Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2022.27993

246 (2022) 212–225
January

Biosorption activity of chemically modified *Trifolium alexandrinum* for the detoxification of Cr(III) contaminated water

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Received 14 July 2021; Accepted 13 November 2021

ABSTRACT

This study aims to explore the adsorption capacities of methanol, formaldehyde and glutaraldehyde modified *Trifolium alexandrinum* for the removal of trivalent chromium. The techniques like FTIR, SEM, pH_{pzc} percentage weight gain, and Boehm's titration is used for the characterization of modified adsorbents. The influence and optimization of various parameters such as dosage, pH and contact time, initial concentration of metal ions and temperature were investigated here. The equilibrium, kinetics, and thermodynamics studies were also explored in this work. The equilibrium study was followed by the isotherm Langmuir with adsorption capacities of up to 94.3, 77.5 and 61.7 mg/g using modified methanol, formaldehyde and glutaraldehyde adsorbents, respectively. The nonlinear isotherm also explains that the study was well observed in Langmuir isotherm. The kinetic study was best described by the pseudo-second-order model. The thermodynamics study explored the endothermic nature of the process and indicate that process was a physisorption adsorption system. These exploration results indicate that methanol modified *T. alexandrinum* works best for the removal of trivalent chromium.

Keywords: Kinetics; Non-linear modelling; Thermodynamics; Biosorption; Trivalent chromium

1. Introduction

Currently because of industrial processes, an extensive variety of pollutants are generated that may be organic or inorganic. Out of all pollutants, heavy metals possessed serious issues to the aquatic ecosystem [1]. The increased use of heavy metals is a serious problem in causing pollution and results in toxicity symptoms which are responsible for causing various diseases, besides this they are also contributing towards global warming by abolishing the natural atmosphere like ozone layer depletion. Most of the heavy metals are assimilated, deposited, and accumulated in the human body causing some long-lasting effects so that's why it is compulsory to eradicate these heavy metals from the industrial wastewater before its exposure to the fresh aquatic environment [2,3]. Due to their poisonous effects and accumulation in the environment and human body, it is a crucial environmental problem. So that's why, the World Health Organization (WHO) and Environmental Protection Agency (EPA) have specified the tolerable parameters of their discharge in water [4]. The discharge of heavy metals In the water is mainly from various sources like plastics, pigments, textile, electroplating, mining and metallurgical processes [5]. The significant environmental

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effect of heavy metals was mainly due to the tannery wastewater [6]. Heavy metals like chromium have been used widely in tannery industries along with dyes [7].

Chromium (Cr) is a redox-sensitive heavy metal and its toxicity, bioavailability and physical properties depend mainly upon its oxidation state [8,9]. In wastewater of industrial methods, chromium (Cr) is found in two oxidation states mostly, that is, trivalent [Cr(III)] and hexavalent [Cr(VI)]. Among these states, hexavalent form is more toxic than the other one [10] but it is also seen that continued contact with the trivalent species causes skin allergies, cancer, and maybe oxidized to mutagenic species by some environmental factors [11]. According to the US EPA (Environmental Protection Agency), the permissible limit of Cr in drinking water should be 0.05mg/L. A report published by the Pakistan Council of Research in Water Resources indicated that in Pakistan groundwater of 23 major cities is contaminated with chromium. Drinking water sample analysis of Kasur, Lahore, Sialkot and Karachi showed that concentration is greater than the allowable limits. The reason of higher chromium concentration in water is mainly because of tanneries industries placed in these localities [12]. Because of the toxicity and severe environmental influences of chromium, it is necessary to eradicate chromium from waste waters before its discharge into the water bodies into the environment.

Many chemical and physical methods have been utilized and developed for the removal of heavy metals like precipitations, solvent extractions, reverse osmosis, ion exchanger [13,14], redox, sedimentation, electrochemical techniques [15,16], cation surfactant, and filtration [17]. Though these methods need more development because of high operational cost, poor removal efficiency at low concentration, and sludge formation which may be toxic and require supplementary treatment. Therefore, researchers focused on the development of new methods which are economical for heavy metals removal. Adsorption is a common method utilized for the removal of heavy metals. This technique is advantageous because of its easy design, simple operational method, and good removal efficiency [18,19]. The strategy for water treatment is generally encouraging in such a manner that it is simpler to deal with and reuse, not creating harmful substances, and harmless to the ecosystem, while the adsorbents can be deliberately custom-made for better effectiveness [20,21]. Biosorption signifies the biological material capacity for the removal of heavy metals. Biomaterials have excellent removal efficiency, affinity, and selectivity towards metals. A wide variety of biosorbents has been used for the removal of heavy metals. According to literature, different biosorbents such as avocado shells [22], peanut shells [23], orange peel [24], immobilized sugarcane bagasse [25], activated carbon [26], pineapple crown leaf [27], sorghum biomass [28] have been utilized for the removal of chromium metal.

This study aims to find a novel low-cost biosorbent that is found locally in Lahore, Pakistan for the removal of Cr(III). In this study, we used *Trifolium alexandrinum* commonly known as Berseem in Pakistan. It is a legume grown for green food. Several methods are used to modify the agricultural waste to improve its metal binding capacity. In the present work, we explore the metal binding capacity of *T. alexandrinum* by using different chemical modifications for the removal of Cr(III). The impact of adsorbent dosage, pH, contact time, temperature, and initial metal ion concentration was also studied in detail. Different equilibrium modeling, that is, Langmuir and Freundlich's models have been employed to get maximum absorption capacities. Kinetic modeling was also employed to get information about the reaction mechanism. The thermodynamics study was also taken into account.

2. Material and methods

2.1. Chemicals

$CrCl_3\cdot 6H_2O$ (Molecular weight: 158.36 $gmol^{-1}$) was purchased from the MERCK. Anhydrous methanol, formaldehyde, glutaraldehyde, formic acid, and hydrochloric acid were purchased from Sigma-Aldrich. Hydrochloric acid (0.1 mol/dm^3) and sodium chloride (0.1 mol/dm^3) were utilized for the pH setting. Sodium carbonate (0.1 mol/dm^3) and sodium bicarbonate (0.1 mol/dm^3) was used for Boehm's titration for characterization of biomass. All the chemicals were of analytical grade.

2.2. Collection of biosorbent

As an agricultural waste *T. alexandrinum* as a whole form, that is, stems and leaves were collected from the agricultural farm of Lahore, Pakistan. It was washed with distilled water to remove dirt particles. Afterward washing it was left for air drying. Dried *T. alexandrinum* was ground as whole biomass and washed again and again to remove the coloration and remaining dust particles. It was then left for oven drying at about 70°C and then utilized for the chemical modification. The chemical modification was one by following the methodology of Jianlong [29] with slight modifications.

2.3. Chemical modification of Trifolium alexandrinum

T. alexandrinum was chemically modified in three different ways to compare the roles of different functional groups for the removal of chromium. *T. alexandrinum* was modified using methanol, formaldehyde ad glutaraldehyde using hydrochloric acid and formic acid as a protonated agent for a respective reagent.

The chemical modifications utilized to enhance the biomass were as follows:

2.3.1. Methanol modification (MMTA)

T. alexandrinum (30 g) was added in anhydrous methanol (1,500 mL) along with concentrated hydrochloric acid (20 mL). This reaction mixture was stirred for 7 h at 150 rpm. The suspension was filtered and washed sequentially with distilled water, sodium carbonate (0.2 mol/dm^3), and finally with distilled water. This modified biomass was dried at 80°C for 10 h and labeled as MMTA.

2.3.2. Formaldehyde modification (FMTA)

T. alexandrinum (30 g) was taken along with formaldehyde (500 mL) and stir for about 30 min on a rotary shaker

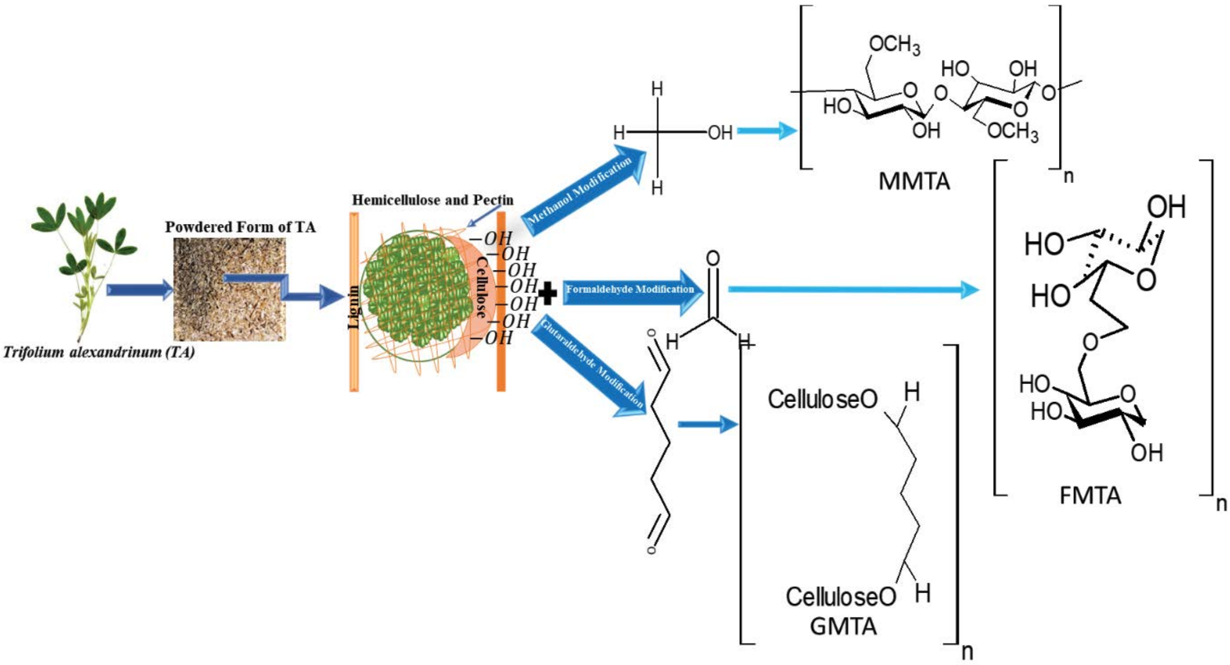

Fig. 1. Modification mechanism of *Trifolium alexandrinum*.

(VORTEX-OSM-747). Later formic acid (1,000 mL) was added, and this reaction mixture was stirred for about 7 h at 150 rpm. After reaction completion, biomass was filtered and washed sequentially with distilled water, sodium carbonate (0.2 mol/dm^3), and finally with distilled water. This modified biomass was dried at 80°C for about 12 h and labeled as FMTA

2.3.3. Glutaraldehyde modification (GMTA)

T. alexandrinum (30 g) was added in the dilute 1,000 mL of hydrochloric acid (0.2 mol/dm^3) along with glutaraldehyde (50 mL). This reaction mixture was stirred for about 4 h and left for 3 d at room temperature. The biomass was filtered and washed sequentially with distilled water sodium carbonate (0.2 mol/dm^3) and finally with distilled water. This modified biomass was dried at 80°C for 10 h and labeled as GMTA.

2.4. Biosorption studies (batch process)

The adsorption process was carried out as a batch process to get the optimum values of different parameters for the adsorption of Cr(III) at an orbital shaker (VORTEX Model-OSM-747). The different influencing parameters like adsorbent dosage, contact time, pH, and temperature were studied in the batch process. A batch experiment was carried out using a 50 mg/L concentration of Cr(III). The fixed amount of adsorbent was added in 50 mL solution of fixed concentration solution of Cr(III), keeping other parameters constant and one variable parameter. After a specific interval, the mixture was filtered, and the filtrate was subjected to atomic adsorption spectrometer (Perkin Elmer-Analyst 100) for analysis.

The adsorbed amount of metal ($q_{\max}$) was calculated by using:

$$q_{\max} = \frac{\left(C_0 - C_e\right)V}{W} \tag{1}$$

where C_0 is the initial Cr(III) concentration (mg/L), C_e is the equilibrium concentration of Cr(III) (mg/L). V is the volume of Cr(III) used (L) and W is the amount of biomass used (g).

The removal percentage (R %) was calculated as:

$$R\% = \frac{\left(C_0 - C_e\right)}{C_0} \times 100 \tag{2}$$

In the same way, we keep investigating the parameters one by one, by keeping one parameter variable and other parameters constant. Isotherm modeling was obtained by using different concentrations of Cr(III) (20–180 mg/L). Kinetic and thermodynamics study was obtained at a different temperature to get the optimum temperature value and mechanism of the reaction.

2.5. Characterization

The Fourier transform infrared (FTIR) was used to explain the main functional groups responsible for binding on the surface of the biosorbent. Scanning electron microscopy (SEM) was used to reveal the surface morphology. Furthermore, pH$_{\text{pzc}}$ and Boehm's titration was used for the point zero charge valuation and concentration of acidic and basic groups on the surface of biosorbents. The percentage weight gain was determined to check the weight gained by raw *T. alexandrinum* after modifications with methanol, formaldehyde, and glutaraldehyde.

3. Results and discussion

3.1. Characterization of chemically modified biomass

All samples were pre-dried at 60°C for 1 h preceding any characterization. The percent weight gain (pwg) after the modifications of groups of *T. alexandrinum* with formaldehyde, methanol, and glutaraldehyde was calculated as:

$$\text{pwg}\left(\%\right)=\frac{W_{\text{MB}}-W_{\text{TA}}}{W_{\text{TA}}}\times 100 \quad (3)$$

where pwg (%) is weight gain percent, W_{MB} is the weights of modified biomasses (MMTA < FMTA and GMTA) and W_{TA} is the weight of original biomass (*T. alexandrinum*). The results are tabulated in Table 1.

Boehm's titration was utilized for the determination of acidic and basic group concentration. The modified adsorbents interacted with bases and acid (NaOH, Na_2CO_3, $NaHCO_3$, and HCl). By interacting with bases and acid, acidic and basic functional groups were calculated by utilizing Boehm's titration calculations [30]. In this way number of acidic groups, that is, phenols, carboxylic groups, lactones, and basic groups can be calculated. HCl will give the number of basic functional groups as it neutralizes NaOH. Sodium hydroxide (NaOH) as a strong base neutralizes all the acidic groups. Sodium carbonate neutralizes carboxylic acid only. Sodium bicarbonate neutralizes both lactones and carboxylic groups. The results of the total number of acidic and basic groups are shown in Table 1 along with comparisons of each group in all adsorbents. The comparison of acid and basic groups is shown in Fig. 2.

This study is an important way to ascertain the binding sites present at the surface of the biomass that is used for the metal ion removal by binding the heavy metal ions with themselves. The modified biosorbents, that is, MMTA, FMTA, and GMTA were subjected to an FTIR spectrophotometer (Aligent Technologies CARY 630 FTIR), and spectra were studied to get information about the enhanced functional groups.

The formaldehyde modified (FMTA) biosorbent was subjected to FTIR analysis. Fig. 3a shows the spectrum of FMTA. The broad peak 3,348.37 cm^{-1} showing the presence of hydroxyl groups. The peak that appeared at 1,651.00 cm^{-1} indicated the presence of the carbonyl group that may be attributed to formaldehyde modification. Peaks appeared at 1,156.18 and 1,024.72 cm^{-1} indicated the presence of a carboxyl group. These may be the possible sites for Cr(III) attachment.

Table 1
Characterization of chemically modified *Trifolium alexandrinum*

Characterization		Results		
Adsorbents		MMTA	FMTA	GMTA
Percent weight gain (%)		6.67 ± 0.02	5.33 ± 0.02	8.7 ± 0.02
pH_{pzc}		2.6	4.5	5.9
Acidic and basic groups (mmol/g)	Total acidic groups	16.5 ± 0.02	31 ± 0.02	25.2 ± 0.02
	Carboxylic groups	6.9 ± 0.02	17 ± 0.02	13.9 ± 0.02
	Lactones	2.3 ± 0.02	9.7 ± 0.02	6.7 ± 0.02
	Phenols	7.3 ± 0.02	4.3 ± 0.02	4.6 ± 0.02
	Total basic groups	7.3 ± 0.02	3.4 ± 0.02	8.9 ± 0.02

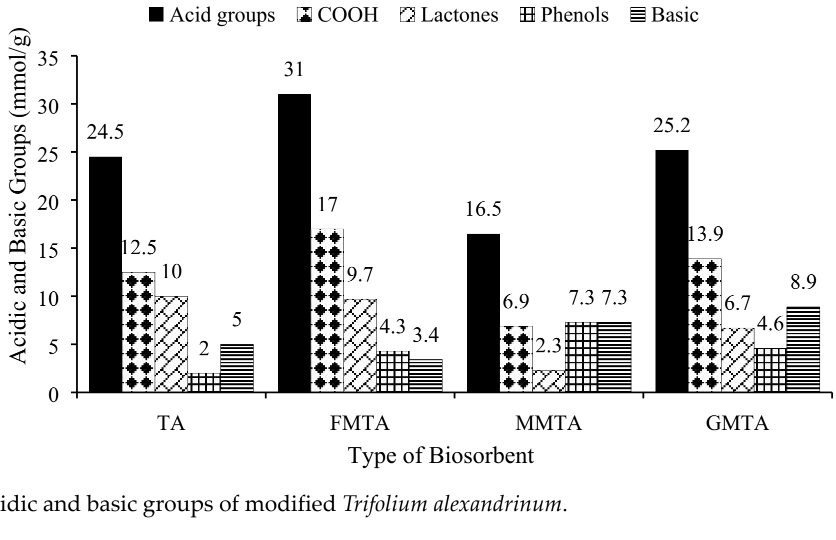

Fig. 2. Comparison of acidic and basic groups of modified *Trifolium alexandrinum*.

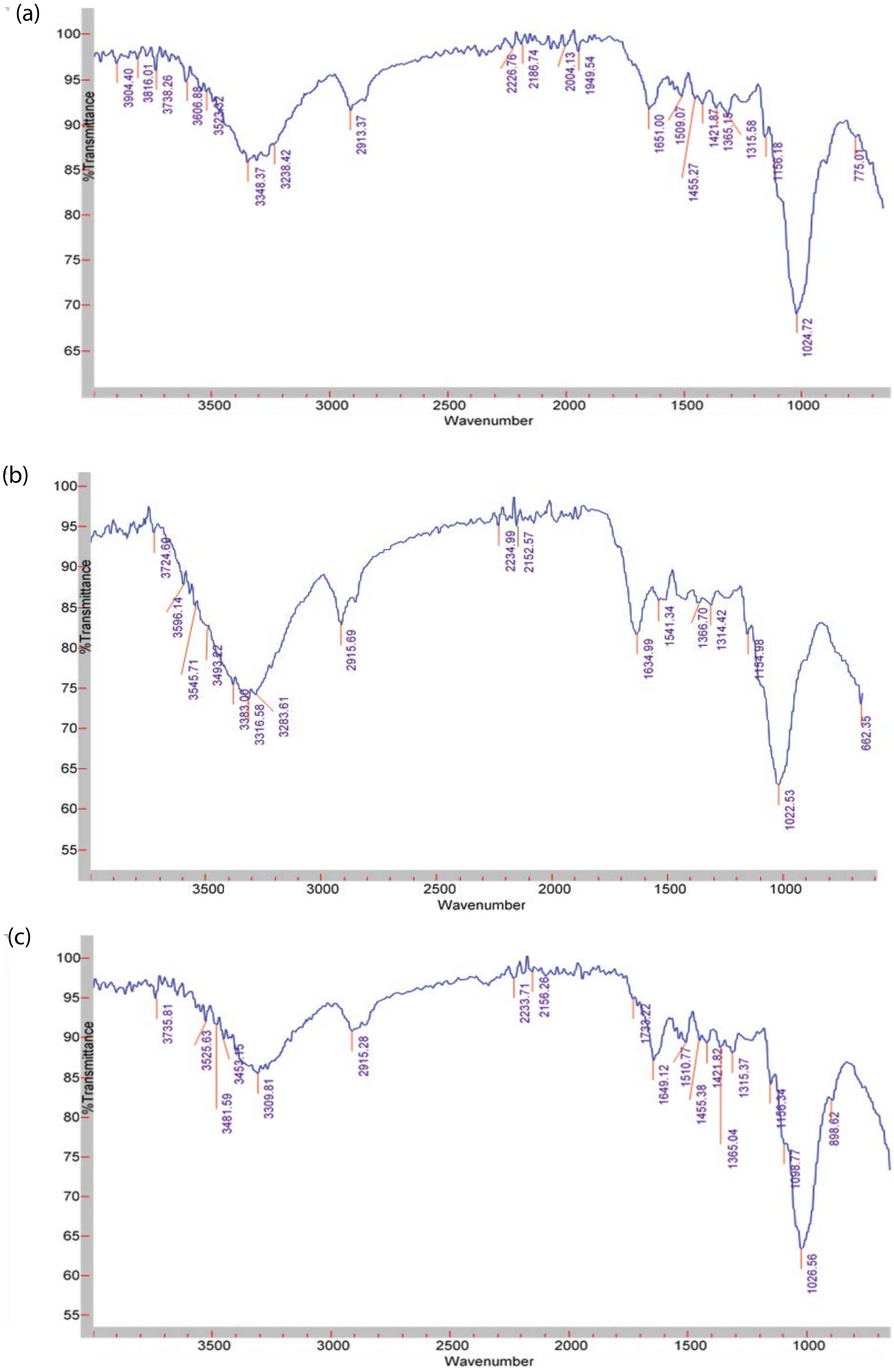

Fig. 3. Spectra of (a) formaldehyde modified *Trifolium alexandrinum* (FMTA), (b) methanol modified *Trifolium alexandrinum* (MMTA), and (c) glutaraldehyde modified *Trifolium alexandrinum* (GMTA).

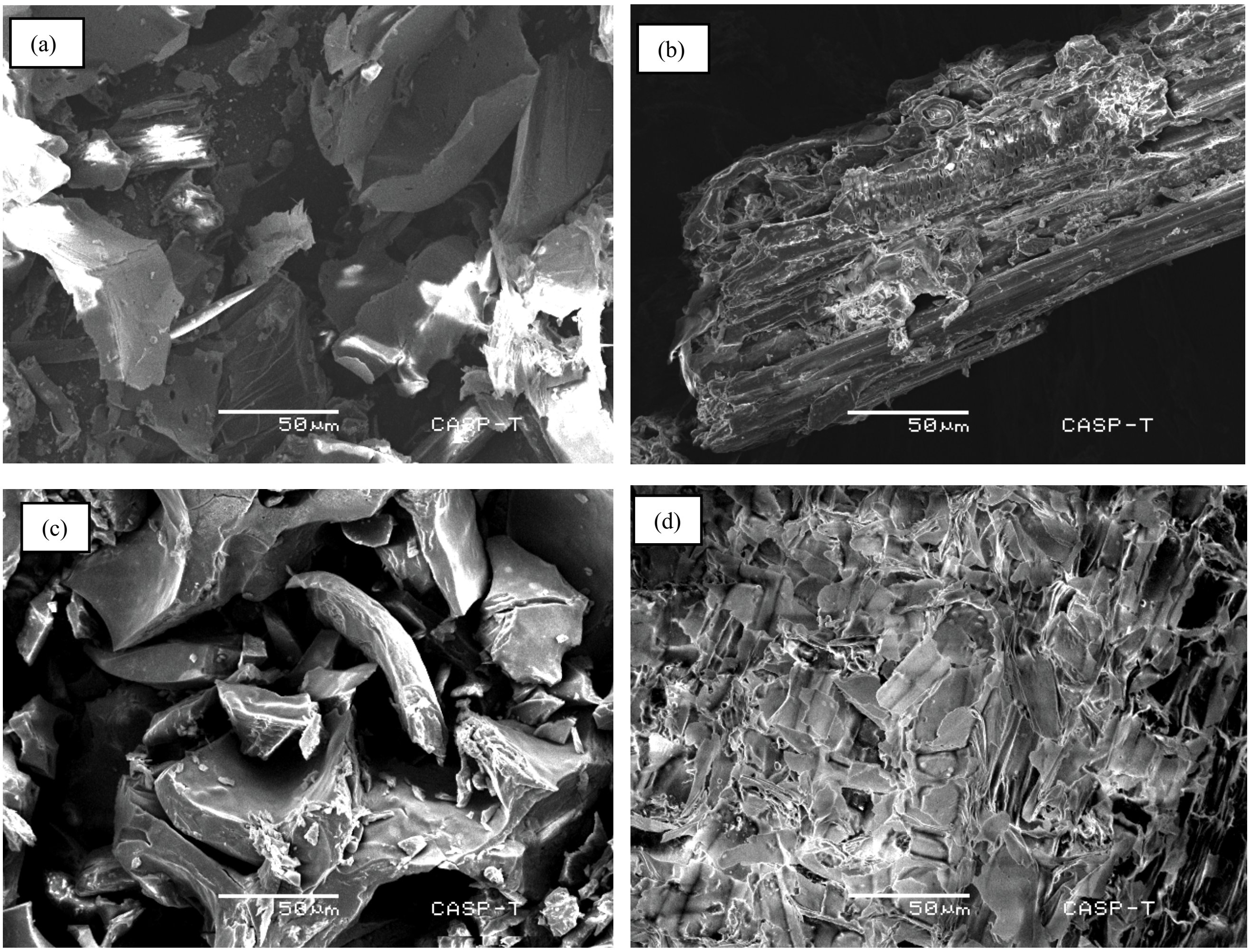

Fig. 4. SEM image of *Trifolium alexandrinum* (a), MMTA (b), FMTA (c) and GMTA (d).

The methanol modified (MMTA) adsorbent was subjected to FTIR analysis. A relatively broader stretching peak was observed at 3,316.58 cm^{-1} depicts the presence of enhanced hydroxyl group (–OH). A peak observed at 2,915.69 cm^{-1} shows the presence of an enhanced alkyl group (–R). The spectrum also indicates the presence of enhanced carbonyl group (C=O) having a peak at 1,639.99 cm^{-1}, and other peaks observed at 1,541.34; 1,154.98 and 1,022.53 cm^{-1}. Thus, the FTIR spectrum indicates that MMTA adsorbent shows the enhanced functional groups. Fig. 3b shows the spectrum of MMTA.

The glutaraldehyde modified (GMTA) adsorbent was subjected to FTIR analysis and Spectrum is shown in Fig. 3c. A broad stretching peak was observed at 3,309.81 cm^{-1} depicts the presence of enhanced hydroxyl group (–OH). A peak observed at 1,649.12 cm^{-1} shows the presence of the carbonyl group and other peaks observed at 1,510.77; 1,098.77 and 1,026.56 cm^{-1}. Thus, the FTIR spectrum indicates that GMTA adsorbent shows the enhanced functional groups.

Raw *T. alexandrinum* and Modified biosorbents (MMT, FMTA, and GMTA) were subjected to the SEM (JEOL-JSM 6480) and their images obtained are shown in Fig. 3. The SEM images of raw and modified biosorbents reveal their surface morphology. The images indicated the high porosity of modified biosorbents which may facilitate the binding of substrate (Cr(III)). The comparison in the figure also reveals the incorporation of different modified agents onto the surface of the biosorbent under study (*T. alexandrinum*). For this reason, further investigations were carried out on the biosorptive characteristics of modified biosorbents (MMTA, FMTA, and GMTA).

The point of zero charges is the pH or point where equilibrium is established between negative and positive sites of the adsorbent surface. The charge is neutral at the surface on this point. When a deviation occurs in pH from the point of pHpzc the balance is disturbed [31]. This characterization is very important for the determination of optimal circumstances for the procedure to carried out at a large scale.

When the pH$_{pzc}$ value is greater than the pH then the system was held to be "below the pzc" indicating that the surface is positive due to protonation. While when the pH value is greater than pzc the system was held to be "above the pzc" indicating the net negative charge due to deprotonation. The results are tabulated in Table 1.

3.2. Influence and optimization of biosorbent dosage on the Cr(III) removal

This influence was studied at an initial concentration of 50 ppm of Cr(III) by varying amounts of adsorbents, that is, MMTA, FMTA, and GMTA. Removal percentage and adsorption capacities as a function of varying adsorbents dosages are shown in Fig. 5. As illustrated from Fig. 5, removal of Cr(III) was rapid initially followed by the equilibrium. Initially, removal percentage by FMTA and GMTA for Cr(III) is 69.09% and 68.64% at 0.1 g/50 mL respectively, and then increase up to 85.54% and 84.55% at 0.9 g/50 mL respectively. By using MMTA adsorbent, it went up to from 74.09% at 0.1 g/50 mL to 91.36% at 0.9 g/50 mL. This increase in adsorption by increasing the dosage amount was mainly due to the maximum adsorption sites available on the surface of the adsorbents [32].

As observed from Fig. 5, adsorption capacity starting to decrease with the increase of adsorbent. This change occurs maybe because after attaining equilibrium, metal ions start to accumulate or aggregate on available sites in a result of which the total surface area decreases and consequently diffusion path length increases. The decrease in the adsorption capacity of Cr(III) may be because of the interventions of the aggregated binding sites at a high dosage amount available for the removal.

Under similar conditions, methanol modified biomass works efficiently for the removal of Cr(III). From this fact, it is illustrated that modification of Trifolium with methanol has the advantage to remove the Cr(III) efficiently from the solution under the optimum conditions.

3.3. Influence and optimization of solution pH on the Cr(III) removal

The pH of a solution is an important factor to study for the batch process of Cr(III) removal [33] as it has a significant effect on the chemical speciation, solubility, and charge of chromium; state of active sites responsible for chromium removal and its competition with other ions present in the medium [34,35]. pH influences the protonation of all the active sites, that is, hydroxyl, amino, and carboxyl group present in the biomass. Cr(III) ions occur as Cr^{3+} in acidic pH (1–6). Afterward, precipitation starts in the solution in the form of $Cr(OH)_3$ and thus it becomes unavailable for removal. At low pH, the concentration of protons increased and H^+ ions contest with the Cr^{3+} and protonate the active sites [36] which in results leads to the free ions in solution.

As illustrated in Fig. 6, adsorption capacity increases with an increase in solution pH up to pH 5 after that, adsorption capacity decreased due to the protonated active sites. As pH increases from 5.5, Cr(III) ions precipitated due to the formation of hydroxyl anions in solution. The maximum uptake of Cr(III) was seen at pH 5 by using MMTA, FMTA, and GMTA but the order of adsorption capacity was MMTA > FMTA > GMTA. This indicates that though the maximum removal has occurred at optimum pH 5 methanol modified works best in this condition for the Cr(III) removal.

3.4. Influence and optimization of time of contact on the Cr(III) removal

The contact time is a very decisive step in the process as this describes the minimum time, we required for the removal of the metals by biosorbent. It has been observed that adsorption occurs in two phases [37]. Initially, a sharp increase was observed in the adsorption capacity because of the high number of active sites present initially after that capacity change becomes constant as sorbent becomes enriched with the metal ions.

As illustrated in Fig. 7, the adsorption data for Cr(III) removal was plotted against the contact of time using all the modified adsorbents, that is, MMTA, FMTA, and GMTA biosorbents. In all cases, we can see that adsorption capacity increases by increasing the contact time and after a specific time, it becomes constant depicting that no further removal of Cr(III) was taking place from the medium. At this state, a dynamic equilibrium has been established between the amount of absorbed Cr(III) by adsorbents and the amount of removed Cr(III) by the adsorbents. The time of contact needed to attain equilibrium for Cr(III) adsorption by using MMTA, FMTA, and GMTA for an initial

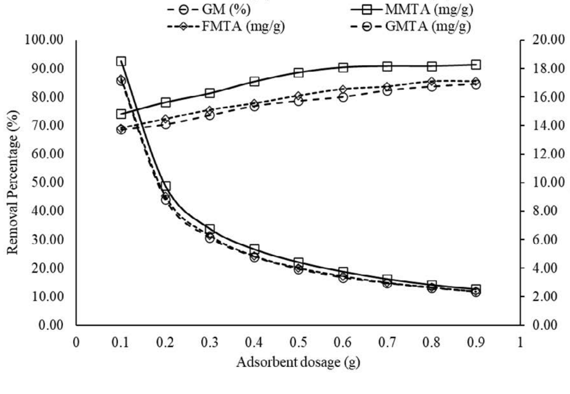

Fig. 5. Effect of adsorbent dosage on Cr^{3+} removal with the initial concentration of 50 mg/L varying adsorbent dose (0.1–0.9 g) at 25 min.

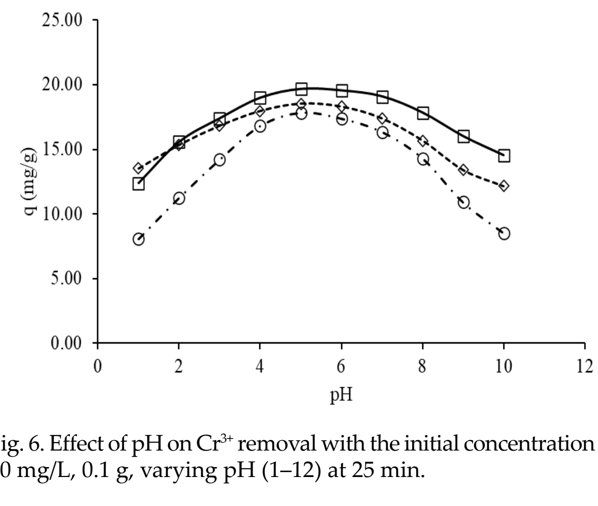

Fig. 6. Effect of pH on Cr^{3+} removal with the initial concentration of 50 mg/L, 0.1 g, varying pH (1–12) at 25 min.

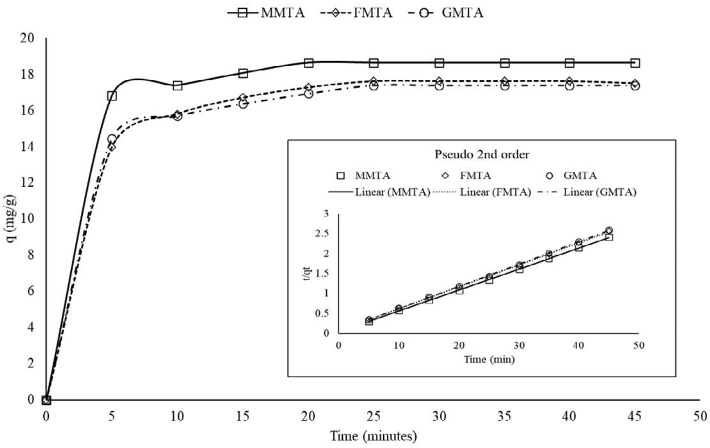

Fig. 7. Effect of contact time on Cr^{3+} removal with initial concentration of 50 mg/L, 0.1 g at pH 6 varying time from 5 to 45 min.

concentration of 50 mg/L was 15 min for MMTA and 20 min for FMTA and GMTA both.

3.4.1. Kinetic modelling

The mechanism of adsorption was determined by using kinetic modeling and it is also helpful to determine the rate steps and scheming of a large batch reactor for Cr(III) removal. This mechanism of the adsorption process can be examined by using pseudo-first and second-order rate equations.

The linear rate equation for the pseudo-first-order reaction was given by Lagergren [38] and expressed as:

$$\ln\left(q_e - q_t\right) = \ln q_e - k_1 t \tag{4}$$

where q_e adsorbed amount of adsorbate (mg/g), q_t is amount adsorbed at time t (mg/g), k_1 rate constant (g/mg/min) and t is contact time (min). The values of k_1 and q_e for the biosorption of Cr(III) were evaluated from the linear plot between $\ln(q_e–q_t)$ and t.

The pseudo-second-order linear rate equation was determined by Ho and McKay [39] and expressed as:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \tag{5}$$

where q_e is adsorbed amount of adsorbate (mg/g), q_t is the amount adsorbed at time t (mg/g), k_2 is the rate constant (g/mg/min) and t is contact time (min). The plot between t/q_t and t gives a linear relationship. The value of intercept gives $q_{e(\text{cal})}$ and from slope rate constant (k_2) can be calculated.

The percentage difference for adsorption capacity can be calculated as:

$$D\% = \frac{q_{e(\text{cal})} - q_{e(\text{exp})}}{q_{e(\text{exp})}} \times 100 \tag{6}$$

The calculated kinetic data from the above equations and linear plots are tabulated in Table 2. The correlation coefficient (R^2) for the pseudo-first-order model (0.9597 for MMTA, 0.9917 for FMTA, and 0.9882 for GMTA) was less than the pseudo-second-order model (0.9998 for MMTA, 0.9995 for FMTA, and 0.9997 for GMTA). The R^2 value for the pseudo-second-order approaching unity indicates that kinetics data fitted well in the pseudo-second-order rate equation as compared to the pseudo-first-order. Besides this $D\%$ value for the pseudo-first-order is greater than the pseudo-second-rate equation which also shows that kinetic data is being followed by the pseudo-second-order rate equation. From Table 2, it can be seen that theoretical q_e values for the pseudo-second-order rate equation are closer to the experimental q_e for the adsorption of Cr(III) for MMTA, FMTA, and GMTA.

3.5. Influence and optimization of initial metal ion concentration on Cr(III) removal

In batch mode, initial metal ion concentration plays a vital role and acts as a powerful strength that offers resistance between the liquid and solid phases during the mass transfer which indicates that amount of ions absorbed should be high at higher concentrations till the binding sites are enriched with them [40]. The effect of initial ion concentration on Cr(III) removal was evaluated in the range of 20–200 mg/L. In this study, we observed that in start removal percentage was higher due to the availability of active sites present for binding, and after that, it started to decrease, and no significant changes occurs due to the augmentation of all the binding sites. The same trend was observed in all three cases. The removal percentage decrease observed as 98.8% to 94.43%, 96.59% to 75.90% and 95.45% to 57.04% for MMTA, FMTA and GMTA correspondingly. The maximum adsorption capacity $q_{\max}$ for Cr(III) removal was found to be 94.43, 75.91, and 57.50 mg/g by MMTA, FMTA, and GMTA biosorbents respectively.

3.5.1. Adsorption isotherms

Adsorption isotherms are the graphical representations of the relationship between the amount adsorbed by unit weight amount of adsorbent and the left amount of

Table 2
Kinetic parameters for the removal of Cr(III) using chemically modified *Trifolium alexandrinum*

Models	Parameters	Adsorbents		
		MMTA	FMTA	GMTA
Pseudo-first-order	k_1 (min^{-1})	0.1159	0.1565	0.1221
	$q_{e(\text{cal})}$ (mg/g)	3.48	8.43	5.71
	$q_{e(\text{exp})}$ (mg/g)	18.64	17.61	17.39
	R^2	0.9597	0.9917	0.9882
	D%	81.32	52.15	–67.16
Pseudo-second-order	k_2 (min^{-1})	0.082	0.043	17.99
	$q_{e(\text{cal})}$ (mg/g)	18.97	18.18	17.98
	$q_{e(\text{exp})}$ (mg/g)	18.64	17.61	17.39
	R^2	0.9998	0.9995	0.9997
	D%	1.80	3.25	3.43

adsorbate present the medium at equilibrium [41]. They give information about the capacity of the adsorbent. Adsorption isotherms give a constant value which gives the surface properties and affinity of adsorbent. Therefore, they are necessary for the batch mode adsorption design at a larger mode [42]. For the investigations, two models, that is, Langmuir and Freundlich Isotherms were studied.

Langmuir model quantitively defines the monolayer formation of adsorbate outside the adsorbent, later on, no adsorption takes place further [43]. This isotherm signifies the distribution of adsorbate between the solid and liquid phases at equilibrium. The linear form of this model is represented as:

$$\frac{1}{q_e} = \frac{1}{bq_{\max}} \cdot \frac{1}{C_e} + \frac{1}{q_{\max}} \tag{7}$$

While non-linear form is expressed as:

$$q_e = \frac{b \cdot q_m \cdot C_e}{1 + b \cdot C_e} \tag{8}$$

where $q_{\max}$ is the maximum adsorbent capacity (mg/g), b refers to the Langmuir constant, q_e is uptake capacity at equilibrium (mg/g) and C_e is the concentration of species in solution. The parameters, that is, b and $q_{\max}$ can be determined from the linear plot of $1/q_e$ vs. $1/C_e$ from slope and intercept (Fig. 8).

For the favorable or unfavorable description of the adsorption process a separation factor constant R_L which is dimensionless is calculated by the following equation:

$$R_L = \frac{1}{1 + bC_0} \tag{9}$$

where b is the Langmuir constant and C_0 is the initial concentration. This value shows the favorability of isotherm, that is, $0 < R_L < 1$, unfavorable ($R_L > 1$), Linear ($R_L = 1$) or irreversible ($R_L = 0$) [5].

The Langmuir parameters are shown in Table 3. It was observed that $q_{\max}$ is greater for Cr(III) removal by using MMTA adsorbent, that is, 94.34 mg/g while in the case of FMTA and GMTA it was 77.52 and 61.73 mg/g respectively. It indicates that MM adsorbent is best for the removal of Cr(III) ions from the medium. From the correlation coefficient approaching unity, the experimental data of all the three cases fitted well in the Langmuir isotherm. This study shows that monolayer adsorption occurs by using MMTA, FMTA, and GMTA adsorbents. The R_L values for all the adsorbents used show that favorable adsorption occurs here.

The Freundlich isotherm model [44] is an experimental equation that is utilized for the multilayer system of adsorption due to the interactions of molecules adsorbed [41]. The linear form of the model is expressed as:

$$\log q_e = \log K_f + \frac{1}{n} \log C_e \tag{10}$$

where the non-linear form is written as:

$$q_e = K_f \cdot C_e^{1/n} \tag{11}$$

where K_f is Freundlich constant giving adsorption capacity (mg/g), $1/n$ refers to the intensity of adsorption, q_e is uptake capacity of adsorbent at equilibrium (mg/g) and C_e is ionic concentration in solution (mg/L). The Freundlich constant n indicates the intensity of adsorption if $1/n < 1$ shows the favorable adsorption and if $1/n > 1$ then it will be an unfavorable system. When n increases adsorption, the bond becomes stronger. Furthermore, $1/n$ is the heterogeneity factor, and n shows the deviation measure from linearity. If $n > 1$ then adsorption is favorable by physisorption and if $n < 1$ then adsorption is favored by chemisorption [45]. Linear adsorption is observed when $n = 1$ or near to unity.

As observed from the correlation coefficient value compared to the Langmuir model, it is indicated that equilibrium data did not follow the Freundlich isotherm. As seen from values tabulated in Table 3 Freundlich constant '*n*' shows that deviation from the linearity occurs here as values are higher than the unity. The *n* values indicate the good adsorption characteristics for adsorbents.

Table 3
Equilibrium modelling for removal of Cr(III) using chemically modified *Trifolium alexandrinum*

Isotherms	Parameters	MMTA	FMTA	GMTA
Langmuir model	$q_{\max}$ (mg/g)	94.34	77.52	61.73
	b (L/mg)	0.524752	0.214642	0.19708
	R_L	0.01–0.09	0.96–0.99	0.02–0.20
	R^2	0.9919	0.9905	0.9921
	RMSE	2.415737	1.773276	1.304959
Freundlich model	K_F	27.77794	15.29678	13.94762
	n	1.77683	2.136752	13.94762
	$1/n$	0.5628	0.468	0.385
	R^2	0.976	0.9537	0.8553
	RMSE	2.045542	2.241074	48.96734

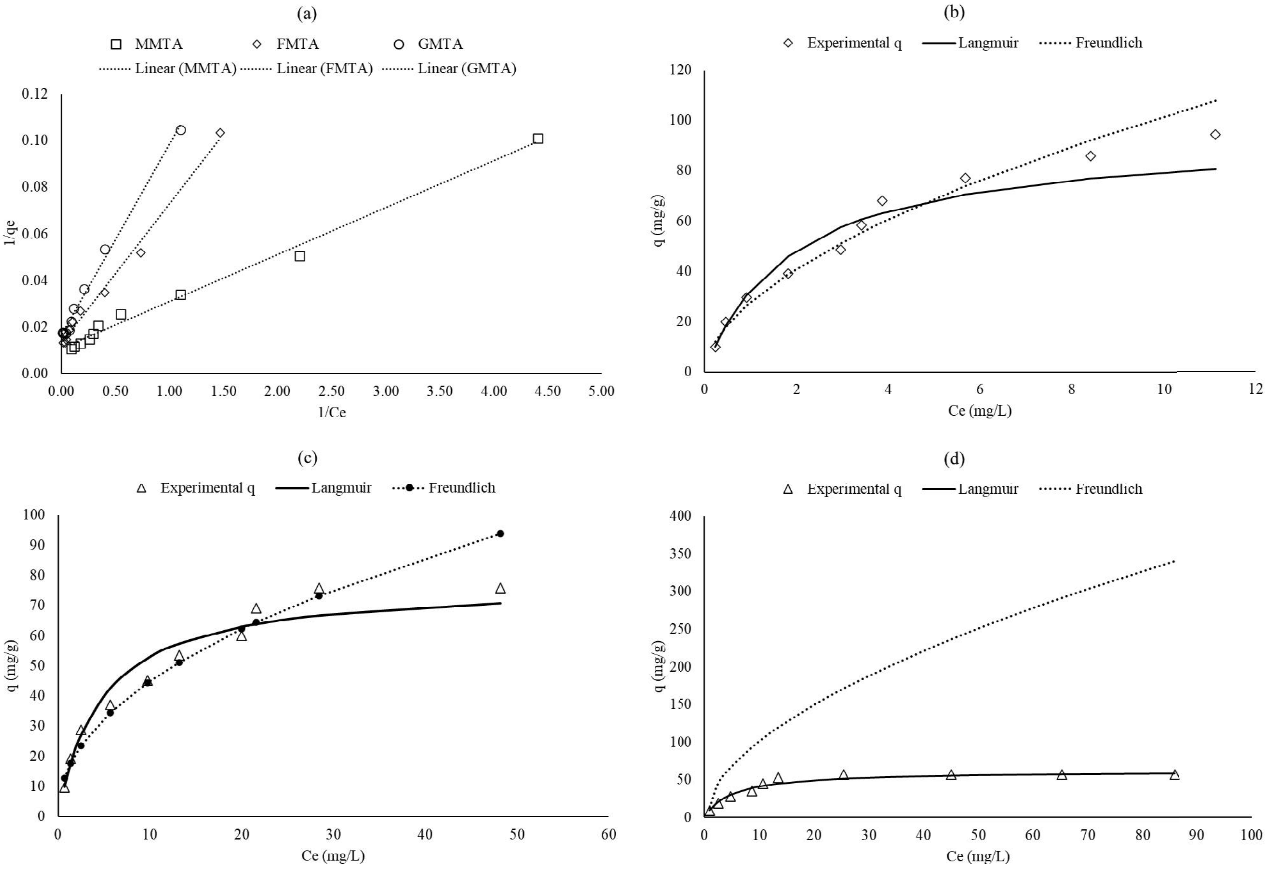

Fig. 8. (a) Linear isotherms plot for MMTA, FMTA, and GMTA. Non-linear isotherm plot for (b) MMTA adsorbent, (c) FMTA adsorbent and (d) GMTA biosorbent depicting the Langmuir and Freundlich models.

The above-explained models were related by using the RMSE-the root mean square error values calculated as:

$$\text{RMSE} = \sqrt{\frac{\Sigma\left(q_{e(\text{cal})} - q_{e(\text{exp})}\right)^2}{N}} \tag{12}$$

This value shows the error for linearity. As seen from Table 3 RMSE value is lower in the case of the Langmuir model which shows that equilibrium data fitted well in this model.

3.6. Influence and optimization of temperature on the Cr(III) removal (thermodynamics)

Thermodynamics study is beneficial for the possibility of the adsorption system and its capability for the removal of Cr(III) removal from the medium. Thermodynamics parameters calculated for the study are enthalpy ($\Delta H°$), entropy ($\Delta S°$), and free energy ($\Delta G°$). As seen from figure Cr(III) removal increases with an increase in temperature. This trend maybe because of the opening of binding sites by temperature increase. All thermodynamics parameters are shown in Table 4 calculated from:

$$\Delta G° = \Delta H° - T\Delta S° \tag{13}$$

$$\Delta G° = -RT\ln K_D \tag{14}$$

$$\ln K_D = \frac{\Delta S°}{R} - \frac{\Delta H°}{RT} \tag{15}$$

where $\Delta G°$ standard free energy, $\Delta S°$ is the entropy change, $\Delta H°$ is the enthalpy of the process, T contact time (min), R is the universal gas constant (8.3134 J/mol/K) and K_D is the distribution coefficient calculated as:

$$K_D = \frac{C_0 - C_e}{C_e} \tag{16}$$

The values of $\Delta S°$ and $\Delta H°$ can be determined from intercept and slope from the linear plot of $\Delta G°$ vs. T (K). The values of free energy are calculated by using the value of K at different temperatures (Table 4).

As from Table 4, $\Delta G°$ values are negative at all temperature ranges which indicates the spontaneous and viable process. The positive value of enthalpy ($\Delta H°$) represents that process is endothermic. From one assumption extent of enthalpy is also used for the adsorption classification as physisorption and chemisorption. If bonding strengths are less than 84 kJ/mol it is considered as physisorption and bond strengths between ranges of 84–420 kJ/mol referred to as chemisorption. Henceforth value of enthalpy, that is, 12.394 for MMTA, 15.016 for FMTA, and 16.563 for GMTA shown that the adsorption process is physisorption. The positive value of $\Delta S°$ for all the adsorbents shows that adsorption is mainly because of randomness.

3.7. Adsorption mechanism

The methanol modified Biomass shows greater binding for the Cr(III). The treatment of *T. alexandrinum* with methanol actually causes the esterification of the carboxylic acid groups of cellulose which results in the binding of Cr(III). The reaction occurs as follows:

$$\mathrm{RCOOH} + \mathrm{CH_3OH} \xrightarrow{\mathrm{H^+}} \mathrm{RCOOCH_3} + \mathrm{H_2O} \tag{17}$$

Table 4
Thermodynamic parameters for removal of Cr(III) using chemically modified *Trifolium alexandrinum*

Adsorbents	Temperature (K)	$\Delta G°$ (kJ/mol)	$\Delta S°$	$\Delta H°$
MMTA	283.16	–1.6963	0.05	12.394
	293.16	–2.33504		
	303.16	–2.89275		
	313.16	–3.25411		
	323.16	–3.64894		
	333.16	–4.33882		
FMTA	283.16	–0.64608	0.0551	15.016
	293.16	–0.98825		
	303.16	–1.66161		
	313.16	–2.43558		
	323.16	–2.82297		
	333.16	–3.24831		
GMTA	283.16	0.128442	0.0588	16.563
	293.16	–0.66889		
	303.16	–1.46014		
	313.16	–2.14992		
	323.16	–2.4533		
	333.16	–2.7806		

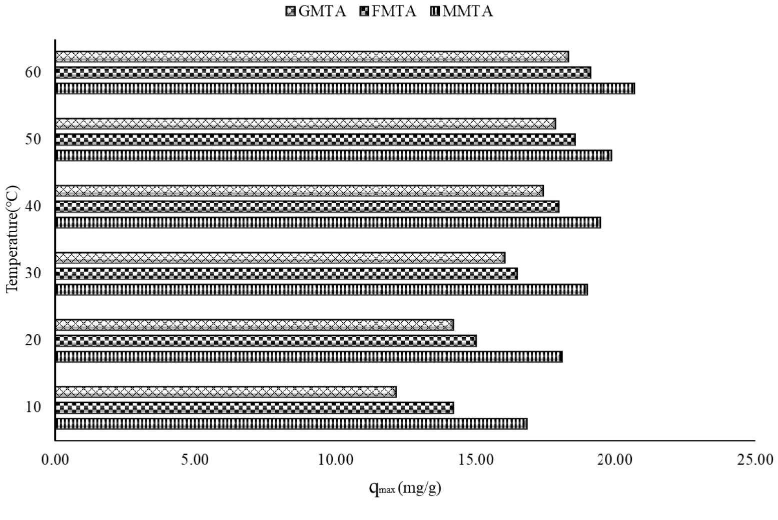

Fig. 9. Effect of temperature on Cr^{3+} removal with initial concentration of 50 mg/L, 0.1 g varying the temperature at 25 min.

Table 5
Comparison of biosorption capacity with other biosorbents

Biosorbents used for Cr(III) removal	Adsorption capacities (mg/g)	References
Methanol modified *Trifolium alexandrinum*	94.34	This study
Formaldehyde modified *Trifolium alexandrinum*	77.52	This study
Glutaraldehyde modified *Trifolium alexandrinum*	61.73	This study
Urea-modified *Sorghum bicolor* L. biomass	16.36	[28]
Peanut shells	27.86	[23]
Pre-treated orange peels	9.43	[24]
Green alga *Ulva lactuca*	10.61	[46]
Biacetyl treated wheat straw	24.69	[40]
Orange (*Citrus cinensis*) waste	79.03	[47]
Vineyard pruning waste	12.45	[48]
Surface modified pineapple crown leaf	5.195	[27]
Sorghum bicolor	25.64	[49]
Coaca shell	2.52	[50]
Cassia fistula	85.71	[51]
Pine bark	31.40	[52]
Cabomba caroliniana	19.61	[53]
Bone char	78.59	[54]
Salvinia auriculata biomass	72	[55]

In case of formaldehyde and glutaraldehyde, they acted as a cross linking agents between two cellulose monomers, resulting the decrease of biosorption capacities.

3.8. Comparison with other biosorbents for Cr(III) removal

Our modifications were also compared with other biosorbents capacities for Cr(III) removal. The comparison is tabulated in Table 5. Methanol modification and other modifications in this study cause a noteworthy variation in the biosorption capacity than the other biosorbent used for the removal of Cr(III). This increase was majorly shown by the methanol modification used with variation in this study. Hence, we can say that out of all modifications methanol modified biomass can be used as a potential adsorbent for Cr(III) removal from the medium.

4. Conclusion

A novel adsorbent *T. alexandrinum* was used for the removal of Cr(III) from the aqueous medium. Three modifications were utilized to check the adsorption capacities for Cr(III) removal. The study was conducted in batch mode and different parameters were optimized. All the results indicate that methanol modified adsorbent works best for the removal of Cr(III) from aqueous solution. The optimum conditions were found to be 0.1 g/50 mL by using all three adsorbents for Cr(III) removal. The optimum pH was found to be 5 for all the adsorbents but methanol modified adsorbent works best in this condition removing maximum Cr(III) ions from aqueous solution. The contact time was found to be in the range of 15–20 min for Cr(III) removal. Kinetics modeling exposed that biosorption of Cr(III) by using MMTA, FMTA, and GMTA adsorbents were followed by a pseudo-second-order rate equation signifying the limited rate step mechanism. Equilibrium data fitted well in the Langmuir isotherm model making this system monolayer adsorption. The parameters, that is, R^2, R_L, and RMSE supported the Langmuir model fitness to equilibrium data. The thermodynamics study revealed the endothermic nature of the process.

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