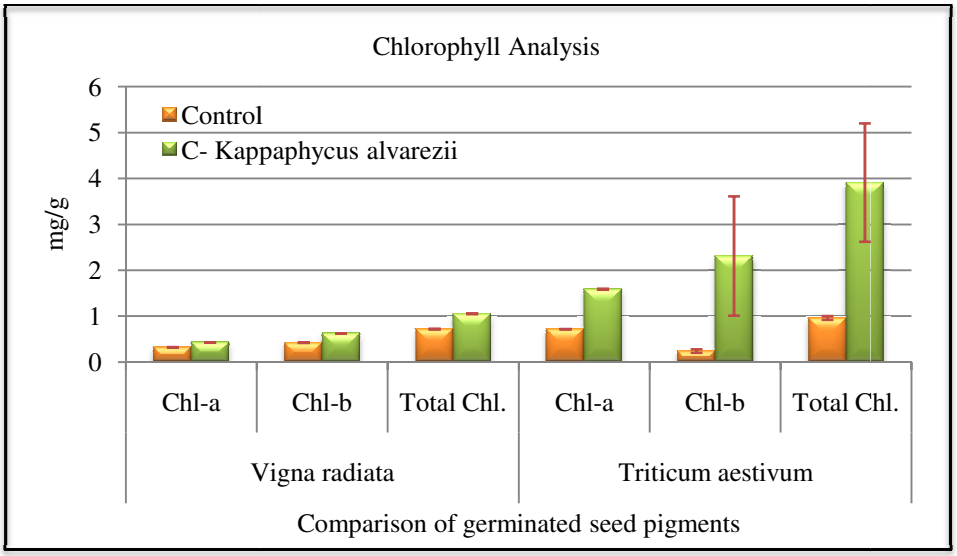


Figure-12: Biofertilizing potential on germination of *Vigna radiata and Triticum aestivum* (Chlorophyll content).

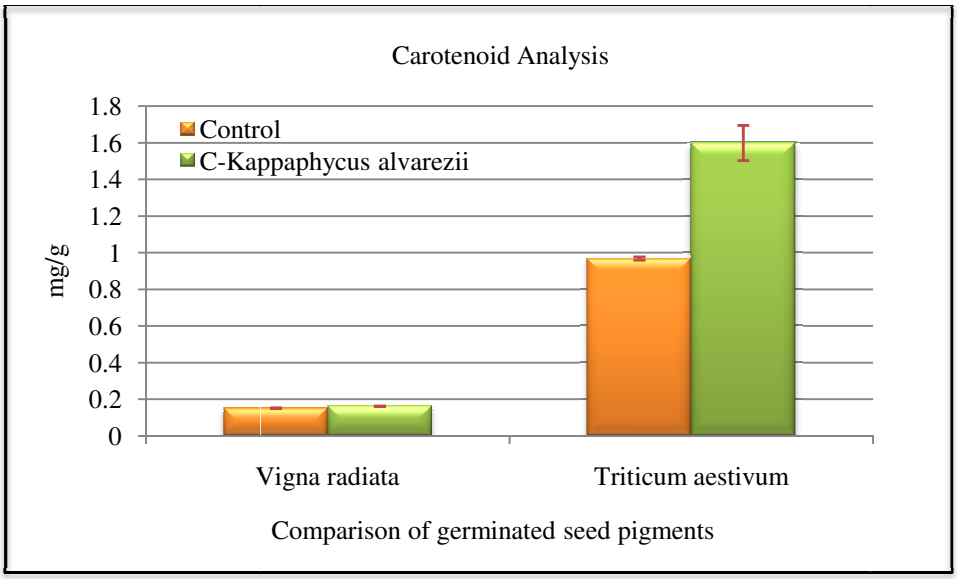


Figure-13: Biofertilizing potential on germination of *Vigna radiata and Triticum aestivum* (Carotenoid content).

Control ***Vigna radiata*** | Biocomposted ***Vigna radiata*** | Control ***Triticum aestivum*** | Biocomposted ***Triticum aestivum***

Figure-14: Images of germination of *Vigna radiata and Triticum aestivum.*

Conclusion

This present study shows the potential of dry biomass of seaweed-C (*Kappaphycus alvarezii*) for the dye solution treatment in the laboratory scale which gives excellent result in decolorization and degradation of dyes indicates this seaweed biomass can be utilized in further waste water treatment systems in industrial scale. The FTIR study concluded that the some of the dye functional groups were removed resulted the transformation or breakdown of the molecules of the dye, accumulates inside the active sites of the seaweed biomass creates potential result for the biodegradation as well as biodecolorization. This is the first attempt shown in paper to study degradation of reactive azo dyes by seaweed biomass *Kappaphycus alvarezii* generates environment friendly treatment option as the waste biomass can be used as biofertilizer creates sustainable approach, still the further study required to implement this technique in the field level.

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