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Investigation on biosorption of acidic dye from an aqueous solution by marine bacteria, *Planococcus* sp. VITP21

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Abstract: The marine microorganism *Planococcus* sp.VITP21 from Kumta costal region of Karnataka, India was used as biosorbent for the sorption of acidic industrial dye, Black TSB (BT). The adsorbent efficiency of the dye was studied by varying the different parameters such as pH, initial dye concentration, initial salt (NaCl) concentration and initial Cr (III) concentration. The isotherm studies were performed by agitating 0.5 gm (wet weight) of biomass with 50 ml of dye solution with different concentration (10 – 40 mg/l). The parameters were analyzed using Langmuir, Freundlich and Temkin adsorption isotherms. On comparison, Freundlich was found best fitted with regression coefficient value of .99 and the maximum biosorption capacity was found to be 9.6 mg/gm. The FT-IR and SEM were also used for characterizing the biosorption process by *Planococcus* sp. VITP21. The results showed that marine biosorbent, *Planococcus* sp. VITP21 has very good potential for removing acidic dye from aqueous solution under different operating conditions.
Keyword: *Planococcus* sp., marine microorganism, synthetic dye, adsorption isotherms, FT-IR, SEM and biosorbent.

Introduction

A huge amount of dyes containing waste water are generated from textile, paper, cosmetic, pharmaceutical or leather industry. These industrial effluents when released into the environment lead to pollution and affect human health[1]. The dye incorporated industrial effluents are one of the hardest wastewaters to be treated because of their high chemical and biological oxygen demand [2]. The amount of dyestuff and dye intermediate production of the entire world is estimated to be about 7.10^8 kg per annum[3]. Water effluents containing dyes have a very hazardous impact on environment and thus causing chronic and acute diseases [4]. Dyes with unfavourable compound like chlorides and aromatic compounds affect the photosynthetic ability by decreasing the light penetration power and also cause toxicity to different aquatic life[4]. Different classes of dyes are used in industries namely anionic, cationic, reactive and direct dyes. Anionic dyes are water soluble and bind covalently to the materials and hence they cannot be removed easily by conventional treatment techniques[5]. They also differ from the other classes of dyes because of their stable nature against heat, light, biodegradation and oxidizing agents[5]. Thus the receiving water is destroyed and contaminated, urging for alternate treatment methods[6]. The conventional or physico chemical methods of dye removal are of low efficiency and high operational cost but methods like adsorption and bio sorption has proved to be effective with high adsorption capacity[7]. Among the various adsorbents available, activated carbons are one of the most competent methods but the main disadvantage of using activated carbon is that it is not cost effective[8]. Bio sorption differs from adsorption in the way that the adsorbent material being used is living or dead microorganism or its derivatives. Many biomasses were reported for biosorption such as *Aspergillus niger*[9], *Aspergillus foetidus*[10], *Phanerochaete chrysosporium*[11] and *Rhizopus arrhizus*[12]. However biosorption by marine bacteria have not been reported extensively though they have wider application for bioremediation of dye effluent rich in NaCl. Henceforth in the present study an investigation was performed for biosorption of anionic industrial dye, Black TSB from aqueous solution by using marine bacteria, *Planococcus* sp.VITP21 under different operating conditions.

Experimental

Biosorbent

Marine bacteria, *Planococcus* sp. VITP21[13], an isolate from Kumta coast of Karnataka, India was used as adsorbent in the present study. Bacterial culture was grown for 24 hours in Luria Bertani media under optimal condition (35ºC, 7 pH, 120 rpm and 4 % w/v NaCl), centrifuged at 10000 rpm for 15 minute and the biomass (pellet) was used as biosorbent material. 0.5 gm (wet weight) of biosorbent was used in all the studies for biosorption of dye.

Dye solution preparation

The acidic industrial dye, Black TSB (Able Advanced Chemicals, Taiwan) was purchased from local shop and used for the present investigation. The aqueous solution of dye was prepared by dissolving 1000 mg of appropriate dye in 1 litre of distilled water and used as stock solution for all the experiments.

Batch biosorption experiment

The batch adsorption studies were carried out by treating 50 ml of dye solution with 500 mg (wet weight) of biomass in Erlenmeyer flask for 22 hours of incubation at 120 rpm and 298 K in an orbital shaker for all the experiments. The effect of pH on biosorption was studied at 3, 5, 7 and 9 pH with initial dye concentration of 40 mg/l. The effect of initial dye concentration (10 to 40 mg/l) on biosorption and adsorption isotherm study for different initial dye concentration (10 to 40 mg/l) was carried out. The study on effect of different salt concentration (2, 4, 6 and 8 % (w/v) NaCl) on biosorption of 40 mg/l of dye concentration by biosorbent was investigated. Cr (III) salt effect on biosorption of anionic dyes was studied in the presence of different doses of chromium (50,100,150 and 200 mg/l) for the removal of 40 mg/l of dye. Further all the experiments were carried out at pH 3 and pH was adjusted using 0.1 N HCl and NaOH solutions, prior to addition of biosorbent. U-2800 Spectrophotometer was used to determine the maximum wavelength for Black TSB and the wavelength was observed to be at 493nm for Black TSB[14]. The initial and final absorbance was estimated at 493nm, after samples were centrifuged at 10000 rpm for 15 minutes. The percentage removal of dye and amount of dye adsorbed (in mg/g) were calculated using the following relationships:

$$\text{Percent Removal} = \frac{\text{Initial absorbance} - \text{final absorbance}}{\text{initial absorbance}} \times 100$$

$$\text{Amount adsorbed } (q_e) = \frac{C_o - C_e}{m}$$

Where C_o and C_e are the initial and the final dye concentration (mg/l) respectively, m is the mass of biosorbent (mg/l).

Scanning electron microscopy and FT-IR analysis

The morphology of the surface of biomass of *Planococcus* sp.VITP21 (biosorbent) before and after adsorption of dye were investigated[15] using the scanning electron microscope (SEM, ZEISS model). FT-IR analysis was done in a range of 500-4000 cm^{-1} to identify the active sites present on the surface of the bio sorbent and their interaction[16]. The pure biosorbent and biosorbent loaded with dye were dried at 50ºC for a time period of 24 h and mixed with KBr and spectrum was recorded[17].

Results and Discussion

Biosorption experiment

Effect of pH

pH is an important factor to increase the rate of sorption of industrial dye, Black TSB from the aqueous solution. The investigation on the effect of initial pH on biosorption was performed for pH 3, 5, 7 and 9 with the initial dye concentration of 40 mg/l (Figure 1). The results showed that the maximum removal was 76 % at pH 3 and from pH 5 to pH 9, removal efficiency decreased from 75 to 69 % at equilibrium time of 22 hours. The possible reason for the effectiveness of the lower pH in the dye biosorption onto *Planococcus* sp.VITP21 biosorbent may be due to the fact that at lower pH, more protons will be available for the protonation of the adsorbent surface which resulted in increasing the electrostatic interaction between the positively charged adsorbent sites and the negatively charged anionic dyes [18].

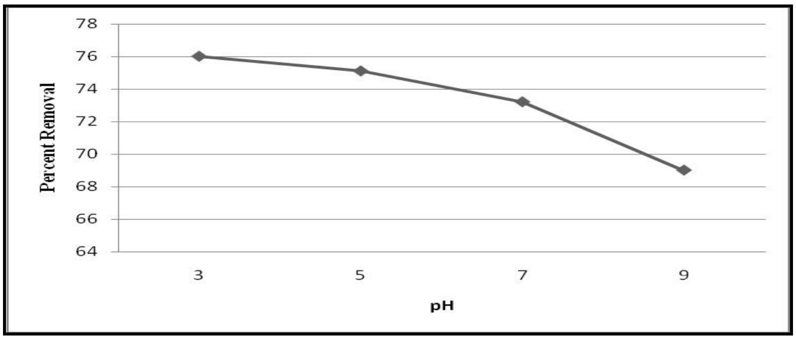


Figure 1: Effect of pH (3, 5, 7, and 9) on adsorption of Black TSB (40 mg/l) from aqueous solution by biosorbent, *Planococcus* sp. VITP21 at 298^{0}K. Condition: Time - 22 hours, agitation rate -120 rpm

Effect of Initial dye concentration

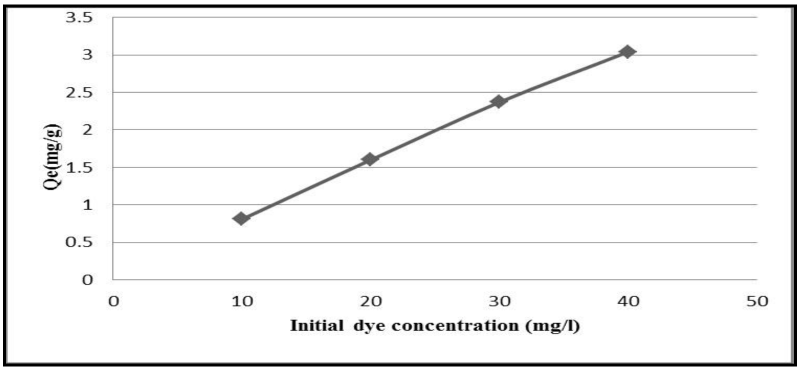


Figure 2: Effect of initial dye concentration (10 - 50 mg/l) on biosorption of Black TSB by biosorbent, *Planococcus* sp. VITP21 at 298^{0}K. Condition: Time - 22 hours, pH 3, agitation speed -120rpm.

The effect of initial dye concentration (10 to 40 mg/l) on biosorption of Black TSB is shown in figure 2. The amount of dye adsorbed per amount of adsorbent (q_e) value increased with increase in dye concentration (10 to 40 mg/l) from 0.81 to 3.04 mg/g. The adsorption capacities of biosorbent increased because of an increase in the driving force associated with concentration gradient with an increase in initial dye concentration[19, 20].

Effect of different salt concentration

The effect of varying initial salt concentration (NaCl) on percentage removal of dye was carried out by adding different NaCl concentration (2%, 4%, 6% and 8% (w/v)) to 40 mg/l of dye solution, Black TSB (Figure 3). The experimental results revealed that the percent removal increased from 78 to 88% with the increasing salt concentration (2 to 8% w/v). The increase in biosorption under saline condition may be due to increase in ionic strength and the electrostatic interaction [16, 21].

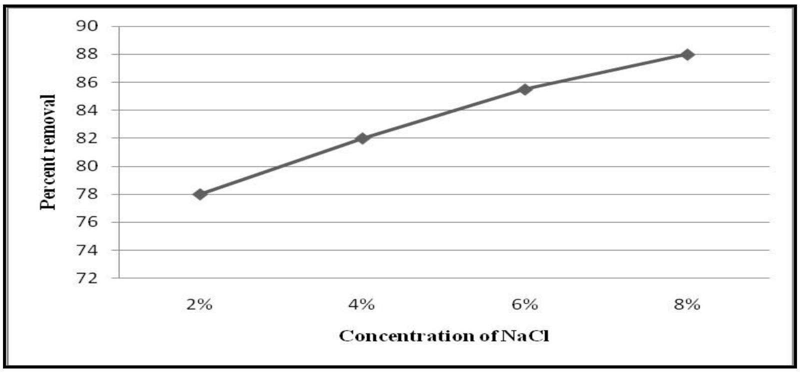


Figure 3 : Effect of initial salt concentration (2 – 8% w/v) on biosorption of Black TSB dye (40 mg/l) from aqueous solution by biosorbent, *Planococcus* sp. VITP21 at 298^0K. Condition: Time - 22 hours, pH 3, agitation speed- 120 rpm.

Effect of different chromium (III) concentration

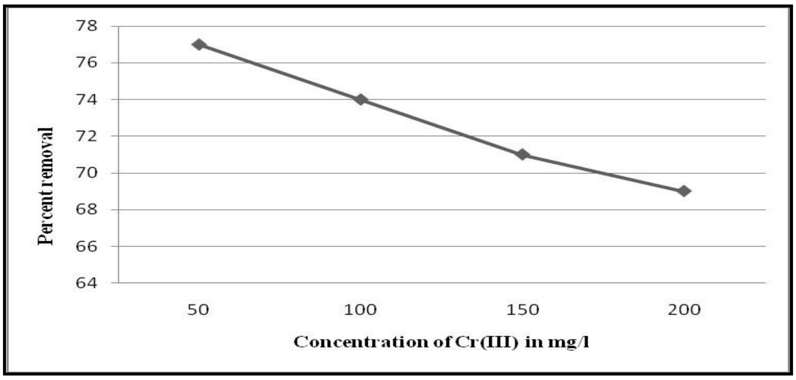


Figure 4: **Effect of initial Chromium (III) concentration (50 -200 mg/l) on biosorption of Black TSB dye (40 mg/l) by biosorbent, *Planococcus* sp. VITP21 at 298^0K. Condition: Time - 22 hours, pH 3.**

The effect of different Cr (III) concentration (50,100,150 and 200 mg/l) on biosorption of Black TSB dye (40 mg/l) was carried out. The results showed that the biosorption decreased from 77% to 69% with increasing chromium (III) concentration from 50 to 200 mg/l (Figure 4). The decrease in biosorption capacity in the presence of Cr (III) ions may be due to higher affinity of these ions on the dye binding sites of adsorbent material[16].

Adsorption isotherm studies

Langmuir adsorption isotherm

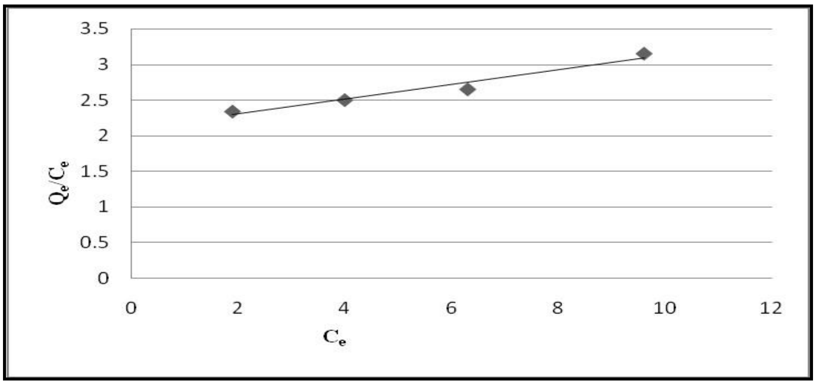


Figure 5: Langmuir isotherm of biosorption of Black TSB (10- 40 mg/l) by biosorbent, *Planococcus* sp. VITP21 at 298^0K. Condition : Time - 22 hours, pH 3.

The theory of Langmuir assumes the formation of saturated monolayer of adsorbate molecules on a surface of adsorbent with no transmigration across the plane of the surface and with constant adsorption energy[23]. The linearized form for the Langmuir adsorption isotherm is:

$$\frac{C_e}{q_e} = \frac{1}{bQ_m} + \left(\frac{1}{Q_m}\right) C_e$$

Where C_e is the solute concentration (mg/l) at equilibrium, q_e is the amount of adsorbate which has been adsorbed at equilibrium (mg/g), b is the Langmuir constant related to the biosorption energy (l/mg), Q_m is the maximum sorption capacity with complete monolayer coverage (mg/g). The linear plot of C_e/q_e vs Ce shows that the adsorption of Black TSB obeys Langmuir adsorption model with the R^2 value of 9.6 (Figure 5). Q_m (9.6 mg/g) and b (0.05 l/mg) were determined from the slope and the intercept of the plot and comparative adsorption capacity (Q_m) of different biosorbents is given in table 1. The analysis of the Langmuir equation is done by estimating a dimensionless equilibrium parameter R_L which is given by the expression [24], $R_L = \frac{1}{1+bC_e}$. The value of R_L is greater than zero and less than one, the adsorption is considered as favourable and if R_L is equal to 1, linear adsorption takes place. Irreversible adsorption and unfavourable adsorption take place respectively when R_L is equal to 0 and greater than 1.The R_L value for the Black TSB is 0.34, which is between 0 and 1 that refers the process as favourable [14, 25, 26].

Table 1: Comparative adsorption capacity (Q_m) of different biosorbents

Biosorbent/adsorbent	Experimental conditions	Adsorption capacity (Q_m in mg/g)	Reference
A. wentii (Methylene Blue)	C_o = 8.0 mg/l, pH=6.3	3.30	[27]
A. niger (Acid Blue 29)	C_o = 50 mg/l, pH=6.0	1.17	[28]
Stoechospermum Marginatum (Acid Black 1)	C_o= 50 mg/l, pH=2.0	6.57	[17]
Streptomyces rimosus (Methylene Blue)	C_o= 50 mg/l	9.86	[29]
Planococcous sp.VITP21 (Black TSB)	C_o= 40 mg/l, pH=3	9.6	This study

Freundlich adsorption isotherm

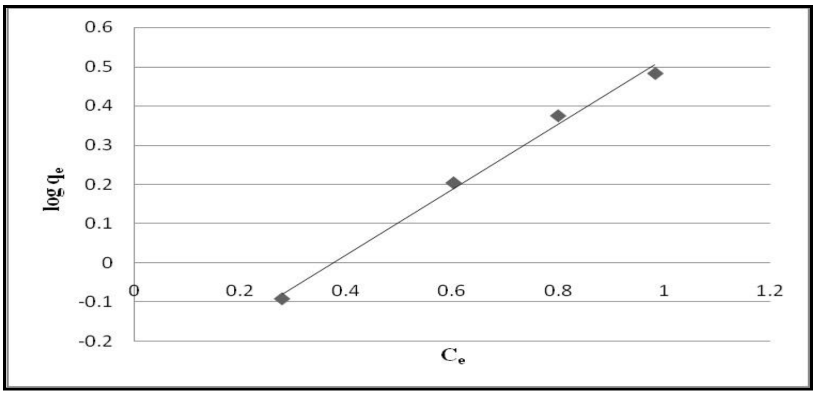


Figure 6: Freundlich isotherm of biosorption of Black TSB (10- 40 mg/l) by biosorbent, *Planococcus* sp. VITP21 at 298^{0}K. Condition: Time - 22 hours, pH 3.

For the study of heterogeneous surface energy systems[30], the Freundlich adsorption isotherm is used. The Freundlich equation assumes that concentration of dye on the surface of adsorbent will increase continuously as long as there is an increase in the dye concentration in the interaction medium[31]. The linearized form for the Freundlich isotherm is:

$$\log q_e = \log K_f + \frac{1}{n}\log C_e$$

Where, K_f (l/mg) is the Freundlich constant and is related to the adsorption capacity of the adsorbent[4]. 1/n is the measure of adsorption intensity or surface heterogeneity and with the value ranging between 0 and 1 is said to be favourable[6]. K_f and n is determined from the linear plot (R^2 = 0.99) of log q_e versus log C_e (Figure 6). In the present study, K_f is 0.489 l/mg and 1/n is 0.8 which indicates a favourable adsorption. All the values are in accordance with the reported literature[32].

4.5.3 Temkin adsorption isotherm

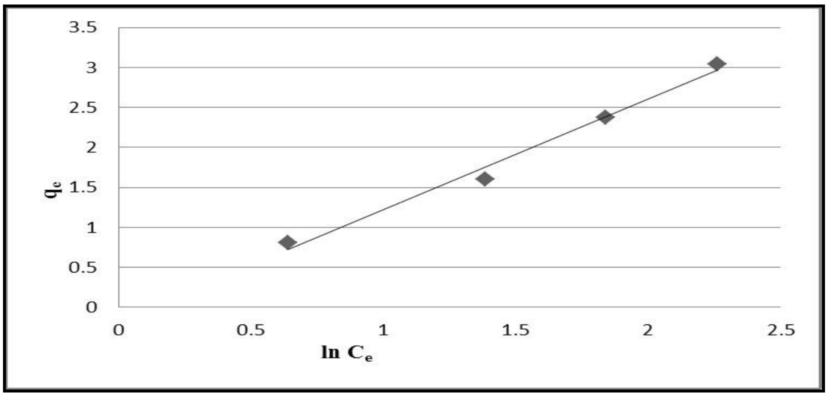
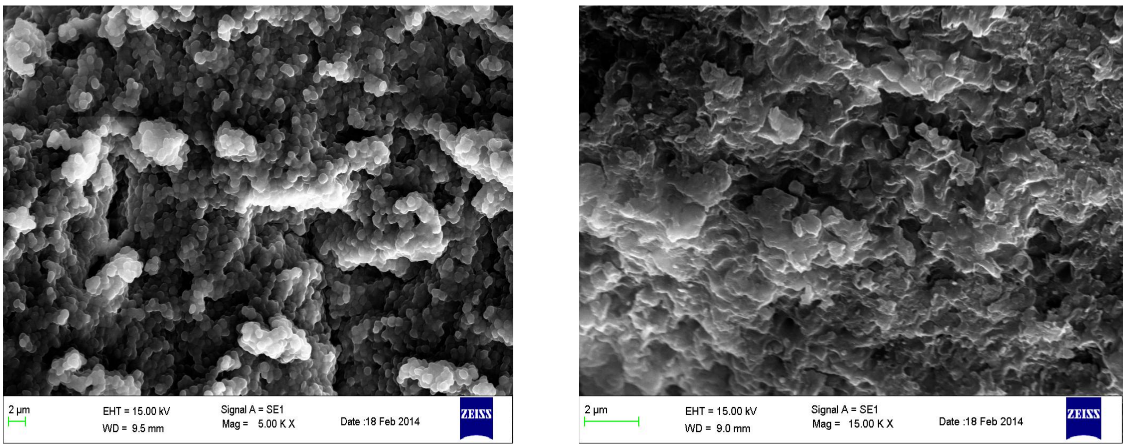


Figure 7: Temkin isotherm of biosorption of Black TSB (10- 40 mg/l) by biosorbent, *Planococcus* sp.VITP21 at 298^{0}K. Condition: Time -22 hours, pH 3.

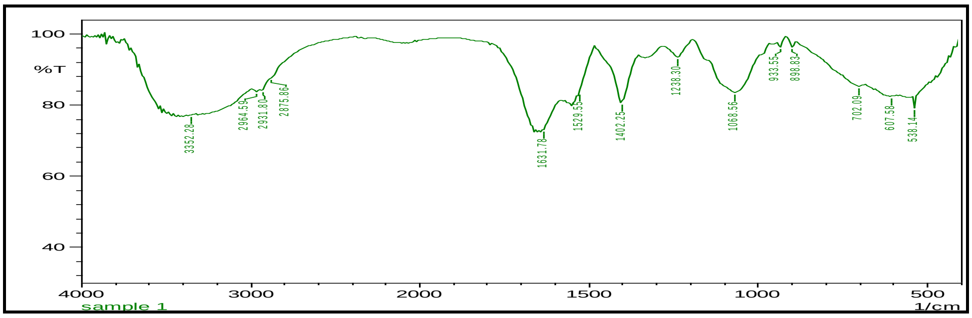
The Temkin adsorption isotherm assumes that the heat of adsorption increases linearly on a surface[30] and the adsorption is assumed to be characterized by uniformly distributed binding energies, up to some binding energy which is maximum[33]. The linearized form of Temkin adsorption isotherms is : $q_e = B\ln A_t + B\ln C_e$, where $B = R.{}^{T}/_{b_t}$, A_t is the Temkin isotherm equilibrium binding constant (l/g), b_t is the Temkin constant related to heat of sorption (J/mol), R is universal gas constant (8.314J/mol K), T (K) is absolute temperature . The plot of q_e versus ln C_e (R^2 = 0.987) is used for calculating constant A_t and B (Figure 7). The value of B is 1.381 J/mol and A_t is 0.89 l/gm and the values obtained are in accordance with the literature [33, 34] and this shows that the adsorption is fitting well in Temkin isotherm. A positive value of b_t (1794.04) indicates that the adsorption process is exothermic[35].

FTIR and SEM analysis

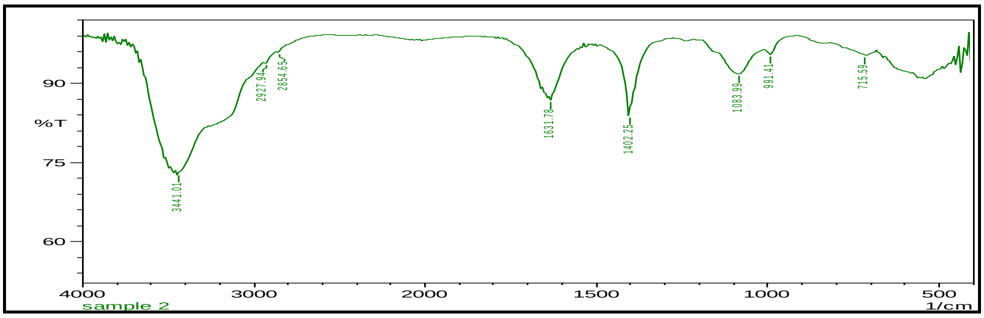


(a) (b)

Figure 8: SEM image of (a) unadsorbed biosorbent,*Planococcus* sp.VITP21 and (b) Black TSB adsorbed biosorbent,*Planococcus* sp.VITP21.



(a)



(b)

Figure 9: FTIR spectrum of (a) unadsorbed biosorbent,*Planococcus* sp.VITP21 and (b) Black TSB adsorbed biosorbent,*Planococcus* sp.VITP21

The SEM (Scanning Electron Microscope) imaging revealed the morphological changes due to biosorption of Black TSB on the surface of biosorbent, *Planococcus* sp.VITP21[36]. Before adsorption, the spherical shaped clusters of cells were observed (Figure 8(a)) but after the adsorption, the surface completely flattened due to adsorption of Black TSB dye (figure 8(b)) as reported in the literature[37].

The FT-IR spectrum of unadsorbed and adsorbed biosorbent, *Planococcus* sp.VITP21 with dyes, Black TSB was performed to understand the possible biosorbent-dye interaction (Figure 9). Table 2 shows the corresponding functional group of IR frequencies. The shift in the band region 3352.28, 2964.59, 2875.86, 1631.78 and 1068.56 indicates the stretching vibration of different functional group, N-H, O-H, CH_3, C=O and C-O due to interaction with the dye (17, 39 and 40). P=O, P-O-C and P-OH stretching was also observed in the region of 933.55[38].

Table 2: FT-IR peaks of unabsorbed and adsorbed biosorbent, *Planococcus* sp.VITP21 with Black TSB, Black TSB

Unadsorbed biosorbent (Wavenumbers in cm^{-1})	**Dye adsorbed biosorbent (Wavenumbers in cm^{-1})**	**Assignment**	**Reference**
3352.28	3441.01	-OH and –NH group	[17]
2964.59	2927.94	C-H stretching	[17]
2875.86	2854.65	CH_3 group interaction	[39]
1631.78	-	C=O group	[17]
1068.56	1083.25	C-O stretching	[40]
933.55	991.41	P=O,P-O-C,P-OH stretching	[38]

Conclusion

The investigation revealed the biosorption potential of biosorbent, *Planococcus* sp. VITP21. The biosorption process in the present study was pH dependent and the presence of NaCl and Cr (III) did not affect biosorption process. Adsorption studies for the dye were analyzed by Langmuir, Freundlich and Temkin adsorption isotherms and on comparison, Freundlich was found best fitted. The FT-IR and SEM were used to characterize biosorption process by *Planococcus* sp. VITP21. The maximum biosorption capacity was found to be 9.6 mg/g for Black TSB. The experimental results revealed that the marine biosorbent, *Planococcus* sp. VITP21 has very good capacity of dye removal from aqueous solution.

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