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RESEARCH ARTICLE

ENTEROMORPHA INTESTINALIS: LOW COST BIOSORBENTS FOR BIOSORPTION METHYLENE BLUE

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ABSTRACT

In the present investigation, the use of low-cost, abundantly available, highly efficient and eco-friendly biosorbent *Enteromorpha intestinalis* (L.) Knee has been reported as an alternative to the current expensive methods of removing of Methylene Blue (MB) dye from aqueous solution. The effects of different variables such as pH, agitation time, adsorbate concentration, adsorbent dose and agitation speed etc. were investigated and optimal experimental conditions were analysed. The Langmuir isotherm model has given a better conformity than the Freundlich model with 197.74mg/g as maximum adsorption capacity at room temperature. The adsorption of MB on dried biomass of *E. intestinalis* was confirmed by FTIR, SEM and EDX study, as it showed the change in characterization before and after adsorption. The pseudo- second order model provided a better approximation to the system's kinetics, while intra particle diffusion study was used to furnish the mechanistic study. Present investigation and comparison with other reported adsorbents concluded that, *E. intestinalis* may be applied as a low-cost attractive option for removal of MB from aqueous solution.

INTRODUCTION

With a rapid development of chemical, polymer and petroleum industries, the amount and variety of chemicals that are discharged into water bodies are also increased. Several synthetic chemical dyes are in use for colouring their products, and their amount produced annually is about $7x10^5$ metric tons per year (Celekli *et al.*,2013). Effluents from cosmetics, printing, dyeing, food colouring and paper making industries are contaminated with dyes which generates wastewater, characteristically high in colour and organic content.

The textile industry alone accounts for two third of the total dye stuff production (Azhar *et al.*,2005; Garg *et al.*,2003). The contaminants in wastewater even at a very small concentration of less than 1ppm of dye are highly toxic, undesirable and may be carcinogenic causing serious hazards to aquatic ecosystem (Banat *et al.*,1996; Robinson *et al.*,2001; Vijayraghavan and Yan, 2008). Physico-chemical methods used for removal of these dyes such as coagulation, ultra filtration, electro-chemical adsorption, photo-oxidation, activated carbon adsorption are not convenient (Kannan and Sundaram, 2001; Bhattacharya and Sharma, 2004; Aksu *et al.*,2008). Use of low cost, easily available biomaterials for the adsorption of dyes is practiced as an alternative method and several botanical, low cost materials have directly been used as an adsorbent for removal of dyes from wastewater (Jayaraj *et al.*,2011; Hameed and El-K Haiary, 2008; Jain and Sikarwar, 2006).

In present study aqueous solution of M.B. was used as a model compound to moniter biosorption using dried biomass of green seaweed *Chaetomorpha media* as adsorbents. The purpose of this work was to evaluate and compare adsorption capacity of selected green seaweed with respect to the effect of pH, contact time, adsorbent dose, initial concentration of dye, agitation speed on the process of dye adsorption.

MATERIALS AND METHODS

Preparation of adsorbent and dye solution

Fresh and mature thalli of *Enteromorpha intestinalis* were collected from Kunakeshwar and Malvan in Sindhudurga District along the West coast of Maharashtra during growing season (August to February). This material was washed, dried in shade at room temperature, powdered using grinder and then

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passed through different sieves to obtain fine (0.1 to 0.84 mm) particles. The powdered material was stored in airtight plastic containers at room temperature and used for batch experiments.

Stock solutions having 1 g/L concentration of Methylene Blue was prepared in distilled water. Biosorption experiment was set up in an Erlenmeyer flask containing weighed biomass and dye solution. Absorbance of dye solution was recorded before and after the batch experiment on a UV-VIS spectrophotometer at the corresponding λ_{max} (663 nm for M.B.). The amount of dye adsorbed was calculated using the difference between the dye concentration in solution before and after the biosorption process.

Batch adsorption experiment

Effect of physico chemical factors was recorded by changing pH, agitation time, adsorbate concentration, adsorbent dose and agitation speed in the batch experiments. Optimum parameters for maximum adsorption were determined for each dye from these experiments.

In order to explain the mechanism of the process of biosorption Langmuir and Freundlich isotherm models, Pseudo first order and Pseudo second order rate kinetics and intra particle diffusion models were followed.

Determination of dye removal efficiency

The percent removal of dye during biosorption process was determined using the following formula.
Dye Removal percent = $(C_i - C_e) / C_i \times 100$

Uptake of dye during the biosorption process was calculated as follows

$q_e = (C_i - C_e) \times V / M$

Where C_i is initial concentration of dye (mg/L),C_e is final concentration of dye (mg/L), M is weight of adsorbent (mg), V is volume of adsorbate (ml), q_e is amount of dye adsorbed (mg/g)

Characterization of adsorbent

Characterization of biosorbent algae was made before and after biosorption using FTIR, SEM and EDX techniques.

RESULTS AND DISCUSSIONS

1 Effect of pH: The range of pH of the dye solution used to observe M.B. adsorption was 1to12. In *E. intestinalis* the removal of M.B. was 26 % which became 81% by increasing pH from 1 to 6. Afterwards removal decreased to 57% by increasing pH to 12. (Fig.1).

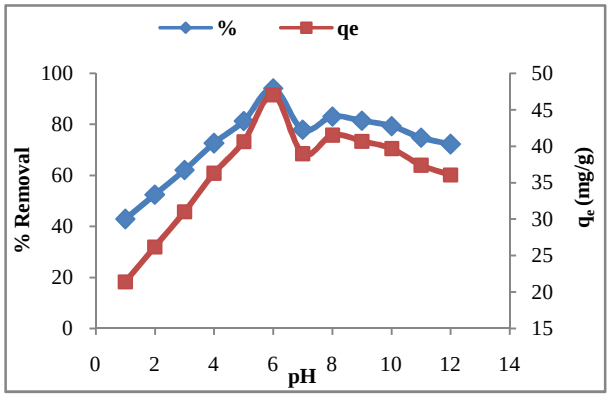


Fig.1 Effect of pH on biosorption of M.B.

Hammud *et al.* (2011), found maximum removal of M.B. by *Carolina sp.* at 6.8 pH. Khaled *et al.* (2005) could see maximum M.B. removal at pH 8 by *Ulva lactuca*. Tahir *et al.* (2008) obtained maximum M.B. removal at pH 7 with *Ulva lactuca* and *Sargassum* species. Rubin *et al.* (2005) also recorded maximum M.B. removal at pH 7 using *Sargassum muticum*. However Vilar *et al.* (2006) found that M.B. uptake was unaffected in the pH range of 4 to10 but was impaired at low pH when algal biomass based material of *Gelidium* was used.

Effect of agitation time

It was recorded using 100 mg biomass in 100 mg/L aqueous M.B. solution having pH 6 at room temperature. It was observed that adsorption of M.B. was rapid during the first 30 minutes for all materials and thereafter the rate of biosorption decreased slowly and became constant after 60 minutes. The maximum removal (86.47%) took place within 60 minutes. (Fig. 2).

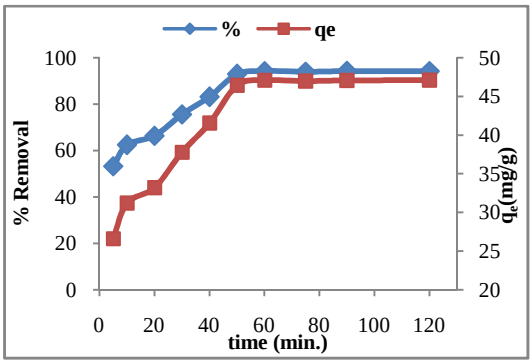


Fig.2 Effect of agitation time on biosorption of M.B.

The time curves showed that the removal of adsorbate was initially rapid but gradually became slow and reached equilibrium after one hour. This is due to a large number of vacant surface sites available for adsorption during the initial stage.

The remaining vacant surface sites are difficult to be occupied after a specific time interval due to repulsive forces between the solute molecules on solid and bulk phases (Ahmad *et al.*, 2009). After attending the equilibrium the percent sorption of M.B. did not change with further increase in time. Thus longer

treatment might not have further effect on biosorption. A similar observation was made for M.B. adsorption onto hazelnut shells by Dogon *et al.* (2009). Caparkaya and Cavas (2008) have reported equilibrium time of 210 minutes for M.B. adsorption on brown alga *Cystoseira barbatula*.

Effect of adsorbate concentration

Effect of initial concentration of dye on adsorption was analyzed at optimum pH 6, using 100mg biomass and keeping 60 minutes agitation time. The concentration of dye was varied from 100 to 1000 mg/L. It was observed that the adsorption was dependent on initial concentration of dye. As the concentration of M.B. increased the uptake (q_e) also increased however removal of dye was decreased by 25 to 30%. Maximum removal and uptake occurred at 100 mg/L concentration from 17.38 to 197.74 mg/g in *E. intestinalis*

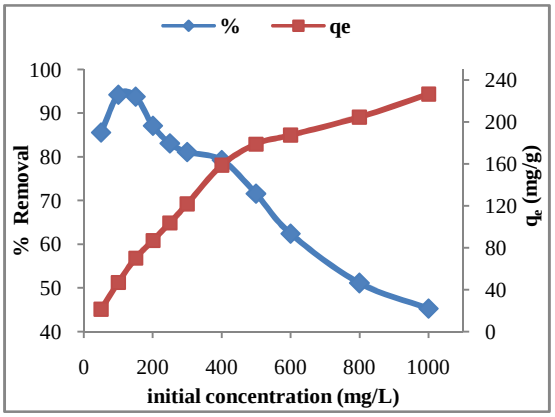


Fig.3 Effect of adsorbate concentration on biosorption of M.B.

From the present study it is evident that as the dye concentration increased, its uptake was increased due to an increase in the driving force. Initial concentration provides an important driving force to overcome all mass transfer resistance of dye between the aqueous and solid phases. Hence initial concentration of dye will enhance the adsorption process (Hameed, 2009).

Khaled *et al.* (2005) have reported increased removal of M.B. by *Ulva lactuca* with increasing dye concentration. Tahir *et al.* (2008) also observed improvement in removal of M.B. by *Ulva lactuca* and *Sargassum* species, when M.B. concentration was increased from $3x10^{-6}$ to $3x10^{-4}$ mole/ L.

Effect of adsorbent dosage

Effect of biomass dose on M.B. removal was carried out by increasing the amount of biomass from 50 to 500mg at optimum pH, using 100mg/L dye solution at room temperature. Agitation time was fixed to 60 minutes. Dye removal increased when amount of biomass was increased from 50 to 100 mg. Further increase in biomass did not improve the removal and uptake of M.B. Maximum biosorption occurred with 100 mg biomass in the algae. Percentage of dye removal varied from 68.86 to 83.9 in *E. intestinalis* was recorded (Fig. 4).

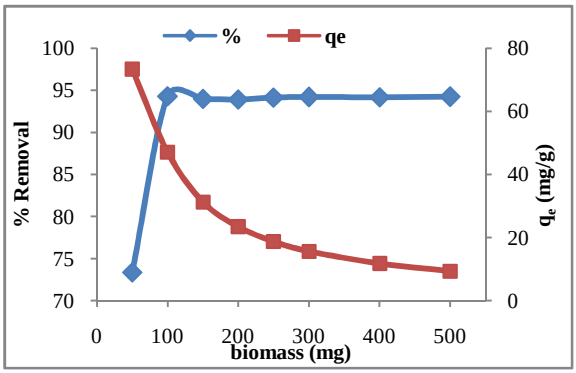


Fig.4. Effect of adsorbent dosage on biosorption of M.B.

These result is due to the fact that the active sites are effectively utilized when the biomass is less. With increased amount of biomass, some of the available active sites may remain uncovered because of limited number of adsorbate molecules leading to lower specific uptake (Ponnusami *et al.*, 2009).

According to Franca *et al.* (2009), an increase in adsorbent mass enhances surface area, thereby increasing the number of active adsorption sites. The amount of dye adsorbed per unit mass of adsorbent decreases with increasing adsorbent mass. Cengiz and Cavas (2008) recorded a sharp increase in the adsorbed dye by increasing dose of *Caulerpa racemosa var. cylindracea*

Effect of agitation speed

In the batch experiment, agitation speed acts as an important factor by affecting the external boundary film and distribution of the solute in the bulk solution. The batch experiment was carried out at an optimum pH using 100mg/L dye concentration and 100mg biomass. Agitation time was kept 60minutes and the shaking speed was increased from 50 to 250 rpm. Results are represented in Fig. 5.

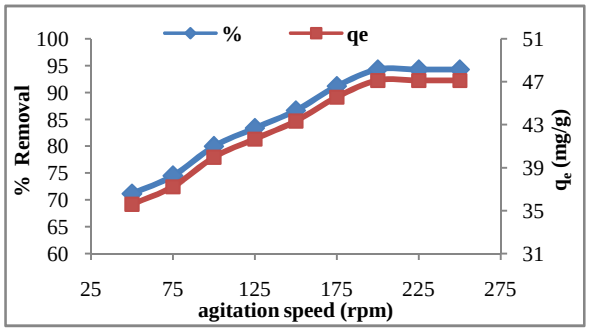


Fig.5 Effect of agitation speed on biosorption of M.B.

The removal of M.B. and its adsorption efficiency increased slowly with respect to speed up to 200 rpm. Thereafter percentage and efficiency remained constant. In *E. intestinalis* removal of dye was about 84 %.

Khaled *et al.* (2005) reported maximum removal of M.B. (40.2 mg/g) by *Ulva lactuca* at agitation speed of 200 rpm. Kumar *et al.* (2006) recorded optimum agitation speed of 175 rpm for M.B. removal by *Pithophora* species. According to Ong *et al.* (2007) higher agitation speed boosts up the uptake of dyes by

adsorbent as the film resistance adjacent to the sorbent particles decreases and enhances the adsorption process.

Adsorption isotherms: Adsorption isotherms provide qualitative information of the nature of solute - surface interaction at constant temperature. They explain distribution of the adsorption molecules between the liquid phase and solid phase at equilibrium state of an adsorption process (Yang and Qiu, 2010)

Langmuir and Freundlich are the most frequently employed to describe the relationship between the amount of dye adsorbed and its equilibrium concentration.

Langmuir Isotherm*:* Langmuir adsorption isotherm is plotted as C_e versus C_e/q_e where C_e and q_e are equilibrium adsorbate concentrations in the aqueous and solid phases respectively. b is the equilibrium constant related to the energy of adsorption. The values of q_m (mg/g), b (L/mg) and R^2 (Regression correlation coefficient) are evaluated from equation of Langmuir plot and presented in Table 1. The fitness of isotherm models describing the type of adsorption was determined by correlation coefficient values R^2. Higher R^2 value (near to unity) indicates the fitness of the isotherm model.

R_L is an important characteristic of the Langmiur isotherm described as the separation factor. The values of R_L ranged from 0.873 to 0.997 in *E. intestinalis.* As these values lie between 0 to1, the process of adsorption was said to be favourable.

Freundlich isotherm

Freundlich model suggests a multilayer adsorption involving heterogenous sorption with different classes of adsorption sites (Arvindhan *et al*., 2007; Singh *et al*., 2006).

A plot of log C_e versus log q_e produced a straight line with 1/n and K_f values determined from slope and intercept respectively. The K_f value is related to adsorption capacity and 1/n value is related to the adsorption intensity. A higher value of K_f represents a greater adsorption capacity. In the present study, the value of correlation coefficient (R^2) was 0.87 in *E. intestinalis.* The values of K_f, n and R^2 of Freundlich are given in Table 1.

Table1 Isotherm constants for biosorption of M.B. by ***E. intestinalis***

Experimental q_m (mg/g)		197.74
Graphical q_m (mg/g)		200
Isotherm Constants		
Langmuir	**b**	0.011
	R^2	0.970
	R_L	0.998 - 0.873
Freundlich	**n**	1.901
	K_f	8.035
	R^2	0.873

The values of correlation coefficient obtained from Langmuir isotherm were found more than 0.991indicated a good agreement between the parameters and confirmed a monolayer adsorption of M.B. with adsorbent surface. By the comparison of calculated and experimental values of q_e and R^2 it was concluded that Freundlich model was not suitable for experimental data in the present study. The calculated and experimental values of q_e and R^2 obtained for the Langmuir model were in correlation with each other.

Adsorption Dynamics

In order to investigate the mechanism in adsorption process pseudo first order and pseudo second order kinetic models were used.

Pseudo first order kinetic model

A linear plot of log (q_e -q_t) versus t suggests pseudo first order kinetics. The value of kinetic constant (K_1) was calculated from the slope of the plot. The calculated values of K_1 and q_e for algal biosorbent are presented in the Table 2. By comparing the calculated and experimental values, it was seen that the pseudo first order models was not applicable to this study.

Pseudo second order kinetic model

As pseudo first order model was not applicable, the adsorption data were analyzed for pseudo second order kinetics. The rate parameters K_2 & q_e were directly obtained from intercept and slope of plot t/q_t versus t.

In present study the calculated values of K_2 and q_e for initial concentration of 100 mg/L of M.B. and experimental values of q_e are given in the Table 2. The value of correlation coefficient obtained from plots was 0.964 for *E. intestinalis.* The results suggested that the pseudo second order model provided a better approximation to the experimental kinetic data than the pseudo first order model.

Table 2 Kinetic parameters for biosorption of M.B.

q_e (Experimental)		43.235
Pseudo first order kinetics	**q_e (Graphical)**	29.7166
	K_1	$18x10^{-3}$
	R^2	0.658
Pseudo second order kinetics	**q_e (Graphical)**	47.619
	K_2	$0.44x10^{-3}$
	R^2	0.964

Based on pseudo second order kinetics, uptake of M.B.by seaweeds was assumed to be controlled by chemical process or chemisorptions which involved valence forces through sharing or exchange of electrons (Hameed, 2009; Thinakaran *et al*., 2008). The occurrence of chemisorptions is controlled by strong intra particle bonding such as ionic and covalent bonding and contributes to the irreversible adsorption process. (Allen and Koumanova, 2005).

Intra particle diffusion study

According to Dogan *et al.* (2004), the adsorbate species are most probably transported from the bulk of the solution into the solid phase through intra particle diffusion of transport process which is the rate limiting step in many adsorption processes.

The intra particle diffusion rate constant (K_{id}) was determined from the slope of the linear gradient of plot q_e versus $t^{1/2}$. The values of intercept indicated a greater boundary layer effect. The R^2 values were close to unity representing the application of this model.

The rate constants of intra particle diffusion are $\mathbf{K_{id}}$ (5.686) and $\mathbf{R^2}$ **(**0.854). According to Bhattacharyya and Sharma (2004), if graph line of plot q_t versus $t^{1/2}$ passes through the origin then it is assumed that the intra particle diffusion is the sole rate limiting step. Since this was not observed in the present investigation, it may be concluded that the surface adsorption and intra particle diffusion were concurrently operating during the biosorption of M.B.

Confirmation of biosorption

Fourier Transform Infrared Spectroscopy

In the present investigation infra red spectra of algal biomass before and after biosorption of M.B. were obtained. The main functional groups involved in biosorption process and present on the algal materials were characterized with this analysis. Adsorption peaks from FTIR spectra suggested a complex nature of algal biomass. Presence of several groups and compounds such as amine, carboxyl, carbonyl, phosphoryl, hydroxyl, amide, sulfate, disulfide, lignin, ester, pectin, cellulose, cutin, phosphine were involved in the process of biosorption. The FTIR spectra of algal biomass (before and after biosorption) are shown in Fig. 6.

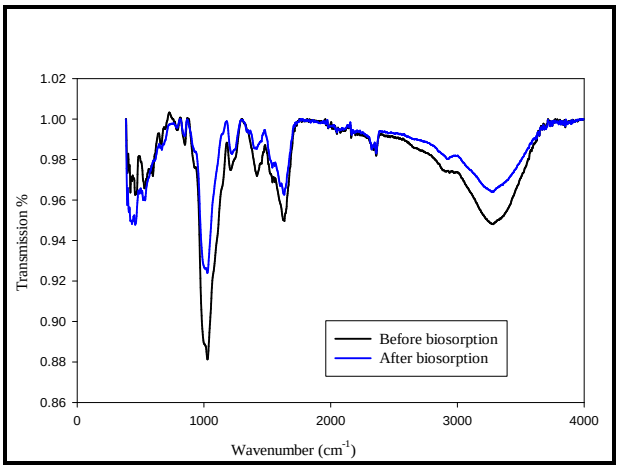


Fig.6 FTIR spectrum of *E. intestinalis* biomass before and after biosorption of M.B.

The variation in IR spectra due to shifting and merging was observed in *Carolina* sp. during biosorption of M.B. (Hammud *et al*., 2006). Sarwa and Verma (2013), observed that some peaks are shifted and some new peaks are emerged after adsorption of dye on micro algae. El-Jamal and Ncibi (2012) confirmed the participation of aromatic ring and C-N aromatic tertiary amine groups in M.B. removal by *Chaetophora elegans*. According to Kannan and Senthamilselvi (2014), carboxyl, phosphoryl, hydroxyl are the prime constituents of seaweeds which are involved in the uptake mechanism. According to Tan (2011), N-H, C=O, S=O and O-H belonging to amino, carboxyl, sulfonic acid were found to coincide with the alginic acid and sulfonate from fucoidon on the surface of seaweed which help in removal of basic dyes from aqueous solution.

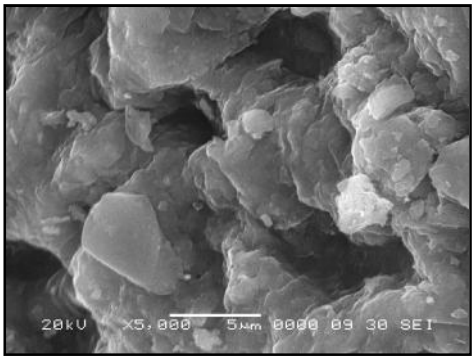
In present study phosphine, phosphate, polysaccharide, amino acids, sulfates, disulphide, ester, pectin, cutin, sulfates, cellulose, carbohydrates, sulfonides and glucose were found actively involved during biosorption of M.B.

Scanning Electron Microscopy

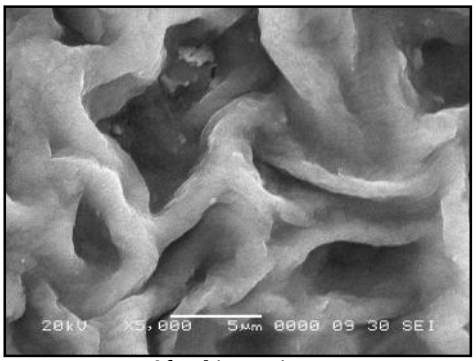
Scanning Electron Microscopy technique was used for analysis of surface structure characteristics. The photomicrographs were recorded using SEM JEOL model JSM6360A at 5000x magnification with accelerating voltage of 20 kv.

Significant morphological differences were observed in the surface structure of adsorbent before and after biosorption of M.B. In *E. intestinalis*, the surface before biosorption appeared porous and rough. After biosorption a smooth but thick layer was seen deposited on the pores (Plate.I).

Alginate has a considerable number of pores where there is a good possibility for dyes to be trapped and adsorbed in dye removal (Mahmoodi, 2011). According to Fakhry (2013) a high surface porosity with numerous macropores and mesopores with rough surfaces in *Padina pavonica* accelerate dye adsorption. Vilar *et al.* (2006) have recorded changes in surface porosity in *Gelidium* due to M.B. adsorption.



Before biosorption



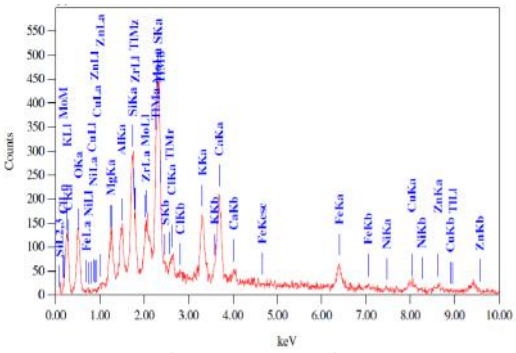
After biosorption

Scanning electron photomicrographs showing biosorption of M.B.

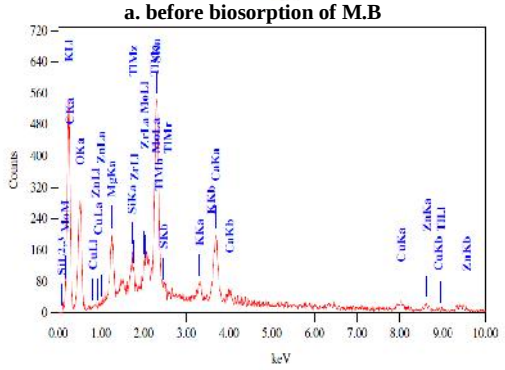
Energy Dispersive X-ray Analysis

In the present study various elements were identified from biomass before and after the biosorption. The peaks of carbon, oxygen and sulfur were recorded in EDX spectrum.

Fig.16. EDX spectra of *E. intestinalis*



a. before biosorption of M.B



b. after biosorption of M.B.

The amount of C, O, Cu, Mo, Fe, Zn, Zr and Ti was increased while that of Mg, Cl, K, Ca, S, Na was found decreased after biosorption in all biomaterials. Therefore it can be said that chemical bonding may be the main mechanism in the adsorption process rather than the ion exchange. Yang *et al.* (2011) also concluded that chemical bonding was the main mechanism in metal adsorption by red alga *Palmaria palmate*.

CONCLUSIONS

Dye adsorption was favorable at weakly acidic pH by *E. intestinalis*. Maximum uptake of dyes took place within one hour in *E. intestinalis*. The shifting of peaks in FTIR spectrum confirmed the MB dye adsorption onto *E. intestinalis*. The SEM study also made support to it by observing difference in surface morphology of adsorbent before and after adsorption of MB. The adsorption equilibrium data showed good fit to the Langmuir isotherm model as compared to the Freundlich isotherm model. The adsorption kinetics followed pseudo second-order kinetic equation for sorption of MB onto *E. intestinalis*. Finally it is concluded that, the present adsorbent could be a good alternative for the removal of MB from aqueous solution very effectively and economically.

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