

Removal of lead and cobalt using lignocellulosic fiber derived from *Citrus reticulata* waste biomass

Haq Nawaz Bhatti^{*,†}, Iqra Ijaz Bajwa^{*}, Muhammad Asif Hanif^{*}, and Iftikhar Hussain Bukhari^{**}

^{*}Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad-38040, Pakistan

^{**}Department of Chemistry, University of Sarghoda, Sarghoda

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Abstract—The present investigation explored the use of *Citrus reticulata* waste biomass (CWB) for the removal of Pb(II) and Co(II) from the aqueous solutions. The Pb(II) and Co(II) biosorption was found to be dependent on pH of the solution, biosorbent dose, biosorbent particle size, temperature, shaking speed, contact time and initial concentration of metal ions. A metal uptake capacity of 41.16 and 52.64 mg/g was observed at pH 5 and 7 for Pb(II) and Co(II), respectively. The biosorption data followed the Freundlich model for both metals. The overall biosorption process was best described by pseudo-second order kinetics. The effect of several pretreatments on the biosorption efficiency of CWB was also investigated. The results demonstrated that pretreatments influenced the biosorption capacity of the biomass for the both metals significantly. Maximum biosorption capacity of 83.77 and 95.55 mg/g was observed for Pb(II) and Co(II) with sodium hydroxide treated and simply heated biomass, respectively. FTIR spectrum indicated the presence of -OH, -NH, -COOH groups in the biomass. The surface structure of CWB was analyzed by JEOL JMT 300 scanning electron microscope (SEM), and the existence of metal ions on the surface of biosorbent was determined by energy dispersive X-ray (EDX) spectroscopy.

Key words: *Citrus reticulata*, Pb(II), Co(II), Biosorption, Kinetics, Treatments

INTRODUCTION

Toxic heavy metal ions get introduced to the aquatic ecosystems by means of various industrial activities, such as mining, alloy making, electroplating, tanneries, batteries, fertilizer industries, paper industries, pesticides etc., and pose a serious threat to aquatic ecosystems as well as human beings. Heavy metal remediation of aqueous systems is of special concern due to recalcitrance and persistency of heavy metals in the environment [1]. Such industries, due to low returns, constrain themselves from investing in the area of effluent treatments [2]. Lead is the most commonly used metal by these small-scale industries, while cobalt is generally associated with this as a contaminating material. Lead (II), like all other heavy metals, constitutes a threat to public health and can find its way into the human food chain. It has also been reported that Pb(II) inhibits plant and microbial growth by influencing the pH of the substrate and inactivating the cell enzymes [3,4]. Industrial exploitation of Co(II) in alloy production, electroplating, generation of gas turbines and petrochemical industries results in discharge of Co(II) wastes into the environment, leading to a variety of toxic effects on all living forms including plants, animals and microorganisms. Although Co(II) is potentially less toxic, special care needs to be taken in order to prevent the accumulation of this metal in the environment.

Conventional methods such as chemical precipitation, ion exchange, chemical oxidation/reduction, reverse osmosis, solvent extraction, electrodialysis, ultrafiltration etc., were used until recently [5,6]. However, these methods have their own limitations such as incom-

plete metal removal, sensitive operating conditions, generation of toxic sludge and its disposal [7,8]. Furthermore, strict environmental protection legislation and public environmental concerns lead researchers to invent new technologies for the treatment of wastewater. The use of biomaterials, such as agricultural wastes, for the removal of heavy metals from wastewater and industrial effluents has emerged as a potential alternative method to conventional methods [9]. The agricultural waste materials, particularly those containing cellulose, show potential metal biosorption capacity due to the presence of a variety of functional groups that facilitate metal complexation [10].

Citrus reticulata is a widely grown variety of citrus fruit that is deep yellow, apex round and flattened rind orange, smooth and glossy. The pulp is very juicy, sweet acidic and rich in flavor. The pulp left after the extraction of juice is a waste material having no commercial value. In continuation of our previous work on the removal of toxic metals using *Citrus reticulata* waste biomass [11], the present project is undertaken to test the biosorption efficiency of this waste biomass for Pb(II) and Co(II) from the aqueous solutions.

MATERIALS AND METHODS

1. *Citrus reticulata* (Kinnow) Waste Biomass

Citrus reticulata waste biomass (CWB) used in this work was harvested, processed and stored as described earlier [11].

2. Reagents

All reagents used in these studies were of analytical grade and were purchased from E. Merck Company (Darmstadt, Germany) except *Moringa Olifera* seed powder (purchased from Jhang Bazaar, Faisalabad, Pakistan). The chemicals used in this study were Pb(NO₃)₂, Co(NO₃)₂, H₂SO₄, HCl, HNO₃, Al(OH)₃, Triton X-100, NaOH, κ-

[†]To whom correspondence should be addressed.

E-mail: hnbhatti2005@yahoo.com

carrageen, CH₃OH, C₂H₅OH, PEI, glutaraldehyde, HCOH, (CH₃)₂SO₄, (NH₄)₂SO₄, EDTA, C₆H₆, H₂O₂, CaCl₂, potash alum, sodium alginate, calcium alginate, lead and cobalt atomic absorption spectrometry standard solutions (1,000 mgL⁻¹).

3. Preparation of Pb(II) and Co(II) Solutions

Stock Pb(II) and Co(II) solutions (1,000 mgL⁻¹) of pH 4.75 and 5.60, respectively, were prepared by dissolving 1.598 g Pb(NO₃)₂ and 3.10 g Co(NO₃)₂ in 100 mL of deionized distilled water (DDW) and diluting it upto 1,000 mL quantitatively using DDW. Solutions of different concentrations were prepared by adequate dilution of the stock solution with DDW. Glassware and polypropylene flasks used were overnight immersed in 10% (v/v) HNO₃ and rinsed several times with DDW. Both lead and cobalt solutions were separately used, and independent optimization of process parameters was investigated.

4. Treatments of Biomass

Treatments of the biomass with different reagent were performed under specific conditions as described earlier [11].

5. Metal Biosorption Studies

A batch equilibration method was employed to study the biosorption of Pb(II) and Co(II) by *Citrus reticulata* waste biomass. A set of 250 mL Erlenmeyer flasks containing 100 mL of the metal solution (100 mg/L) was used and 0.1 g of biosorbent (i.e., CWB) was contacted with the metal solutions by incubating the flasks on a rotating shaker (PA 250/25.H) with constant shaking at 100 rpm for 24 h. The contents of the flasks were filtered through filter paper (Whatman No. 40, ashless) and filtrates analyzed for residual metal concentration. To study the effect of different experimental parameters such as pH [1-5 for Pb(II) and 1-7 for Co(II)], biosorbent dose (0.05, 0.1, 0.2, 0.3 and 0.4 g/100 mL), biosorbent size (<0.250, 0.250-0.350, 0.350-0.500, 0.500-0.710 and 0.710-1.00 mm), initial metal concentration (25, 50, 100, 200, 400 and 800 mg/L), shaking speed (0, 50, 100 and 150 rpm) and contact time (15, 30, 60, 120, 240, 480, 720 and 1,440 min) were used to assess the biosorption of Pb(II) and Co(II) by CWB. All the above experiments were done in triplicate to ensure accuracy of results.

6. Analytical Procedures

The concentrations of Pb(II) and Co(II) in the solutions before

and after equilibrium were determined by flame atomic absorption spectrometry (FAAS), using a Perkin-Elmer AAnalyst 300 atomic absorption spectrometer equipped with an air-acetylene burner and controlled by Intel personal computer.

The Pb(II) and Co(II) uptake was calculated by the simple concentration difference method. The initial concentration C_i (mg/L) and metal concentrations at various time intervals C_e (mg/L) were determined and the metal uptake q_e (mg metal adsorbed/g adsorbent) were calculated from the mass balance equation as follows:

$$Q_e = (C_i - C_e) V / 1000w \quad (1)$$

Where V is the volume of the solution in mL and w is the mass of the sorbent in g.

7. FTIR, EDX and SEM Studies

The functional groups of *Citrus reticulata* waste biomass were analyzed by Bruker Tensor 27 Fourier transform infrared (FTIR) spectrometer with the samples prepared as KBr discs. The surface structure of CWB was analyzed by JEOL JMT 300 scanning electron microscope (SEM) and the existence of metal ions on the surface of biosorbent was determined by energy dispersive X-ray spectroscopy (EDX, Perkin-Elmer, USA). The CWB sample was covered with a thin layer of gold, and an electron accelerated voltage of 20 kV was applied.

8. Statistical Analysis

All experiments were performed in triplicate to ensure reproducibility. All results are reported as mean \pm SD.

RESULTS AND DISCUSSION

1. Effect of pH

Effect of solution pH on the removal of Pb(II) and Co(II) was studied by using CWB, and the results are shown in Fig. 1. As the pH of the solution was increased, the adsorption of both Pb(II) and Co(II) ions increased. It was observed that the maximum percentage removal of Pb(II) and Co(II) was achieved at pH of 5 and 7, respectively. At low pH protons can compete effectively with Pb(II) and Co(II) for the binding sites in the biomass. The protonated binding sites are thus no longer available to bind Pb(II) and Co(II). As

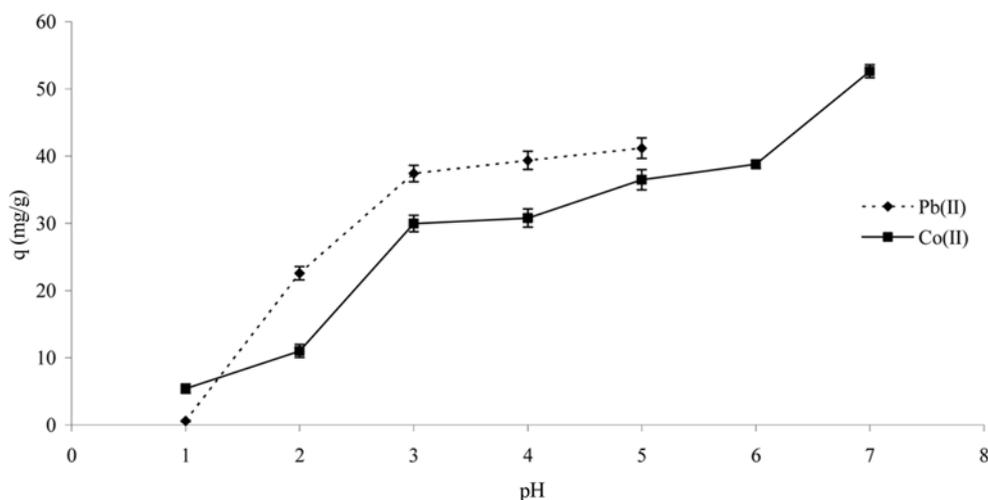


Fig. 1. Effect of pH on the biosorption of Pb(II) and Co(II) by *Citrus reticulata* waste biomass.

the pH increases, the Pb(II) and Co(II) ions become more competitive with hydrogen ions, which in turn results in an increased metals uptake. Solution pH is an important parameter for adsorption of metal ions from aqueous solution because it affects the solubility of the metal ions, concentration of the counter ions on the functional groups of the adsorbent and the degree of ionization of the adsorbate during reaction. The solution chemistry of the heavy metals such as hydrolysis, complexation by organic and/or inorganic ligands, redox reactions and precipitation is also strongly influenced by pH [12]. In a previous study, maximum biosorption capacity of rose waste biomass for Pb(II) and Co(II) was observed at pH 5 and 6, respectively [12]. At pH higher than optimum, the precipitation of insoluble metal hydroxide takes place restricting the true biosorption study. The biomass of marine macro algae has been reported to have a high metal uptake capacity for a number of heavy metal ions and is strongly influenced by the value of solution pH [13]. Gupta et al. [14] studied kinetic and isotherm modeling of lead (II) sorption onto some waste plant materials and found that the plant materials sorbed Pb(II) optimally at pH 4 or 5. Similarly, Gupta and

Rastogi [15] observed a maximum uptake of Pb(II) by two algal biomass *Oedogonium* sp. and *Nostoc* sp. at pH 5.

2. Effect of Biosorbent Dose

The removal of Pb(II) and Co(II) by CWB was investigated by varying the dose of biosorbent while keeping constant the pH (pH 5 for Pb(II) and pH 7 for Co(II), biosorbent size (0.25 mm), initial metal concentration (100 mg/L), shaking speed (100 rpm) and temperature (30 °C). The results regarding the effect of biosorbent dose are shown in Fig. 2. The results indicated that biosorption capacity decreased after a dosage of 0.1 and 0.05 g for Pb(II) and Co(II), respectively. Similarly, the percentage removal decreased significantly in both metals with the increase in biosorbent dosage. On increasing biomass dose further, the removal of Pb(II) and Co(II) decreased, which may be related to overlapping of adsorption sites resulting in a decrease in total adsorbent area; hence the adsorption capacity of CWB decreased with increase in the biosorbent dosage. In an earlier investigation from this laboratory, Javed et al. [12] reported that the biosorbent dose influences the Pb(II) and Co(II) uptake capacity of rose waste biomass, and a dose of 0.5 g/L was optimum

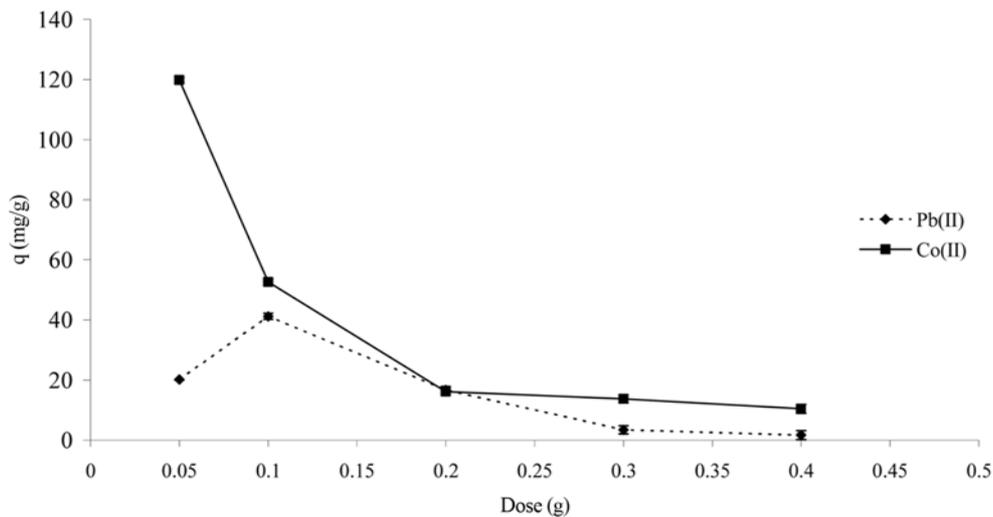


Fig. 2. Effect of biosorbent dose on the biosorption of Pb(II) and Co(II) by *Citrus reticulata* waste biomass.

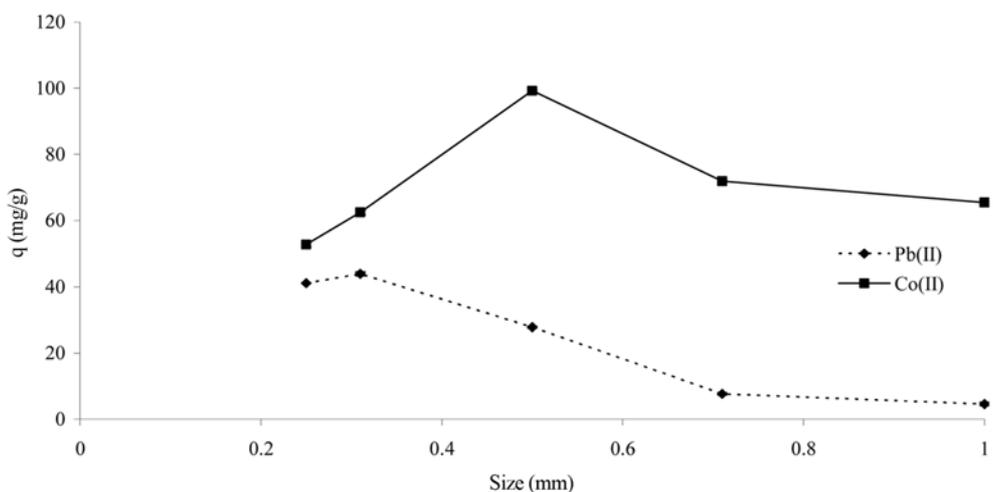


Fig. 3. Effect of biosorbent particle size on the biosorption of Pb(II) and Co(II) by *Citrus reticulata* waste biomass.

for maximum uptake of these metals.

Biosorbent dose is a significant factor to be considered for effective metal sorption from aqueous systems. It determines the sorbent-sorbate equilibrium of the system. Dose of biomass added in to the solution determines the number of binding sites available for adsorption [16]. At low dose level, all binding sites are exposed and adsorption on the surface is faster, but at higher biosorbent doses the availability of higher energy sites decreases with a larger fraction of low energy sites occupied, resulting in low q_e value [15].

3. Effect of Biosorbent Size

The effect of biosorbent size on the biosorption capacity of CWB is depicted in Fig. 3. It is evident from the figure that Pb(II) biosorption capacity increases with an increase in particle size of CWB, reaches maximum (43.99 mg/g) with a particle size of 0.31 mm and then decreases sharply. While in case of Co(II), biosorption capacity also shows an increasing trend with an increase in particle size. But maximum biosorption capacity is observed with the particle size of 0.50 mm. Further increase in the particle size shows a steady decrease in the biosorption capacity of CWB. This was most probably due to the increase in the total surface area, which provided more binding sites for metal ions and hence biosorption increased. Biosorption generally decreases with an increase in biosorbent size, but there is an optimum particle size required for effective metal uptake. Too small or too large particle size decreases interaction of biomass with metal, which results in reduction in biosorption capacity of a biomass [17]. Secondly, there is a definite relationship between hydrated metal ion radius and biomass particle size as shown by Iftikhar et al. [18] in a previous study. In an earlier study, we observed optimum biosorption of Pb(II) and Co(II) using rose waste biomass with a particle size of 0.25 mm [12]. Different biomasses contain different types of compounds having a variety of functional groups which are generally exposed during biosorption. A particular size of a biomass provides particular number/types of functional groups and hence binding sites for the metal ions.

4. Effect of Initial Concentrations of Pb(II) and Co(II)

The biosorption of Pb(II) and Co(II) by CWB increased with increasing metal concentration in the solution (Fig. 4); however, the percentage removal of both the metals decreased with the increase

in concentration. Maximum biosorption capacity of Pb(II) and Co(II) was observed in a solution with initial metal concentration of 800 mg/L. This was probably due to the fact that at high initial concentration the number of metal ions available to surface area was high, so biosorption became dependent on initial concentration. This biosorption characteristic indicated that surface saturation was dependent on the initial metal ion concentration [16]. Various steps are involved in the biosorption mechanism, which deals with the transfer of metal ions from the solution to the surface of biosorbent, e.g., bulk transport of metal ions in the solution phase, film transport involving diffusion of metal ions and the actual adsorption of the metal ions by the active site of the biosorbent. In the case of Pb(II) and Co(II) biosorption by CWB a good mixing of adsorbate and biosorbent in the system suppresses the limitation due to bulk transport of metal ions and hence kinetics seems to be more affected by the transfer of metal ions from the solution to the active site of the biosorbent surface. Thus initial concentration provides an important driving force to overcome all mass transfer resistance of metal ions between the aqueous and solid phases.

5. Adsorption Isotherms

The adsorption isotherms are the plots of solute concentration in the adsorbed state as a function of its concentration in the solution at constant temperature. When an isotherm is plotted using the data at equilibration of the experimental system, it gives valuable information that is useful for the selection of an adsorbent. It also facilitates the evaluation of the feasibility of the adsorption process for a given application. The equilibrium data was modeled by using the most widely used Langmuir and Freundlich isotherm models. The Langmuir isotherm considers sorption as a chemical phenomenon. In the Langmuir model, maximum monolayer adsorption capacity, q_{max} (mg/g) and other parameters were determined from linearizing the given equation:

$$\frac{C_e}{q_e} = \frac{1}{q_{max}K_L} + \frac{C_e}{q_{max}} \quad (2)$$

Where q_e is the metal ion sorbed (mg/g), C_e the equilibrium concentration of metal ions solution and K_L is the Langmuir adsorption constant. The heterogeneous adsorption capacity, q_L (mg/g) of

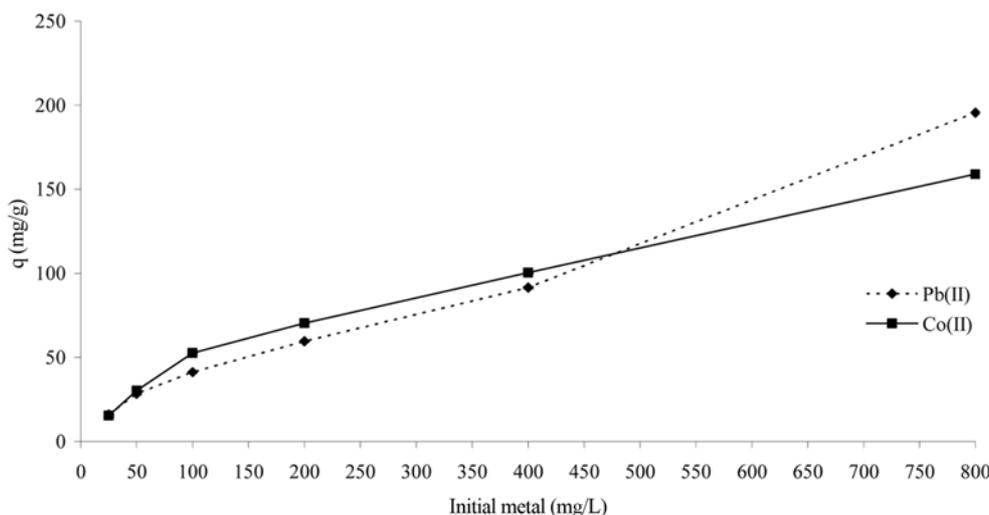


Fig. 4. Effect of initial metal concentration on the biosorption of Pb(II) and Co(II) by *Citrus reticulata* waste biomass.

Citrus reticulata biomass for Pb(II) and Co(II) was determined by the following equation of the Freundlich isotherm:

$$\log q_e = \frac{1}{n} \log C_e + \log k \tag{3}$$

Value of K_L and n indicated that Langmuir isotherm model is not fitted well to the biosorption data, because R^2 value is 0.70 and 0.93 for Pb(II) and Co(II), respectively. On the other hand, the data fitted well to the Freundlich isotherm for both Pb(II) and Co(II), as a higher value of regression coefficient ($R^2=0.97$) was observed (Figs. 5 & 6; Table 1). This indicated that physical adsorption occurred in this

situation. The Freundlich relationship is an empirical equation. It does not indicate a finite uptake capacity of the sorbent and can thus only be reasonably applied in the low to intermediate concentration ranges. However, it is easier to handle mathematically in more complex calculations (e.g., in modeling the dynamic column behavior) where it may appear quite frequently. In our previous study, we observed that biosorption of Pb(II) and Co(II) by rose waste biomass followed the Langmuir model [12].

6. Kinetic Study

A kinetic study with different time intervals with fixed metal solution, biosorbent amount and biosorbent particle size was performed.

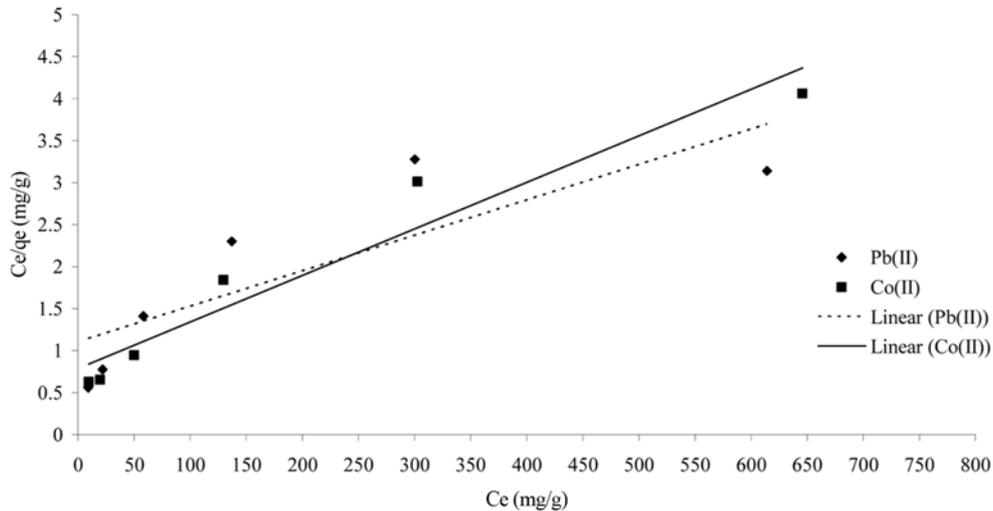


Fig. 5. Linearized Langmuir isotherm model for the biosorption of Pb(II) and Co(II) by *Citrus reticulata* waste biomass.

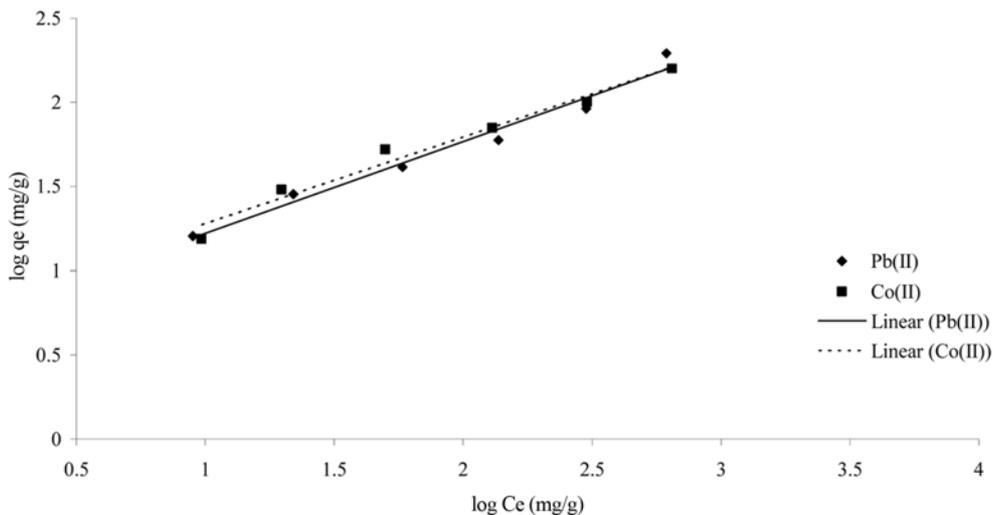


Fig. 6. Freundlich isotherm model for biosorption of Pb(II) and Co(II) by *Citrus reticulata* waste biomass.

Table 1. Langmuir and Freundlich isotherms parameters

Metal	Experimental q_{max} (mg/g)	Langmuir isotherm parameters			Freundlich isotherm parameters			
		q_{max} (mg/g)	K_L	R^2	q_{max} (mg/g)	K	1/n	R^2
Pb(II)	195.55	238.09	3.7×10^{-3}	0.70	155	4.72	0.54	0.97
Co(II)	158.9	181.18	6.98×10^{-3}	0.93	162	2.20	0.51	0.97

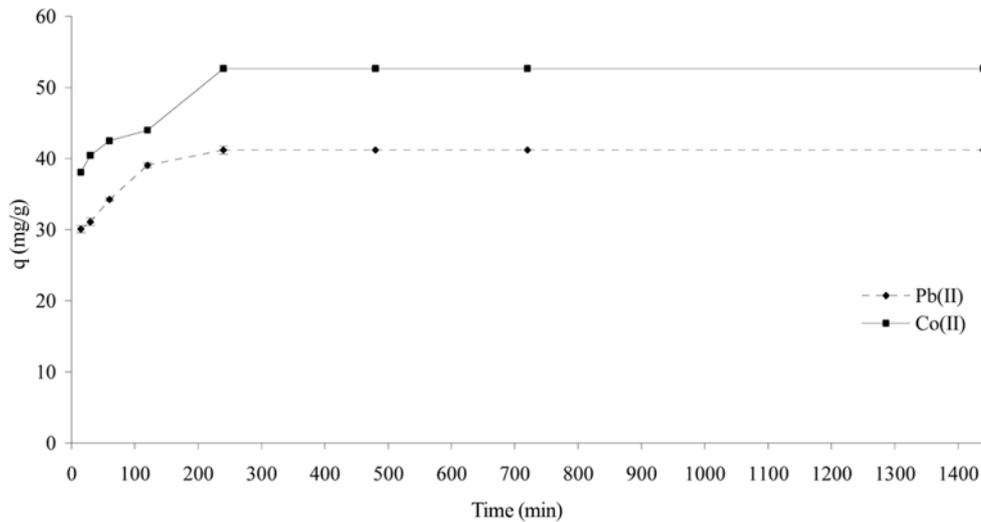


Fig. 7. Effect of contact time on the biosorption of Pb(II) and Co(II) by *Citrus reticulata* waste biomass.

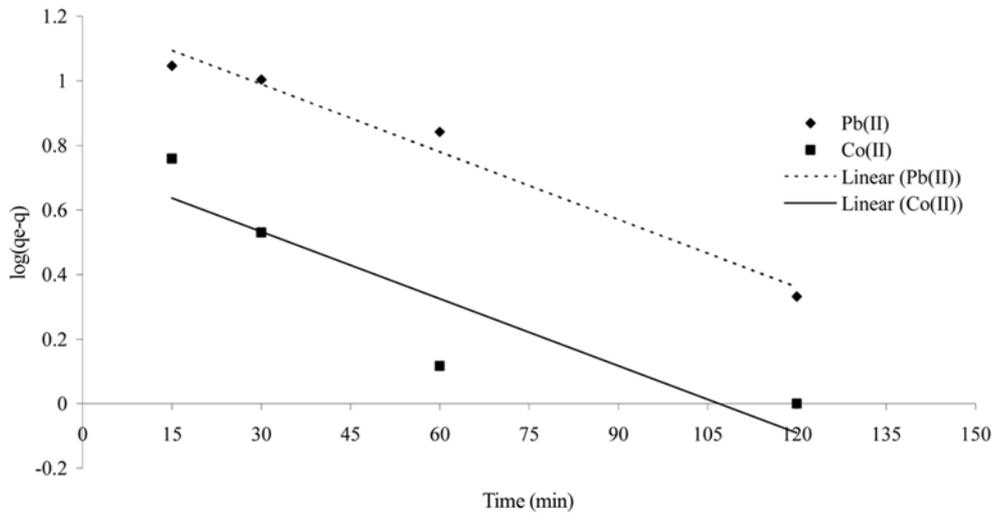


Fig. 8. Lagergren pseudo-first order kinetic model for biosorption of Pb(II) and Co(II) by *Citrus reticulata* waste biomass.

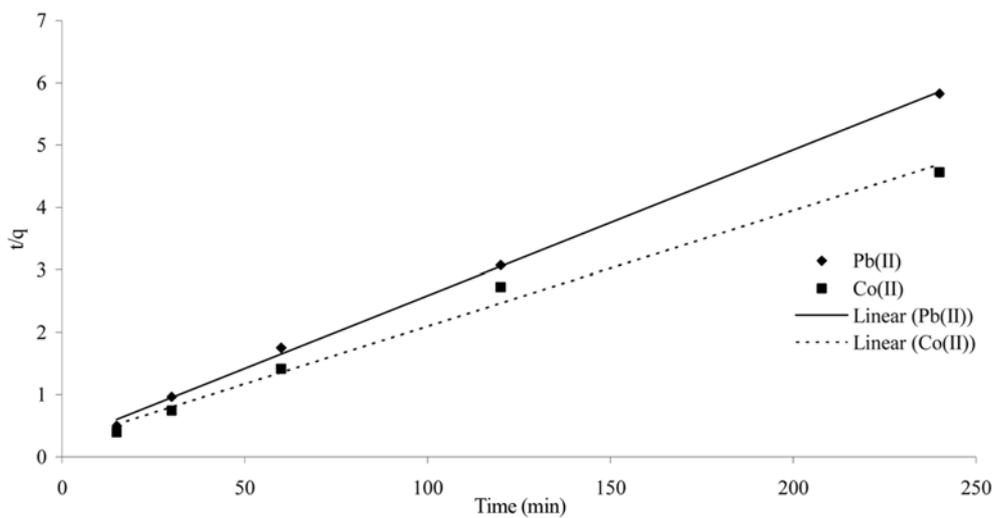
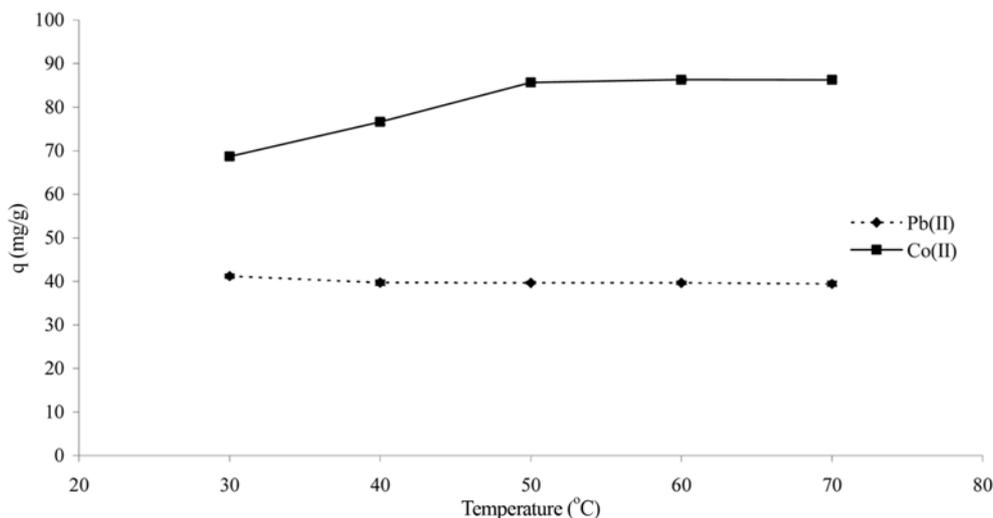


Fig. 9. Pseudo-second order kinetic model for the biosorption of Pb(II) and Co(II) by *Citrus reticulata* waste biomass.

Table 2. Comparison between adsorption rate constants, q_e estimated and coefficient of correlation associated the Lagergren pseudo-first order and to pseudo-second order kinetic models

Sample	Experimental q_{max} (mg/g)	Pseudo-first order			Pseudo-second order		
		q_e	$K_{1, ads}$	R^2	q_e	$K_{2, ads}$	R^2
Pb(II)	41.19	15.81	0.016	0.97	42.37	2.18×10^{-3}	0.99
Co(II)	43.81	13.24	0.0158	0.82	54.05	1.3×10^{-3}	0.99

**Fig. 10. Effect of temperature on the biosorption of Pb(II) and Co(II) by *Citrus reticulata* waste biomass.**

The adsorption capacity (q) by CWB for Pb(II) and Co(II) corresponding to different time intervals is shown in Fig. 7. Experiments were conducted to determine the time required by the biomass to bind the metal ions. Metal solutions (100 mL) were taken in 250 mL conical flasks. The solutions were agitated on an orbital shaking incubator at 100 rpm at 30 °C. Kinetic analysis was performed by withdrawing samples at selected time intervals ranging from 15 to 1,440 min (15, 30, 60, 120, 240, 480, 720 and 1,440) by disposable syringes (5 cc). The samples taken were filtered through 0.45 μ m membrane filter (Millipore) and stored in eppendorff tubes. The optimum sorption time was 240 min for Pb(II) and Co(II). Equilibrium was reached in a contact time of 240 min. Under equilibrium conditions, the biosorption capacity of Pb(II) and Co(II) was 41.19 and 52.64 mg/g, respectively.

Kinetic data was fitted by using the Lagergren pseudo-first order model and pseudo-second order model. The results are depicted in Figs. 8 and 9 and the numerical values of constants are shown in Table 2. The linearized form of the first order Lagergren equation is given as:

$$\log(q_e - q) = \log q_e - \frac{k_{1, ads} t}{2.303} \quad (4)$$

The pseudo-second order equation is

$$\frac{t}{q} = \frac{1}{k_{2, ads} q_e^2} + \frac{t}{q_e} \quad (5)$$

Where q_e is the mass of metal adsorbed at equilibrium (mg/g), q the mass of metal at time t (min.), $k_{1, ads}$ the first order reaction rate of adsorption (per min), $k_{2, ads}$ the pseudo-second order rate constant

of adsorption mg/g min^{-1} . The kinetic data suggest that the sorption of Pb(II) and Co(II) ions followed the second kinetic model, which relies on the assumption that biosorption may be the rate-limiting step. Sorption of Pb(II) and Co(II) by rose waste biomass also followed the second order kinetic model [12].

7. Effect of Temperature

The effect of temperature on the biosorption of Pb(II) and Co(II) onto CWB was investigated at five different temperatures (30-70 °C), and the results are shown in Fig. 10. The biosorption capacity of CWB for Pb(II) ions did not significantly change and appears to be temperature independent over the temperature range tested. However, biosorption capacity of Co(II) increases with rise in temperature, reaching maximum (86.3 mg/g) at 60 °C and then shows a slight decrease. Co(II) uptake by CWB is an endothermic process. The increase with temperature could cause enhanced ion-exchange and a change in the size of biosorbent pores. However, in certain cases at higher temperature, metal ions adsorbed earlier on the surface of biosorbent tend to desorb from the surface at elevated temperatures [18]. The temperature of the adsorption medium could be important for energy-dependent mechanisms in metal ion biosorption by different biosorbents. Generally, adsorption is an exothermic process, although some examples of endothermic adsorption have also been reported [19]. In our previous laboratory investigation, we observed that biosorption of Pb(II) and Co(II) onto rose waste biomass is an exothermic process [12]. Tüzün et al. [20] observed that biosorption of Pb(II) ions onto microalgae *Chlamydomonas reinhardtii* did not change significantly in the temperature range of 5-35 °C.

8. Effect of Pretreatments

The effects of different pretreatments on the biosorption capac-

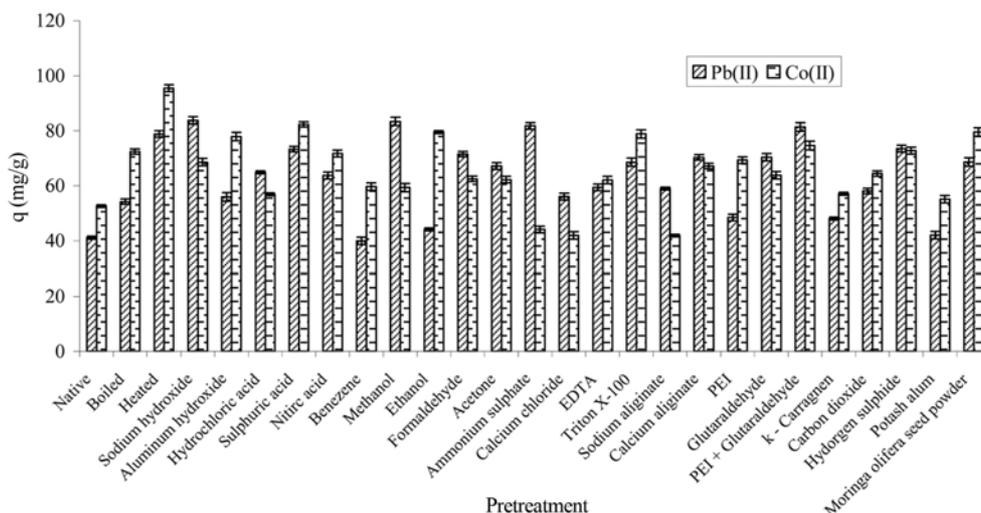


Fig. 11. Effect of pretreatments on the biosorption of Pb(II) and Co(II) by *Citrus reticulata* waste biomass.

ity of CWB are shown in Fig. 11. Biosorption capacity (mg/g) of non-treated, physically and chemically modified CWB were found in the following order for Pb(II): sodium hydroxide (83.77)>methanol (83.39)>ammonium sulphate (81.73)>PEI+glutaraldehyde (81.41)>heated (78.85)>hydrogen sulphide (73.52)>hydrogen sulfide (73.35)>formaldehyde (71.51)>glutaraldehyde (70.38)>Ca-alginate (70.32)>*Moringa Olifera* seed powder (68.66)>Triton X-100 (68.55)>acetone (67.18)>HCl (64.88)>HNO₃ (63.74)>EDTA (59.45)>Na-alginate (58.98)>CO₂ (58.14)>CaCl₂ (56.12)>aluminum hydroxide (55.96)>boiled (54.24)>PEI (48.47)>κ-carrageen (48.04)>ethanol (44.18)>potash alum (42.07)>native (41.19)>benzene (39.97). On the other hand, the following order for Co(II) was observed: heated (95.55)>H₂SO₄>ethanol (79.58)>*Moringa Olifera* seed powder (79.57)>Triton X-100 (78.88)>aluminum hydroxide (77.9)>PEI+glutaraldehyde (74.65)>hydrogen sulfide (72.75)>boiled (72.45)>HNO₃ (71.73)>PEI (69.28)>sodium hydroxide (68.6)>Ca-alginate (67.2)>CO (64.45)>glutaraldehyde (63.83)>formaldehyde (62.5)>acetone (62.23)>EDTA (62.1)>benzene (59.7)>methanol (59.35)>κ-carrageen (57.08)>HCl (56.95)>potash alum (55.1)>native (52.64)>ammonium sulfate (44.08)>CaCl₂ (41.95)>Na-alginate (49.93).

Pretreatments showed a significant effect on the uptake capacity of CWB. Physical and chemical pretreatments either increased or decreased the uptake capacity of CWB. Physical modification of biomass removes mineral matter from biomass and introduces more sorption sites on the biomass surface. Heating of biomass results in decomposition of organic matter, while boiling removes mineral matter by dissolution, resulting in the introduction of more sorption sites on biomass surface, so biosorption capacity increases [21,22].

Fig. 11 illustrates that pretreatment with all of the reagents except benzene enhanced the sorption of Pb(II) ions. Whereas, on the other hand, sorption of Co(II) decreased when treated with (NH₄)₂SO₄, CaCl₂ and Na-alginate. Therefore, it can be stated that the mechanism of biosorption varies from metal to metal. For Pb(II) and Co(II), acidic pretreatments increased the sorption capacity of the CWB. In our previous study, we observed that physical and chemical treatments of CWB resulted in an enhanced biosorption capacity for Cr(III) and Cr(VI) [11]. Specific pretreatment shows a specific be-

havior that may be due to the specific interaction between the biomass and the chemical used for that treatment [23]. Acids can enhance uptake capacity of biomass by increasing the surface area and porosity of original sample [24,25].

Alkali pretreatment can increase the biosorption of heavy metals because it may destroy autolytic enzymes causing putrefaction of biomass, remove lipids and proteins that mask binding sites and could release certain biopolymers from the cell wall that have a high affinity towards heavy metal ions [26]. Removal of surface impurities, rupture of cell membrane and exposure of available binding sites for metal biosorption after pretreatment might be responsible for the increase in metal biosorption. The residual alkalinity in biomass may also result in increased hydrolysis of certain metals and thereby enhance overall metal removal by the treated biomass [27]. The pretreatment may release polymers such as polysaccharides that have a high affinity towards certain metal ions [28,29]. Therefore, if Pb(II) is to be removed from aqueous solution, the use of pretreatment with alkaline chemicals may improve removal efficiency. After basic pretreatment, adsorption capacity of CWB was increased for Pb(II) and Co(II).

The alginates exhibited the best overall ability to remove heavy metals. Change in the cell wall composition of CWB caused by these chemicals may attribute the difference in results after alginic pretreatment. Calcium stabilizes the biomass by binding alginate and converting it to the gel state, so the sorption capacity was increased. Calcium alginate is a polymer, and the carboxyl group of this alginate has been identified as the main binding site [30]. By comparison, pretreatment using Na-alginate caused a decrease in the Co(II) sorption capacity, which may be explained as after treatment Na⁺ may remain on the cell wall of biomass and may be in competition with the Co(II) on the binding sites of the cell wall. The obtained results are in the accordance with the results reported earlier [31,32].

As mentioned earlier, the difference in results after a specific chemical pretreatment might be due to the change in the nature of cell wall composition of biomass. Triton X-100 removes oily phase from the cell surface, hence sorption capacity was increased. Triton X-100 is a non-ionic surfactant. Surfactants are the substances with lyophilic and lyophobic groups capable of adsorbing at interfaces.

The adsorption of heavy metals onto the biomass from solutions can be enhanced in the presence of surfactants due to reduced surface tension and increased wetting power [17]. Similarly, an increase in the sorption capacity of CWB was observed after pretreatment. Pretreatment with benzene resulted in decrease in the sorption capacity of CWB for Pb(II), whereas for Co(II), sorption capacity was increased after pretreatment except ammonium sulphate, CaCl₂ and Na-alginate.

Sorption capacity of CWB increased after being treated with EDTA. EDTA, is a strong complexing agent and could assist in the elution of metal ion sequestration already present on CWB [33].

9. FTIR, EDX and SEM Studies

The FTIR technique is an important tool to identify some characteristic functional groups that are involved in the adsorption of metal ions. The spectrum of CWB in the range of 400–4,000 cm⁻¹ was obtained to determine main functional groups present on biomass cell surface. The strong asymmetrical stretching band at 3,506.59 cm⁻¹ indicated the presence of -OH and -NH groups. The broad, intense -OH stretching absorption from 3,300 to 2,500 cm⁻¹ suggested the presence of -COOH group on cell surface of CWB. Fig. 12 represents the EDX-spectrum of virgin CWB. The spectrum shows that CWB contains only carbon (45.99%) oxygen (52.64%) and potassium (1.37%) by weight. No other element or silica (SiO₂) contents was indicated. Fig. 13 shows the SEM photograph graph of CWB, which reflects that the biomass consists of heterogeneous

fibers of various lengths which were considered helpful for mass transfer of metal ions to biosorbent.

CONCLUSIONS

It was concluded that *Citrus reticulata* waste biomass has an affinity to Pb(II) and Co(II) from the aqueous solutions. Co(II) biosorption capacity of CWB strongly depends on temperature. A maximum adsorption capacity of 86.30 mg/g was obtained at 60 °C. The Freundlich adsorption model adequately described the biosorption of both metals by CWB. The overall biosorption process was best described by pseudo-second order kinetics for both metals. Maximum biosorption capacity of 83.77 and 95.55 mg/g was observed for Pb(II) and Co(II) with sodium hydroxide treated and simply heated biomass respectively.

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REFERENCES

1. D. Sud, G. Mahajan and M. P. Kaur, *Bioresour. Technol.*, **99**, 6017 (2008).
2. B. Volesky, *Biosorption of heavy metals*, CRC Press Inc., Boca Raton, FL (1990).
3. E. V. Soares, A. P. R. S. Duarte, R. A. Boaventura and H. M. V. M. Soares, *Appl. Microbiol. Biotechnol.*, **58**, 836 (2002).
4. T.-Y. Kim, S.-K. Park, S.-Y. Chu, H.-B. Kim, Y. Kang, S.-D. Kim and S.-J. Kim, *Korean J. Chem. Eng.*, **22**, 91 (2005).
5. J. W. Patterson, *Industrial wastewater treatment technology*, 2nd ed., Butterworth Publisher, Stoneham, MA (1985).
6. L. Zhang, L. Zhao, Y. Yu and C. Chen, *Water Res.*, **32**, 1437 (1998).
7. B. Volesky and Z. R. Holan, *Prog.*, **11**, 235 (1995).
8. S. S. Ahluwalia and D. Goyal, *Eng. Life Sci.*, **5**, 158 (2005).
9. E. Harry, *Trends Biotechnol.*, **17**, 462 (1999).
10. A. Hashem, E. S. Abdul-Halim, K. F. Al-Tahlawy and A. Hebeish, *Adsorp. Sci. Technol.*, **23**, 367 (2005).
11. A. Zubair, H. N. Bhatti, M. A. Hanif and F. Shafqat, *Water Air Soil Pollut.*, **191**, 305 (2008).
12. M. A. Javed, H. N. Bhatti, M. A. Hanif and R. Nadeem, *Sep. Sci. Technol.*, **42**, 3641 (2007).
13. Q. Yu and P. Kaewsarn, *Korean J. Chem. Eng.*, **16**, 753 (1999).
14. S. Gupta, D. Kumar and J. P. Gaur, *Chem. Eng. J.*, DOI: 10.1016/j.cej.2008.08.019 (2008).
15. V. K. Gupta and A. Rastogi, *Colloid Surf. B.*, **64**, 170 (2008).
16. M. A. Hanif, R. Nadeem, H. N. Bhatti, N. R. Ahmad and T. M. Ansari, *J. Hazard. Mater.*, **139**, 345 (2007).
17. H. N. Bhatti, B. Mumtaz, M. A. Hanif and R. Nadeem, *Process Biochem.*, **42**, 547 (2007).
18. A. R. Iftikhar, H. N. Bhatti, M. A. Hanif and R. Nadeem, *J. Hazard. Mater.*, **161**, 941 (2009).
19. G. Bayramoğlu, S. Bektas and M. Y. Arica, *J. Hazard. Mater.*, **101**, 285 (2003).

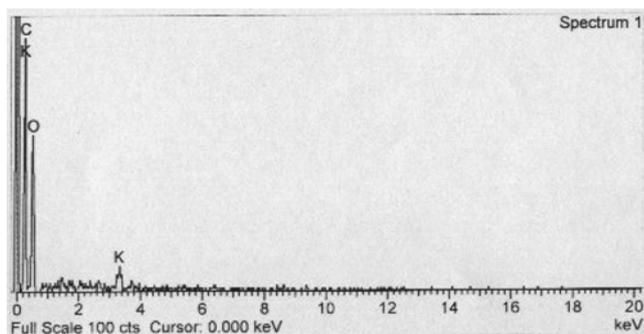


Fig. 12. EDX of *Citrus reticulata* waste biomass.

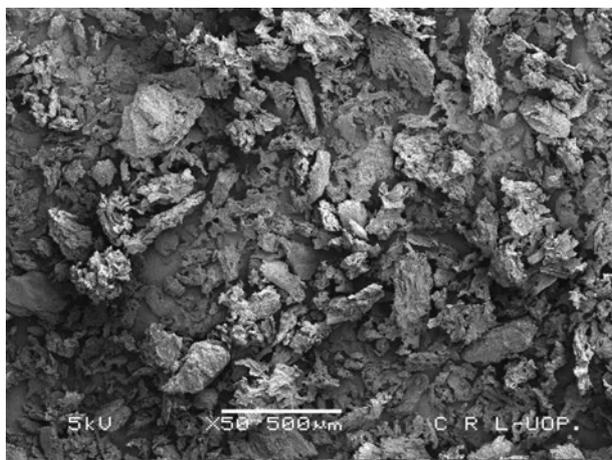


Fig. 13. Scanning electron micrograph of *Citrus reticulata* waste biomass.

20. I. Tüzün, G. Bayramoğlu, E. Yalçın, G. Başaran, G. Celik and M. Y. Arica, *J. Environ. Manage.*, **77**, 85 (2005).
21. R. Nadeem, M. A. Hanif, F. Shaheen, S. Perveen, M. N. Zafar and T. Iqbal, *J. Hazard. Mater.*, **150**, 335 (2008).
22. F. Shafiqat, H. N. Bhatti, M. A. Hanif and A. Zubair, *J. Chil. Chem. Soc.*, **54**, 1565 (2008).
23. H. N. Bhatti, R. Khalid and M. A. Hanif, *Chem. Eng. J.*, DOI: 10.1016/j.cej.2008.09.028 (2008).
24. A. Kapoor and T. Viraraghavan, *Bioresour. Technol.*, **63**, 109 (1998).
25. A. Moreno-Castilla, F. Carrasco-Marin, F. J. Maldonado-Hodar and J. Rivera-Utrilla, *Carbon*, **36**, 145 (1997).
26. G. Yan and T. Viraraghavan, *Water SA*, **26**, 119 (2000).
27. C. L. Brierley, *Geomicrobiol. J.*, **8**, 1 (1990).
28. M. W. Mittelman and G. G. Geesey, *Appl. Environ. Microbiol.*, **49**, 846 (1985).
29. M. Loaec, R. Olier and J. Guezennec, *Water Res.*, **31**, 1171 (1997).
30. E. Fourest and B. Volesky, *Environ. Sci. Technol.*, **30**, 277 (1996).
31. F. Veglio, A. Esposito and A. P. Reverberi, *Process Biochem.*, **38**, 953 (2003).
32. M. Tsui, K. C. Cheung, N. F. Y. Tam and M. H. Wong, *Bioresour. Technol.*, **97**, 2321 (2006).
33. A. Pal, S. Ghosh and A. K. Paul, *Bioresour. Technol.*, **97**, 1253 (2006).