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BIOSORPTION OF AMARANTH DYE FROM AQUEOUS SOLUTION BY ROOTS, LEAVES, STEMS AND THE WHOLE PLANT OF *E. crassipes*

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Abstract

In the present work, the kinetics of amaranth dye biosorption onto the roots, stems, leaves and the whole plant of water hyacinth (*Eichhornia crassipes*) were studied for differential evaluation of the plant's biosorptive potential to remove the toxic dye from aqueous solutions. *E. crassipes´* leaves showed the highest level of amaranth dye biosorption (43.1 mg/g), followed by the entire plant (31.18 mg/g), the roots (28.51 mg/g), and finally by the stems (23.97 mg/g). The same differential trend was observed for the initial volumetric rate of amaranth dye biosorption. The kinetics modeling of amaranth dye biosorption by the roots, stems, leaves and entire *E. crassipes* plant showed good agreement of experimental data with the pseudo-second-order model, which indicates that the rate-limiting step of the biosorption process is the most probably chemisorption. FTIR analysis results suggest that amaranth dye molecules interact with the amide I and amide II functional groups, which are present in the proteins of the vegetative organs and entire aquatic plant. Proximate chemical analysis revealed higher content of total protein in *E. crassipes´* leaves than in other vegetative organs. A linear relationship was found between total protein content and amaranth biosorption capacity at equilibrium, which indicates that the proteins play a crucial role in amaranth dye biosorption from aqueous solution by *E. crassipes*. *E. crassipes'* leaves may be used as a low-cost, effective and environmentally friendly biosorbent to detoxify amaranth dye-polluted wastewaters.

Key words: Amaranth dye, biosorption, *Eichhornia crassipes*, FTIR

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1. Introduction

Water source contamination by xenobiotic organic compounds is a major factor in global environmental pollution. Azo dyes are an important class of xenobiotic pollutants, and their presence in aquatic environments is of great concern because of their potential health hazards associated with their toxic, allergenic, mutagenic, carcinogenic and genotoxic properties (Akar et al., 2008; Zaharia et al., 2012). They also show adverse effects on the natural equilibrium of aquatic ecosystems resulting from reduced oxygenation of the water bodies, and low photosynthetic activity and viability of aquatic plants due to water coloration (Bhatnagar and Minocha, 2010; Hasani-Zonoozi et al., 2008; Pandey et al., 2007).

Amaranth [trisodium 2-hydroxy-1-(4-sulphonato-1-naphthylazo) naphthalene-3,6-disulphonate; acid red 27; E123] is an anionic azo dye, which is widely used for coloring natural and synthetic fibers, paper, wood, leather, cosmetics, phenol-formaldehyde resins, as well as in photography, and as a food and beverage additive (Anjaneya et al., 2013; Shahmoradi et al., 2011). However, there is growing concern regarding the use of this dye because it can cause acute and chronic toxicity. It has been well established that high

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concentrations of amaranth dye can adversely affect human and animal health and cause tumors, allergic and respiratory problems, as well as birth defects in the human being (Mittal et al., 2005). This dye is also suspected to be a mutagenic, carcinogenic and genotoxic agent (Jabeen et al., 2013; Jadhav et al., 2013).

In vivo, amaranth dye is degraded by intestinal microorganisms, and its toxic or carcinogenic effects may derive from the microorganisms' degradation products (Ahmad and Kumar, 2011). Considering its potential for hazardous toxicity, amaranth dye has been banned or restricted in several countries (Jadhav et al., 2013; Karkmaz et al., 2004). Therefore, the removal of amaranth dye from contaminated water and wastewater is crucial to protect the environment as well as public health and well-being.

However, the treatment of azo dyes-polluted wastewater by physicochemical methods is a difficult task because these dyes are extremely soluble in water and stable to light, heat and oxidizing agents (Bhatnagar and Minocha, 2010; Colar et al., 2012; Mittal et al., 2005). In addition, the physicochemical treatment methods have several drawbacks in terms of operational problems, high cost, and chemical sludge production (Anjaneya et al., 2013).

Biotechnological processes have received increasing attention as viable alternatives for the detoxification of azo dyes-containing wastewaters owing to their cost-benefit, effectiveness, lower sludge production and environmental friendliness (Chen et al., 1999). In this context, biodegradation of azo dyes by microbial populations has been the basis of many conventional and emerging treatment technologies. Nevertheless, azo dyes are recalcitrant organic molecules, highly resistant to aerobic degradation, and the products of their biodegradation (aromatic amines) under anaerobic conditions may be more toxic and carcinogenic than the parent dyes themselves (Ahmad and Kumar, 2011; Asgher and Bhatti, 2010).

In contrast, biosorption has been demonstrated to be potentially effective for removing dyes from aqueous solutions. There are several advantages in using biosorption processes for the treatment of dye-contaminated wastewaters, such as simplicity of operation, low cost, reliable treatment efficiency, and absence of by-products. A number of non-conventional low-cost biosorbents, including by-products and wastes from industry, agriculture and forestry, have been proposed for this purpose (Asgher and Bhatti, 2010; Crini, 2006).

The water hyacinth (*Eichhornia crassipes*) is a free-floating aquatic plant belonging to the family Pontederiaceae, found abundantly throughout the year in most tropical and subtropical countries of the world. This plant is considered a nuisance because it reproduces rapidly, forming dense mats that interfere with irrigation, fishing, shipping, recreation and power generation, and it is also difficult to eradicate (Hasan et al., 2010; Mahamadi, 2011). In addition, the dense mats of *E. crassipes* reduce light penetration and oxygen transfer into water bodies, thereby affecting the photosynthetic and respiratory activity of aquatic organisms (Carrión et al., 2012). Practical applications for the large amount of available *E. crassipes* biomass have been actively searched for.

A potential beneficial use for the *E. crassipes* biomass is as biosorbent for the removal of toxic pollutants from aqueous solutions, since *E. crassipes* biomass is able to biosorb heavy metals (Hasan et al., 2010; Valipour et al., 2010) and dyes (El-Zawahry and Kamel, 2004); however, a thorough review of the literature showed that its potential as biosorbent for amaranth dye removal from aqueous solutions has not been examined yet.

In the present work, the kinetics of amaranth dye biosorption onto the vegetative organs (root, stem and leaf) and entire *E. crassipes* plant were studied. The aim was to evaluate their differential biosorption potential. Furthermore, the functional groups involved in the biosorption process were identified by Fourier transform infrared (FTIR) spectroscopy and the possible mechanisms of biosorption are discussed.

2. Material and methods

2.1. Biosorbent preparation

Fresh *E. crassipes* plants were collected from the channels in Xochimilco, Mexico, and washed thoroughly with distilled deionized water to remove dirt. The roots, stems and leaves were cut out from some plants; others were kept intact. Afterwards, the roots (REC), stems (SEC), leaves (LEC) and entire plants (EEC) were oven-dried separately at 60°C until dry weight was constant.

Subsequently, they were milled using a Glen Creston mill, and the resulting particles were screened using ASTM standard sieves. The fractions with particle size between 0.3 and 0.5 mm were used in the amaranth dye biosorption experiments performed in this work. The sieved biosorbents (REC, SEC, LEC and EEC) were stored in airtight plastic containers until used.

2.2. Proximate chemical composition of biosorbents

Proximate chemical analysis of REC, SEC, LEC and EEC was performed in triplicate according to the methods described by the Association of Official Analytical Chemists (AOAC, 2005). Total ash (A) was determined by burning samples in a muffle furnace at 600°C until weight was constant. Total protein (TP) was measured by the Kjeldahl method, using 6.25 as conversion factor of total nitrogen into total protein. Ether extract (EE) was determined by the Soxhlet method. Crude fiber (CF) was verified as loss of ignition of dried lipid-free residues after digestion with H_2SO_4 and NaOH standard solutions. Nitrogen-free extract (NFE) was estimated according to Eq. (1):

% NFE = 100 - %A - %TP - %EE - %CF (1)

2.3. Chemicals

Amaranth dye (molecular formula = $C_{20}H_{11}N_2O_{10}S_3Na_3$; molecular weight = 604 g/mol; Fig. 1) was purchased from Sigma-Aldrich Chemicals and all other reagents were of analytical grade. Stock amaranth solution was prepared by dissolving 2 g of the dye in 1 L of distilled deionized water. Test amaranth solutions were prepared by diluting the stock dye solution.

2.4. Kinetic biosorption experiments

Batch kinetic sorption experiments were performed to evaluate and compare the amaranth dye biosorption levels of REC, SEC, LEC and EEC from aqueous solution. All experiments were conducted in 500-mL Erlenmeyer flasks containing 120 mL of test amaranth solution at an initial dye concentration of about 50 mg/L, pH of 2.0 ± 0.1 and 1 g (dry weight)/L of biosorbent with a particle size of 0.3-0.5 mm. Flasks were agitated in an orbital shaker (Cole Parmer Inc.) at 120 rpm and 25 ± 1°C for 48 h to ensure biosorption equilibrium was reached. Care was taken to maintain pH constant (2.0 ± 0.1) in each test solution throughout the course of the experiments by periodically checking and adjusting when necessary with 0.1 N HCl and/or NaOH solutions.

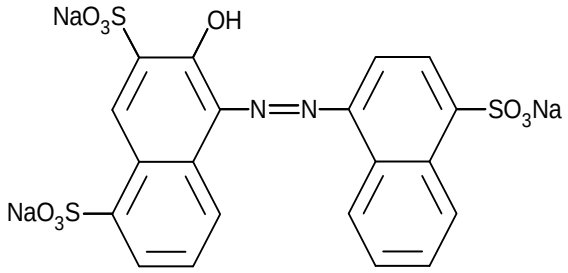

Fig. 1. Amaranth dye chemical structure

Biosorbent-free controls were run concurrently and under exactly the same conditions as used for the amaranth biosorption experiments in order to check for glassware sorption and other potential side effects such as photolysis, precipitation, etc. Throughout the course of the experiments conducted in this work, no measurable change in amaranth dye concentration was detected in the biosorbent-free controls, which indicates that the observed amaranth dye removal in the experiments with REC, SEC, LEC and EEC was exclusively due to the biosorbents.

Samples were collected at different experimental times and centrifuged at 3000 rpm for 5 min. The supernatants were then analyzed spectrophotometrically (Genesys™ 10UV-Visible, Thermo Electron Scientific Instruments Corporation) at 520 nm wavelength to quantify the residual amaranth concentration.

The amount of amaranth dye removed at time t by the unit mass (dry weight) of biosorbent (q_t, mg/g) was calculated according to the mass balance relationship (Eq. 2):

$$q_t = \frac{(C_0 - C_t)V}{W} \quad (2)$$

where: C_o and C_t (mg/L) are the initial and residual amaranth dye concentrations at time t_o=0 h and t=t (h) respectively, V is the solution volume (L) and W is the dry weight of biosorbent (g).

The volumetric rate of amaranth dye removal (ν_R, mg/L·h) was expressed by Eq. (3) (Netzahuatl-Muñoz et al., 2012b):

$$V_R = \frac{C_0 - C_t}{t - t_0} \quad (3)$$

Batch experiments in this work were performed in triplicate and mean values are reported herein. Amaranth dye biosorption data were statistically analyzed by analysis of variance (Tukey´s method; overall confidence level = 0.05) using GraphPad Prism® software version 6.0c.

2.5. Biosorption kinetics modeling

To describe the kinetic profiles of amaranth dye biosorption from aqueous solution and to elucidate the mechanism and potential rate-controlling steps involved in the process of biosorption of amaranth dye onto the biosorbents, four different kinetic models were applied to the experimental data: pseudo-first-order, pseudo-second-order, Elovich and fractional power.

The nonlinear expression of Lagergren´s pseudo-first-order model (Febrianto et al., 2009) is given by Eq. (4):

$$q_t = q_{e1}(1 - e^{-k_1 t}) \quad (4)$$

where: q_{e1} and q_t are the adsorption capacities (mg/g) at equilibrium and at time t (h), respectively, and k_1 is the rate constant of the pseudo-first-order adsorption (1/h).

The pseudo-second-order kinetic model proposed by Ho and McKay (1999) can be expressed by Eq. (5):

$$q_t = \frac{t}{\frac{1}{k_2 q_{e_2}} + \frac{t}{q_{e2}}} \quad (5)$$

where: q_{e2} is biosorption capacity at equilibrium (mg/g), q_t is biosorption capacity at any time t (mg/g)

and k_2 is the rate constant of second-order biosorption (g/mg·h).

The Elovich equation is given by Eq. (6) (Ahmad and Kumar, 2011):

$$q_t = \frac{1}{\beta_e} ln\,(\alpha_e \beta_e) + \frac{1}{\beta_e} ln\,(t) \quad (6)$$

where: α_e is the initial adsorption rate constant (mg/g·h), β_e is related to the extent of surface coverage and to the activation energy for chemisorption (g/mg), q_t is the adsorption capacity (mg/g) at any time t (h), and t is the contact time (h) between the adsorbent and the aqueous solution (Benavente et al., 2011).

The sorption kinetics can be described by a fractional power model as follows (Eq. 7):

$$q_t = kt^{\upsilon} \quad (7)$$

where: υ is the rate constant (1/h) and k is the constant (mg/g) of the power function model. The product of the power function model constants $k\upsilon$ can be calculated to obtain the specific biosorption rate at unit time, i.e. when t = 1 (Netzahuatl-Muñoz et al., 2012a).

In the present work, the kinetic models´ parameters were evaluated by non-linear regression analysis of the experimental data using GraphPad Prism® software version 6.0c. To evaluate the goodness-of-fit of the kinetic models to the experimental biosorption data, the determination coefficient (r^2), the root mean squared error or standard error (*RMSE*) of the estimate and the 95% confidence intervals of the models´ parameters were calculated.

2.6. FTIR analysis

FTIR spectroscopy was used to elucidate the functional groups present on the surface of the biosorbents (REC, SEC, LEC and EEC) that may be involved in the removal of amaranth dye from aqueous solutions. Biosorbent samples (at a concentration of 1 g/L) were brought into contact with amaranth dye solutions at a concentration of 200 mg/L, pH 2.0 ± 0.1 for 24 h, with constant agitation at 120 rpm and 25°C to saturate the binding sites with the dye. All suspensions were subsequently centrifuged for 5 min at 3000 rpm, and the pellets were washed twice with distilled deionized water to remove the unbound dye. Then, after centrifuging the resulting suspensions, the obtained dye-loaded biosorbents were oven-dried at 105°C in order to remove any water retained which could interfere with the observation of hydroxyl groups on the biosorbents' surface.

Dye-unloaded (native biosorbents) and dye-loaded biosorbents were finely ground, mixed with dried KBr at a ratio 1:5 and immediately analyzed with a Perkin-Elmer Spectrum 2000 Fourier transform infrared (FTIR) spectrophotometer equipped with a Perkin-Elmer diffuse reflectance FTIR accessory. The FTIR spectra were obtained from 16 scans over the 4000-400 cm^{-1} range at a resolution of 4 cm^{-1}.

3. Results and discussion

3.1. Chemical composition of biosorbents

The proximate chemical composition of the vegetative organs (root, stem and leaf) and entire *E. crassipes* plant is shown in Table 1. High ash content on dry matter basis was found in all vegetative organs and the entire aquatic plant, with values ranging from 14.93% in the leaves to about 18.55-21.21% in the roots and stems. These results are in agreement with those reported by Wolverton and McDonald (1978) and Okoye et al. (2002), who found a higher content of ash in the roots and stems than in the leaves of the water hyacinth (*E. crassipes*).

Ash represents inorganic matter, mainly including plant minerals. The high content of ash in the *E. crassipes* plant is probably due to accumulation of minerals absorbed from water (Akinwande et al., 2013). The high ash content value obtained for roots may be attributed to their direct contact and accumulation of absorption nutrients from the growing media (Okoye et al., 2002).

Total protein content was higher in the leaves than in other parts of *E. crassipes,* which has also been reported elsewhere (Mishra and Tripathi, 2009; Okoye et al., 2002; Wolverton and McDonald, 1978). Roots showed the lowest ether extract (crude fat/lipid) content (2.98%) while leaves had the highest (4.89%). These findings concur with those found by Gollamudi et al. (1984). The low content of ether extract in the roots of *E. crassipes* has been attributed to the fact that no food synthesized during photosynthesis is stored in this vegetative organ (Okoye et al., 2002).

Table 1. Proximate chemical composition of the vegetative organs and entire *E. crassipes* plant (dry matter basis)

Biosorbent	*% A*	*% TP*	*% EE*	*% CF*	*% NFE*
LEC	14.93 ± 0.01	33.34 ± 0.05	4.89 ± 0.25	12.54 ± 0.35	34.29 ± 0.72
SEC	21.21 ± 0.31	20.78 ± 0.40	3.93 ± 0.42	19.05 ± 0.35	35.02 ± 1.99
REC	18.55 ± 0.65	22.26 ± 0.20	2.98 ± 0.16	21.24 ± 0.58	34.97 ± 1.77
EEC	18.31 ± 0.02	25.26 ± 0.06	3.11 ± 0.66	18.88 ± 0.66	34.44 ± 1.70

The lowest crude fiber percentage was found in the leaves (12.54%) while the highest was found in the roots (21.24%). The crude fiber of *E. crassipes* is predominantly composed of hemicellulose and cellulose, and its lignin content is low (Bhattacharya and Kumar, 2010; Mishima et al., 2008).

No considerable differences were observed in nitrogen-free content between the biosorbents. The differences found in the proximate chemical composition of the leaves, stems, roots and entire *E. crassipes* plant make it reasonable to assume that they would show different performance in the biosorption of amaranth dye from aqueous solutions.

3.2. Removal kinetics of amaranth dye from aqueous solution

Fig. 2 displays the variations in residual concentration, volumetric removal rate and removal capacity of amaranth dye as a function of contact time for the different biosorbents assayed. As the experimental contact time proceeded from 0 to 48 h, the amaranth dye concentration in solution diminished progressively from about 50 mg/L to about 4.22, 22.71, 18.06 and 15.13 mg/L, when LEC, SEC, REC and EEC were used as biosorbents, respectively (Fig. 2A). These results clearly indicate that although the vegetative organs and entire *E. crassipes* plant are capable of biosorbing amaranth dye from aqueous solution, the extent of removal by each biosorbent is distinctive.

The volumetric rates of biosorption of the dye by the assayed biosorbents decreased rapidly during the first 5 minutes of contact; afterwards (from 5 min to 2 h), the decrease was slower, and as time passed (up to 48 h), changes in volumetric rates became negligible (Fig. 2B). These findings indicate that the amaranth dye biosorption processes occurred very fast during the first minutes of contact after which they gradually became slower probably due to the exhaustion of functional groups responsible for amaranth dye biosorption. The highest initial volumetric rate of amaranth dye biosorption was observed in LEC (419.85 mg/L·h), followed by EEC (404.2 mg/L·h), SEC (148.22 mg/L·h) and finally REC (95.71 mg/L·h).

Amaranth biosorption capacity of the vegetative organs and entire *E. crassipes* plant gradually rose as contact time increased, until a maximum constant value was reached which corresponded to the equilibrium biosorption capacity (q_e exp) value (Fig. 2C). The highest amaranth dye biosorption capacity was shown by LEC (43.1 mg/g), followed by EEC (31.18 mg/g), REC (28.51 mg/g) and SEC (23.97 mg/g).

Moreover, biosorption of amaranth dye by the vegetative organs and entire *E. crassipes* plant was a relatively quick process, with equilibrium reached at 7 h for LEC, 6 h for EEC and REC and 4 h for SEM. These results can be explained by the fact that LEC biosorb larger amounts of amaranth dye and therefore require longer periods of contact time to reach equilibrium. The equilibrium times (t_e) reported in this work are lower than those reported for biosorption of amaranth dye by peanut hull (Gong et al., 2005) and bottom ash (Mittal et al., 2005), similar to those found for de-oiled soya (Mittal et al., 2005), and higher than those reported for alumina reinforced polystyrene (Ahmad and Kumar, 2011). It should be emphasized that rapid biosorption kinetics has significant practical importance when considering the process at industrial scale. The biosorption process can be made continuous with short biosorbent residence time, and consequently the dimensions of the biosorber can be small (Michalak and Chojnacka, 2010; Suyamboo and Perumal, 2012).

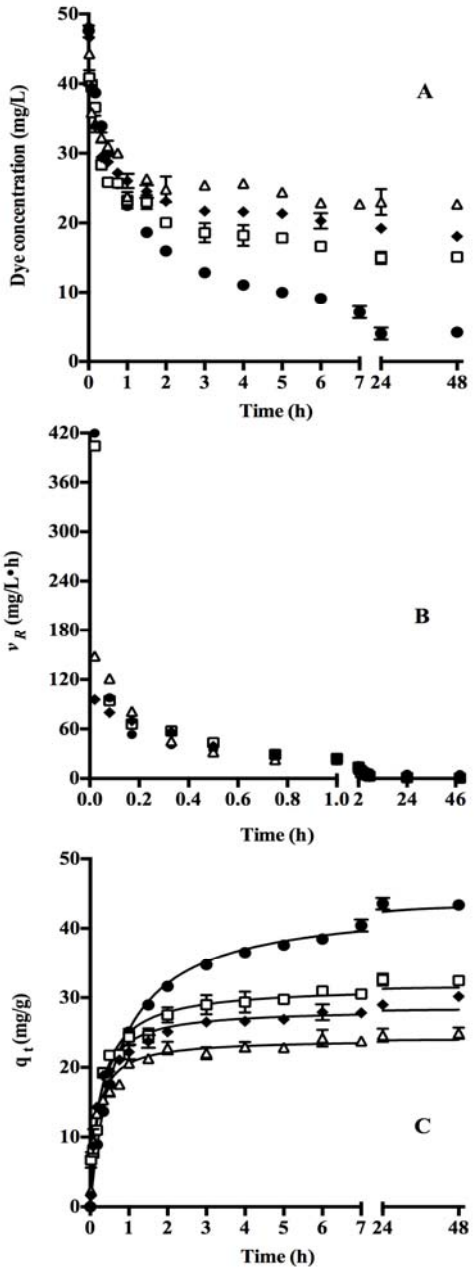

Fig. 2. Variations in residual concentration (A), volumetric removal rate (B) and biosorption capacity (C) of amaranth dye as a function of contact time for the roots (◆), stems (△), leaves (●) and entire *E. crassipes* plant (□)

To the best of our knowledge, this is the first study that evaluates and compares the biosorption characteristics of the vegetative organs and entire *E. crassipes* plant for removal of a toxic pollutant from an aqueous solution. Present results demonstrate that the leaves of *E. crassipes* are more suitable for the biosorption of amaranth dye from aqueous solution than the stems, roots and even the entire aquatic plant.

In addition, present experimental biosorption results were compared with published results to evaluate the application potential of LEC, SEC, REC and EEC as biosorbents for amaranth dye removal from aqueous solutions. Table 2 shows a comparison of experimental amaranth dye biosorption capacity (q_e *exp*) of different biosorbents. The amaranth dye biosorption capacity of LEC is significantly higher than others reported in the specialized literature. Moreover, the EEC, SEC and REC biosorption capacity is also greater than most results from the literature. The high biosorption capacity of the *E. crassipes´* leaves places this biomaterial as one of the best biosorbents currently available for removal of amaranth dye from aqueous solutions and is therefore potentially useful to detoxify water and wastewaters polluted with amaranth dye.

3.3. Amaranth biosorption kinetics modeling

Kinetic profiles of amaranth dye biosorption were characterized to determine the rate-controlling mechanisms involved in the biosorption process. For this purpose, the Elovich, fractional power, pseudo-first-order and pseudo-second-order kinetic models were applied to the experimental biosorption data. Table 3 shows the experimental biosorption capacity at equilibrium (q_e *exp*) of the vegetative organs and entire *E. crassipes* plant, and the kinetic parameter values of the four different kinetic models, together with the corresponding determination coefficients (r^2) and *RMSE* values.

For all the biosorbents tested, the determination coefficients were higher with the pseudo-second-order model, and the *RMSE* values were lower than those obtained with the Elovich, fractional power and pseudo-first-order model. Present experimental data thus show very good compliance with the pseudo-second-order model. Moreover, the pseudo-second-order model suitably described amaranth dye biosorption capacity variations at different experimental times (continuous lines in Fig. 2C) and predicted biosorption capacity values at equilibrium (q_{e2}) very closely compared with the experimentally obtained equilibrium capacities (q_e *exp*; Table 3). In this study, the biosorption kinetics data for all biosorbents are best described by the pseudo-second-order model. The fitness of amaranth dye biosorption kinetics to the pseudo-second-order model suggests that the rate-limiting step in amaranth dye biosorption onto LEC, REC, SEC and EEC is probably a chemical sorption (chemisorption) involving valence forces through the sharing or exchange of electrons between the biosorbents surface and amaranth dye (Febrianto et al., 2009).

Table 2. Comparison of experimental amaranth dye biosorption capacity of some biosorbents

Biosorbent	q_e *exp (mg/g)*	*pH*	*Particle size (mm)*	*Temperature (°C)*	*Reference*
Peanut hull	14.90	2	0.15-0.18	20	Gong et al., 2005
Bottom-ash	7.0	2	≤ 0.3	30	Mittal et al., 2005
De-oiled soya	28.0	2	≤ 0.3	30	Mittal et al., 2005
Alumina reinforced polystyrene	8.281	2	0.15-0.30	30	Ahmad and Kumar, 2011
LEC	43.1	2	0.30-0.5	25	This work
SEC	23.97	2	0.30-0.5	25	This work
REC	28.51	2	0.30-0.5	25	This work
EEC	31.18	2	0.30-0.5	25	This work

Table 3. Parameters of the pseudo-first-order, pseudo-second-order (a), Elovich and fractional power kinetic models (b) for amaranth dye biosorption onto the vegetative organs and entire *E. crassipes* plant

a)

		Pseudo-first-order				*Pseudo-second-order*			
Biosorbent	q_e *exp (mg/g)*	q_{e1} *(mg/g)*	k_1 *(1/h)*	r^2	*RMSE*	q_{e2} *(mg/g)*	k_2 *(g/mg·h)*	r^2	*RMSE*
LEC	43.096 ± 0.23	39.4 ± 0.84	1.036 ± 0.08	0.956	2.98	43.66 ± 0.69	0.032 ± 0.01	0.984	1.82
SEC	23.973 ± 0.29	22.68 ± 0.32	3.689 ± 0.28	0.931	1.99	24.04 ± 0.22	0.245 ± 0.02	0.977	1.15
REC	28.513 ± 0.59	26.51 ± 0.35	3.328 ± 0.23	0.945	2.16	28.38 ± 0.21	0.172 ± 0.01	0.986	1.08
EEC	31.181 ± 0.37	29.43 ± 0.45	2.562 ± 0.19	0.929	2.66	31.62 ± 0.37	0.119 ± 0.01	0.968	1.78

b)

	Elovich				*Fractional Power*			
Biosorbent	α_e *(mg/g·h)*	β_e *(g/mg)*	r^2	*RMSE*	k *(mg/g)*	υ *(1/h)*	r^2	*RMSE*
LEC	379.8 ± 76.89	0.164 ± 0.01	0.929	3.451	23.84 ± 1.02	0.203 ± 0.02	0.851	4.999
SEC	1420 ± 402.2	0.344 ± 0.02	0.875	2.219	17.65 ± 0.43	0.133 ± 0.01	0.752	3.12
REC	993.7 ± 217.1	0.273 ± 0.01	0.906	2.385	20.09 ± 0.51	0.146 ± 0.01	0.777	3.673
EEC	1091 ± 250.4	0.252 ± 0.01	0.898	2.707	21.7 ± 0.54	0.150 ± 0.01	0.794	3.838

The pseudo-second-order model assumes that two reactions are occurring, the first reaction is fast and reaches equilibrium quickly, and the second is a slower reaction that can continue for long periods. The reactions can occur either in series on in parallel (Khambhaty et al., 2009).

The pseudo-second-order model is the most suitable to describe amaranth dye adsorption kinetics by alumina reinforced polystyrene (Ahmad and Kumar, 2011), as well as the biosorption of other anionic azo dyes such as reactive yellow 42 and reactive red 45 by *Citrus sinensis* (Asgher and Bhatti, 2010), direct brown by *Spirogyra* sp. (Sivarajasekar et al., 2009; Venkata-Mohan et al., 2008), reactive brilliant red K-2BP by *Aspergillus fumigatus* (Wang et al., 2008), and brilliant yellow by hen feathers (Mittal et al., 2012), among others.

Present results also show that the pseudo-second-order rate constant (k_2) value depended on the specific biosorbent used for amaranth dye biosorption (Table 3). The k_2 values for the different biosorbents tested showed the following order: SEC (0.245 g/mg·h) > REC (0.172 g/mg·h) > EEC (0.119 g/mg·h) > LEC (0.032 g/mg·h). This trend is opposed to that observed for the experimental equilibrium biosorption capacity of amaranth dye (q_e *exp*) [LEC (43.1 mg/g) > EEC (31.18 mg/g) > REC (28.5 mg/g) > SEC (23.97 mg/g)] and for the equilibrium time (t_e) [LEC (7 h) > EEC, REC (6 h) > SEM (4 h)]. This behavior, which reveals that the greater the values of q_e *exp* and t_e, the lower the value of k_2, may be explained considering that the k_2 constant plays the role of time-scaling factor, i.e., when the k_2 constant value is small, the time required by a biosorption system to reach the equilibrium state is longer because larger amounts of amaranth dye are biosorbed (Plazinski et al., 2009).

3.4. FTIR spectral analysis

Interactions of amaranth dye with the biosorbents functional groups were examined by FTIR analyses. FTIR spectra of amaranth-loaded biosorbents were compared with those of native biosorbents.

Fig. 3 shows the FTIR spectra of unloaded and amaranth-loaded LEC (Fig. 3A), SEC (Fig. 3B), REC (Fig. 3C) and EEC (Fig. 3D). FTIR spectra of native biosorbents revealed a large number of absorption peaks within the interval from 4000 to 400 cm^{-1}, which reflects the complex nature of biosorbents. This is consistent with the results of the proximate chemical analyses, which showed that the vegetative organs and entire *E. crassipes* plant contain proteins, fats, crude fiber, etc., all of which have several functional groups (-COOH, $-NH_2$, -NH, -OH, and/or others).

FTIR analyses of all the native biosorbents exhibited a broad absorption band in the 3800 – 2500 cm^{-1} region, which is assigned to the hydrogen-bonded -OH and -NH groups (Asgher and Bhatti, 2010). The band at approximately 2930 – 2920 cm^{-1} is due to C-H stretching vibration (Sayyah et al., 2002).

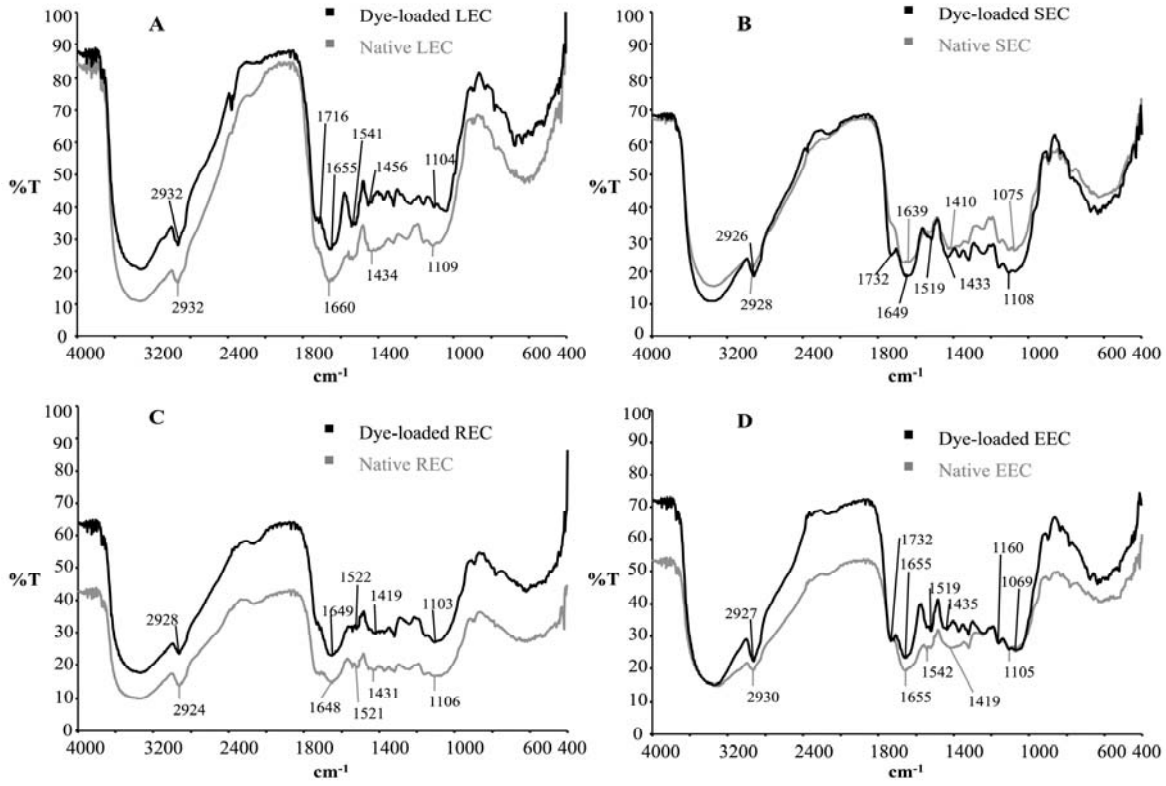

Fig. 3. FTIR spectra of unloaded and amaranth-loaded LEC (A), SEC (B), REC (C) and EEC (D)

The characteristic absorption bands of amide I C=O stretching at around 1660 cm^{-1} and amide II N-H bending at approximately 1520 cm^{-1} were also detected in the spectrum of all the native biosorbents. These absorption bands most probably correspond to the amide groups present in the proteins of *E. crassipes*. Furthermore, absorption bands at 1456 – 1410 cm^{-1} are attributable to the bending of -OH groups and stretching of C-O groups; these functional groups are mainly present in the *E. crassipes* proteins and polysaccharides. The peaks at around 1100 cm^{-1} are due to the vibration of C-O-C groups (Saygideger et al., 2005). Some absorption peaks in the fingerprint region (1500–400 cm^{-1}) are assigned to ionic interactions of biosorbents with minerals (Fong et al., 2006; Saygideger et al., 2005). The spectra of all native biosorbents revealed many absorption peaks in the 800 – 400 cm^{-1} region, which may be related to the high mineral (ash) content of the biosorbents (Table 1).

The absorption spectra of amaranth-loaded biosorbents showed evident changes compared with those of the native biosorbents. Among these changes was the narrowing of the band at the 3800 – 2500 cm^{-1} region, which may be due to a reduction in the number of hydrogen bonds caused by the interaction of the amaranth dye with the –OH and –NH functional groups of the biosorbents. In addition, the relative intensity of the characteristic amide I (1650 cm^{-1}) and amide II (1540 cm^{-1}) bands increased due to involvement of these functional groups in the biosorption of amaranth dye molecules. Better-defined peaks were also observed at 1716, 1732 and 1732 cm^{-1} in the absorption spectra of dye-loaded biomass of LEC, SEC and EEC respectively, and these changes may indicate the interaction of amaranth dye with the carbonyl groups of proteins, lignin and/or other compounds containing carboxyl functional groups (Sundari and Ramesh, 2012). The –OH group peak was shifted from 1410 to 1433 cm^{-1}, and from 1419 to 1435 cm^{-1} for SEC and EEC amaranth-loaded biomass samples respectively, which confirms that –OH groups present in proteins, cellulose, hemicelluloses and/or lignin play a role in amaranth dye biosorption (Suyambo and Perumal, 2012). Minor changes between the absorption spectra of dye-loaded and unloaded biosorbents examined in this work were observed for the roots (REC) and stems (SEC), probably because these biosorbents have the lower amaranth dye biosorption capacity.

FTIR results indicate that chemical interactions between the biosorbents hydroxyl, carbonyl, and amide functional groups and the amaranth dye molecules were involved in dye biosorption. These functional groups are constituents of the polysaccharides, lignin and/or proteins present in REC, SEC, LEC and EEC.

Considering on the one hand that of all biosorbents tested in this study, the leaves of *E. crassipes* (LEC) contain the highest protein concentration (33.34%), that proteins contain amide groups, and, on the other, that FTIR analyses revealed that amide groups are involved in amaranth dye biosorption, and leaves (LEC) showed the highest biosorption capacity of amaranth dye, it is reasonable to assume that *E. crassipes* proteins are involved in the amaranth dye biosorption from aqueous solution. In order to confirm this hypothesis, experimental amaranth biosorption capacity at equilibrium was plotted against total protein content of the biosorbents (Fig. 4).

A linear relationship was found between total protein content and amaranth dye biosorption capacity at equilibrium (r^2 = 0.985), which corroborates that proteins play a major role in the biosorption of amaranth dye from aqueous solution by *E. crassipes*. In contrast, no direct relationship was observed between total ash, ether extract, crude fiber or nitrogen-free extract content and amaranth dye biosorption capacity.

Furthermore, the greater the protein concentration in the vegetative organ or entire plant of *E. crassipes*, the greater the number of amide groups and consequently the greater the AD biosorption capacity (Fig. 4).

The above results are in agreement with that reported by Saeed et al. (2013) and Tal et al. (1985), who proposed that in acidic conditions, amaranth dye and other sulfonic acid dyes bind to proteins as a result of electrostatic attraction between the negatively charged sulfonic groups of dyes and positively charged groups (e.g., amide groups) of proteins, as well as because of a hydrophobic interaction between the dye molecule and the polypeptide backbone that adjoins the positively charged groups of the proteins.

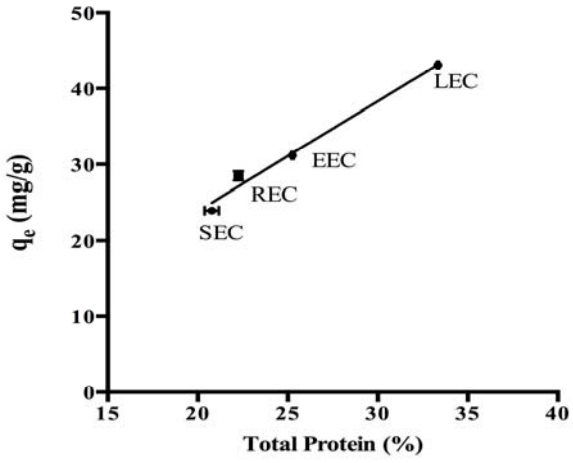

Fig. 4. Relationship between amaranth dye biosorption capacity at equilibrium and total protein content of the biosorbents

4. Conclusions

The potential of the leaves, roots, stems and entire *E. crassipes* plant for amaranth dye biosorption from aqueous solution was investigated in terms of kinetics. *E. crassipes'* leaves showed the best

performance for biosorptive removal of amaranth dye from aqueous solution. Kinetic data agreed well with the pseudo-second-order model. Proteins are strongly involved in amaranth dye biosorption by *E. crassipes*. The plant's leaves possess attractive characteristics to be used as biosorbent to remove amaranth dye from aqueous solutions.

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