



Dynamic biosorption of Zn(II) and Cu(II) using pretreated *Rosa gruss an teplitz* (red rose) distillation sludge

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ARTICLE INFO

Article history:

Received 10 June 2008

Received in revised form

17 September 2008

Accepted 18 September 2008

Keywords:

Red rose

Distillation sludge

Cu(II)

Zn(II)

Isotherms

Pretreatments

Biosorption

ABSTRACT

This work presented conditions for Cu(II) and Zn(II) removal using native, physically and chemically pretreated *Rosa gruss an teplitz* (red rose) distillation sludge. Cu(II) and Zn(II) sorption was found dependent on solution pH, biosorbent dose, biosorbent particle size, shaking speed, temperature, initial concentration of metal ions being sorbed and contact time. Physical and chemical pretreatments of biomass resulted in an increase or decrease in metals uptake capacity. The effect of different pretreatments is discussed in detail. Highest Cu(II) and Zn(II) biosorption capacities were observed for PEI + glutaraldehyde (68.64 mg/g) and NaOH (43.4 mg/g) pretreated biomass, respectively. The native biomass showed Cu(II) biosorption capacity that was adequately described by Freundlich isotherm, whereas Zn(II) biosorption phenomenon was described by Langmuir isotherm. The suitability of a pseudo-first-order chemical reaction for sorption of Cu(II) and Zn(II) ions onto this biomass was apparent as this kinetic model described adequately the largest part of the process.

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1. Introduction

The majority of toxic metal pollutants are waste products of industrial and metallurgical processes. Their concentrations have to be reduced to meet ever increasing legislative standards. According to the World Health Organization (WHO), Zn(II) and Cu(II) are among the metals of most immediate concern. The effluents from metal finishing processes may contain up to 10 mg/L heavy metals [1]. Cu(II) is mainly employed in electric goods industry and brass production. High levels of Cu can cause toxic effects like all other heavy metals although Cu(II) is an essential trace element. Cu(II) may be found as a contaminant in food, especially shellfish, liver, mushrooms, nuts and chocolate. The presence of Cu(II) ions cause serious toxicological concerns, it is usually known to deposit in brain, skins, liver, pancreas and myocardium. According to Environmental Protection Agency (EPA) the concentration of Cu(II) in drinking water should not exceed 1.3 mg/L [2]. Zn(II) may be found in wastewater discharges from acid mine drainage (AMD), galvanizing plants, as a leachate from galvanized structures, natural ores, and from municipal wastewater treatment plant discharges [1]. According to WHO more than 3 mg/L of zinc in drinking water

is unacceptable. Too much intake of Zn(II) can lead to respiratory incapacitation, as indicated by increased respiratory activity such as breathing rate, volume and frequency of ventilation, coughing, decrease in oxygen uptake efficiency [3].

Conventional methods for removing metals from aqueous solutions include chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, membrane technologies and evaporation recovery. These processes may be ineffective or extremely expensive especially when metals in solution are in the range of 1–100 mg/L [4]. Another major disadvantage with conventional treatment technologies is the production of toxic chemical sludge and its disposal/treatment becomes a costly affair and is not eco-friendly. Therefore, removal of toxic heavy metals to an environmentally safe level in a cost effective and environment friendly manner, assumes great importance [5]. Biosorption refers to different modes of non-active metal uptake by biomass, where metal sequestration by cells can take place through biosorption, ion exchange, coordination, complexation, etc. [6]. The major advantages of biosorption over conventional treatment methods include, low cost; high efficiency; minimization of chemical or biological sludge; no additional nutrient requirement; possibility of regeneration of biosorbent and metal recovery [7]. Both living and dead biomasses exhibit biosorption capacity. In addition, living cells are subject to the toxic effects of heavy metals reaching a certain level, resulting in cell death. To

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overcome the disadvantages, non-viable or dead biomass is preferred in removal of metal ions [8]. Modification of a biomass using physical pretreatment methods such as heating, autoclaving, freezing, drying, boiling and chemical pretreatments such as using acids, alkalis and organic chemicals showed enhancement or reduction in metal biosorption [9].

Rosa gruss an teplitz (red rose) belongs to the class Bourbon. It is among one of the most extensively used flowers for steam distillation process. Biomass left after the extraction of rose oil and rose water using steam distillation is a waste material. In continuation of our work on removal of heavy metals from aqueous solutions (1, 7, 8, and 9), the present study is aimed at selection of a low cost biosorbents which can adsorb Zn(II) and Cu(II) from synthetic solutions. Detailed batch studies with the selected biosorbent, red rose distillation sludge, has been carried out in the present investigation. The effect of different experimental parameters such as pH, biosorbent dose, biosorbent size, initial metal concentration, shaking speed and contact time and various pretreatments on Zn(II) and Cu(II) uptake capacity of *R. gruss an teplitz* waste biomass was also investigated.

2. Materials and methods

2.1. Preparation of biosorbent

R. gruss an teplitz (red rose) biomass (distillation sludge) was obtained from Rose Laboratory, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan, and was extensively washed with deionized distilled water (DDW) to remove particulate material from their surface, and sun dried. Dried biomass was ground using food processor (Moulinex, France) and sieved (0.250–1.00 mm) by passing through sieving machine (Octagen Siever OCT-Digital 4527-01). Biosorbent was physically modified using heating (5 g of biosorbent was heated in oven at 60 °C for 30 min) and boiling (5 g of biosorbent/100 mL of H₂O, boiled for 10 min). For chemical pretreatments, 5 g of biomass was soaked in 100 mL of 0.1N HCl, 0.1N HNO₃, 0.1N H₂SO₄, 0.1N NaOH, 0.1N Al(OH)₃, sodium alginate (50 mL), PEI + glutaraldehyde (50 mL each), 1% *Moringa olifera*, 2% calcium alginate (50 mL), 1% glutaraldehyde (50 mL), 1% potash alum, 0.1N EDTA, 0.1N ethanol, 0.1N benzene, 0.1N methanol, 0.1N formaldehyde, 1% PEI (50 mL), acetone, 1% κ-carragenin, 1% Triton X-100 for 2 h. CO₂ and H₂S gas was passed through 5 g of biomass soaked in 100 mL of DDW at the rate of 10 mL/min for 10 min. All pretreated samples were allowed to stand for an hour at 30 °C. Then they were extensively washed with distilled water (DDW) and filtered thoroughly. Finally the biomass was oven dried at 30 °C for 48 h and ground with mortar and pestle and kept in airtight jars. The biomass was digested by wet digestion method (HNO₃ + H₂O₂) to determine its mineral composition.

2.2. Reagents

All the reagents used in this study were of analytical grade, including ZnSO₄·7H₂O and CuSO₄·5H₂O which were purchased from Fluka chemicals except *M. olifera* seed powder which was purchased from Super Market, Faisalabad, Pakistan.

2.3. Zn(II) and Cu(II) solutions

Stock Zn(II) solution (1000 mg/L) was prepared by dissolving 4.41 g of ZnSO₄·7H₂O and stock Cu(II) solution (1000 mg/L) was prepared by dissolving 3.95 g of CuSO₄·5H₂O in 1000 mL of DDW. The pH of stock solution of ZnSO₄·7H₂O was 3.71 and that of

CuSO₄·5H₂O was 3.20. The solutions of different concentrations were prepared by adequate dilutions of stock solution with DDW.

2.4. Batch biosorption studies

All glassware used for experimental purpose was washed with 60% (v/v) nitric acid and subsequently rinsed with DDW to remove any possible interference by other metals. Batch tests were carried out in 250 mL shake flasks to check the influence of starting metal concentration (25–800 mg/L), biomass concentrations (0.05, 0.1, 0.2, 0.3 and 0.4 g per 100 mL), biosorbent particle size (0.250, 0.310, 0.500, 0.710 and 1.00 mm), contact time (15, 30, 60, 120, 240, 480, 720, and 1440 min), temperature (30, 40, 50, 60 and 70 °C), shaking speed (0, 50, 100, and 150 rpm) in order to check the possible maximum removal of metal ions. Control assay was accompanied with each experiment. At the end of each experiment, flasks were removed from the shaker and solutions were separated from the biomass by filtration through filter paper (Whatman no. 40, ashless).

For adjusting pH of the medium 0.1N solutions of NaOH and HCl were used.

2.5. Determination of Zn(II) and Cu(II) contents in solutions

Perkin-Elmer AAnalyst 300 Atomic Absorption Spectrometer equipped with an air-acetylene burner and controlled by Intel personal computer was used to determine concentrations of Cu(II) and Zn(II) in aqueous solutions before and after sorption equilibrium established. The analytical wavelengths used for Zn(II) and Cu(II) were: 213.9 and 327.4 nm, respectively.

2.6. Metal uptake and % sorption

Cu(II) and Zn(II) uptake was calculated by simple concentration difference method. Uptake of Cu(II) and Zn(II) was calculated from the mass balance equation [1]:

$$q_e = \frac{V(C_i - C_e)}{m}$$

where V is the volume of the solution (L), C_i is the initial concentration (mg/L), C_e is the final concentration in solution (mg/L) and m is mass of the sorbent (g).

% sorption is given as

$$\% \text{ sorption} = \frac{C_i - C_e}{C_i} \times 100$$

2.7. Statistical analysis

Mean and standard deviation values were calculated from triplicate sets of experiments. All statistical analysis was performed using Microsoft Excel 2007, Version office Xp.

3. Results and discussion

3.1. Effect of pH

Experiments concerning the effect of pH on sorption were carried out within pH range that was not influenced by metal precipitation (as metal hydroxide). The suitable pH ranges for two metal ions were slightly different, i.e. experiments for Cu(II) sorption were performed at the pH range of 1–5 and for Zn(II) at pH of 1–6. The biosorption of Cu(II) and Zn(II) on the *R. gruss an teplitz* (red rose) distillation sludge biomass was observed to be the function of

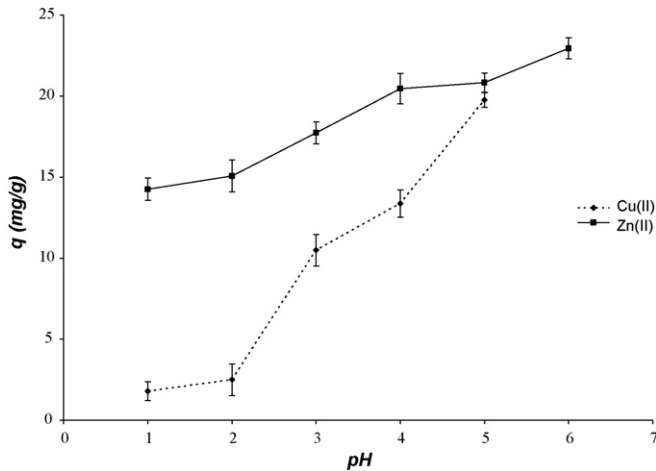


Fig. 1. Effect of pH on uptake of Cu(II) and Zn(II) by *Rosa gruss an teplitz* distillation sludge. Experimental conditions: biosorbent dosage = 1 g/L, biosorbent particle size = 0.250 mm, initial metal conc. = 100 mg/L, contact time = 24 h, shaking speed = 120 rpm, temperature = 30 °C.

solution pH (Fig. 1). The pH is an important parameter in biosorption from aqueous solution because it influences equilibrium by affecting the speciation of the metal ion(s), solubility of the metal ion(s), concentration of counter ions on functional groups of the biomass and degree of ionization of the adsorbate during process [10,11]. In both instances, removal efficiency increased steadily with pH. The most adequate sorption pH was 5 for Cu(II) and 6 for Zn(II). The increase in biosorption with increase in pH can be explained by the fact that at low pH, the biosorbent surface became more positively charged thus reducing attraction between the biomass and metal ions. These bonded active sites thereafter became saturated and was inaccessible to other cations [12]. Moreover, as the pH is increased, the ligands (carboxyl, sulfhydryl, phosphate, etc. groups) would be exposed, increasing the negative charge density on the biomass surface, resulting in greater attraction between metallic ions and ligands [12].

3.2. Effect of biosorbent dosage

For effective metal sorption, biosorbent dose is a significant factor to be considered. It determines the sorbent–sorbate equilibrium of the system [7]. It also determines the number of binding sites available for biosorption [1]. The results demonstrated that the biomass concentration strongly affected the amount of metal removed from aqueous solutions. Maximum biosorption was achieved with a biomass concentration of 0.05 g/L for Cu(II) and 0.1 g/L for Zn(II) (Fig. 2). Moreover, as the biomass concentration rose, maximum biosorption capacity dropped, indicating poorer biomass utilization (lower efficiency). Results could be explained as a consequence of a partial aggregation, which occurred at high biomass concentration giving rise a decrease of active sites. Similar results have been reported by Pal et al. [13] and Bhatti et al. [1].

3.3. Effect of biosorbent size

The effect of altering the sorbents particle size on q_e (mg/g) illustrated that the ground biomass more rapidly up took each metal ion, and equilibrium was reached faster than those achieved with the larger particles of biomass (Fig. 3). This was because particles with smaller particle size allowed a faster contact between the metal ion and the binding sites [12]. The increase in the total surface area provided more sorption sites for metal ions [8].

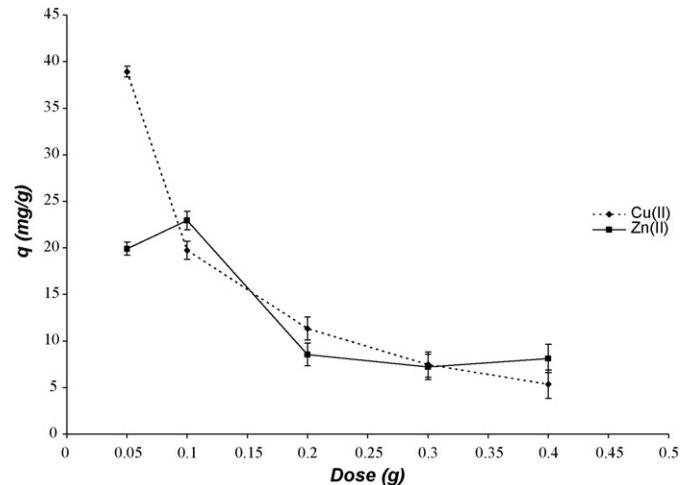


Fig. 2. Effect of biosorbent dosage on uptake of Cu(II) and Zn(II) by *R. gruss an teplitz* distillation sludge. Experimental conditions: pH 5 for Cu(II) and 6 for Zn(II), biosorbent particle size = 0.250 mm, initial metal conc. = 100 mg/L, contact time = 24 h, shaking speed = 120 rpm, temperature = 30 °C.

3.4. Effect of shaking speed

The uptake of Cu(II) and Zn(II) ions by *R. gruss an teplitz* (red rose) distillation sludge was evaluated varying the agitation rate from 0 rpm (without agitation) to 150 rpm, aiming at determining the optimal shaking rate (Fig. 4). The optimal shaking rate for Zn(II) was 100 rpm while Cu(II) showed maximum biosorption without any shaking. Atomic radius of Cu(II) and Zn(II) is 1.27 and 1.33 Å, respectively. Smaller the cation, greater is its radius in aqueous medium. Cu(II) and Zn(II) sorption data revealed that waste rose biomass sorption capacity increased with decrease in hydrated radius of metals ion in aqueous solutions. This sorption characteristic represented that surface saturation was dependent on the hydrated radius of Cu(II) and Zn(II) cations, at lower hydrated radius of metal ion sorption sites took up the available metal more quickly however, at higher aqueous atomic radius, metal ions need to diffuse to the biomass surface by intraparticle diffusion and greatly hydrolyzed ions will diffusion at a slower rate [14]. That is the reason that Cu(II) was more adsorbed without shaking. It is evident from Fig. 4 that

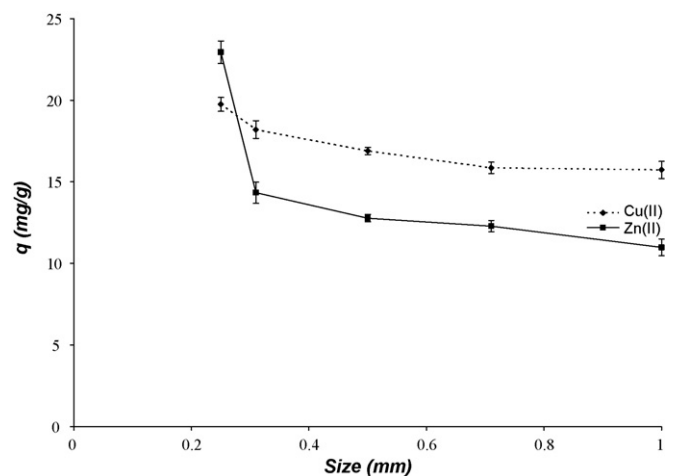


Fig. 3. Effect of biosorbent size on uptake of Cu(II) and Zn(II) by *R. gruss an teplitz* distillation sludge. Experimental conditions: pH 5 for Cu(II) and 6 for Zn(II), biosorbent dosage = 1 g/L, initial metal conc. = 100 mg/L, contact time = 24 h, shaking speed = 120 rpm, temperature = 30 °C.

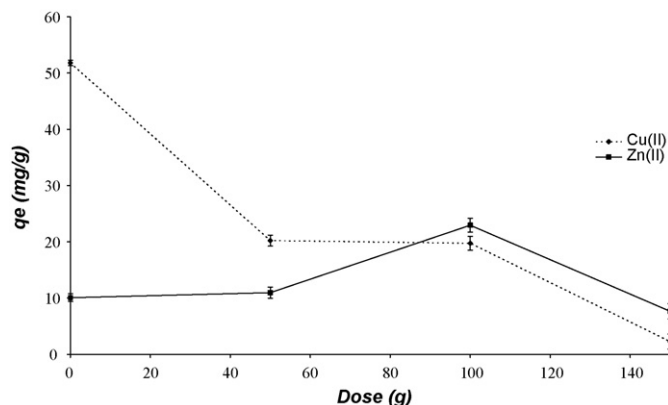


Fig. 4. Effect of shaking speed on uptake of Cu(II) and Zn(II) by *R. gross an teplitz* distillation sludge. Experimental conditions: pH 5 for Cu(II) and 6 for Zn(II), biosorbent dosage = 1 g/L, biosorbent particle size = 0.250 mm, initial metal conc. = 100 mg/L, contact time = 24 h, temperature = 30 °C.

the removal efficiency of Zn(II) increased significantly as agitation speed increased from 0 to 100 rpm. Use of agitation speeds in excess of 100 rpm resulted in decrease in removal efficiency. It is obvious that at lower agitation speed there was insufficient energy for Zn(II) to permeate the intra particular surface for its biosorption by rose waste biomass. Too much increase in agitation speed also regenerated the adsorbed ions from rose waste biomass surface. Hence, it can be postulated that effective agitation of rose waste biomass maintained at a specific mass transfer in the batch reactor was one of the key conditions for improving Zn(II) removal.

3.5. Effect of initial metal concentrations

The rate of biosorption is a function of initial concentration of metal ions, which makes it an important factor to be considered for effective biosorption [1,7]. The apparent capacity of *R. gross an*

teplitz for Zn(II) and Cu(II) metals was determined at the different concentrations. Fig. 5 indicates the relation between capacities and metal ion concentrations, which shows that as metal ion concentration increased the sorption capacity also increased. Maximum removal of Cu(II) and Zn(II) occurred at 800 mg/L. In general, data indicated that sorption capacity increased with increase in initial metal ion concentration for metal on the biomass. This sorption characteristic indicated that surface saturation was dependent on the initial metal ion concentrations.

3.6. Equilibrium modelling

To describe the distribution of the solute in solid phase and liquid phase at equilibrium conditions, it is necessary to express the amount of solute adsorbed per unit weight of sorbent, q , as a function of the residual equilibrium concentration, C_e , of solute remaining in solution. The expression of this relationship is termed as biosorption isotherm, of which the Langmuir and Freundlich equations are most widely used [9]. To get equilibrium data, initial Cu(II) and Zn(II) concentrations were varied while the biomass weight in each sample was kept constant. The Langmuir parameters (Fig. 6a) can be determined from a linearized form of equation [15]:

$$\frac{C_e}{q_e} = \frac{1}{X_m K_L} + \frac{C_e}{X_m} \quad (1)$$

where q_e is the metal ion sorbed (mg/g), C_e the equilibrium concentration of metal ion solution, and X_m and K_L are Langmuir constants.

Freundlich isotherm (Fig. 6b) is represented in the following equation [16]:

$$\log q_e = \left(\frac{1}{n}\right) \log C_e + \log K \quad (2)$$

where q_e is the metal ion sorbed (mg/g), C_e the equilibrium concentration of metal ion solution (mg/L), and K and n are Freundlich constants. The constants K and $1/n$ were determined by linear

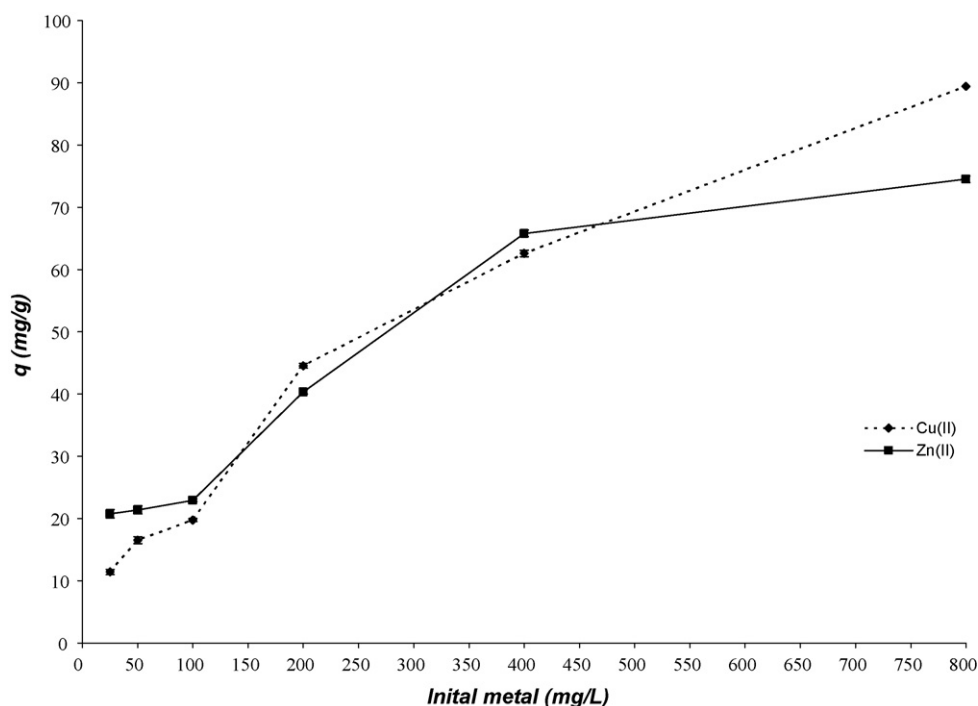


Fig. 5. Effect of initial metal concentration on uptake of Cu(II) and Zn(II) by *R. gross an teplitz* distillation sludge. Experimental conditions: pH 5 for Cu(II) and 6 for Zn(II), biosorbent dosage = 1 g/L, biosorbent particle size = 0.250 mm, contact time = 24 h, shaking speed = 120 rpm, temperature = 30 °C.

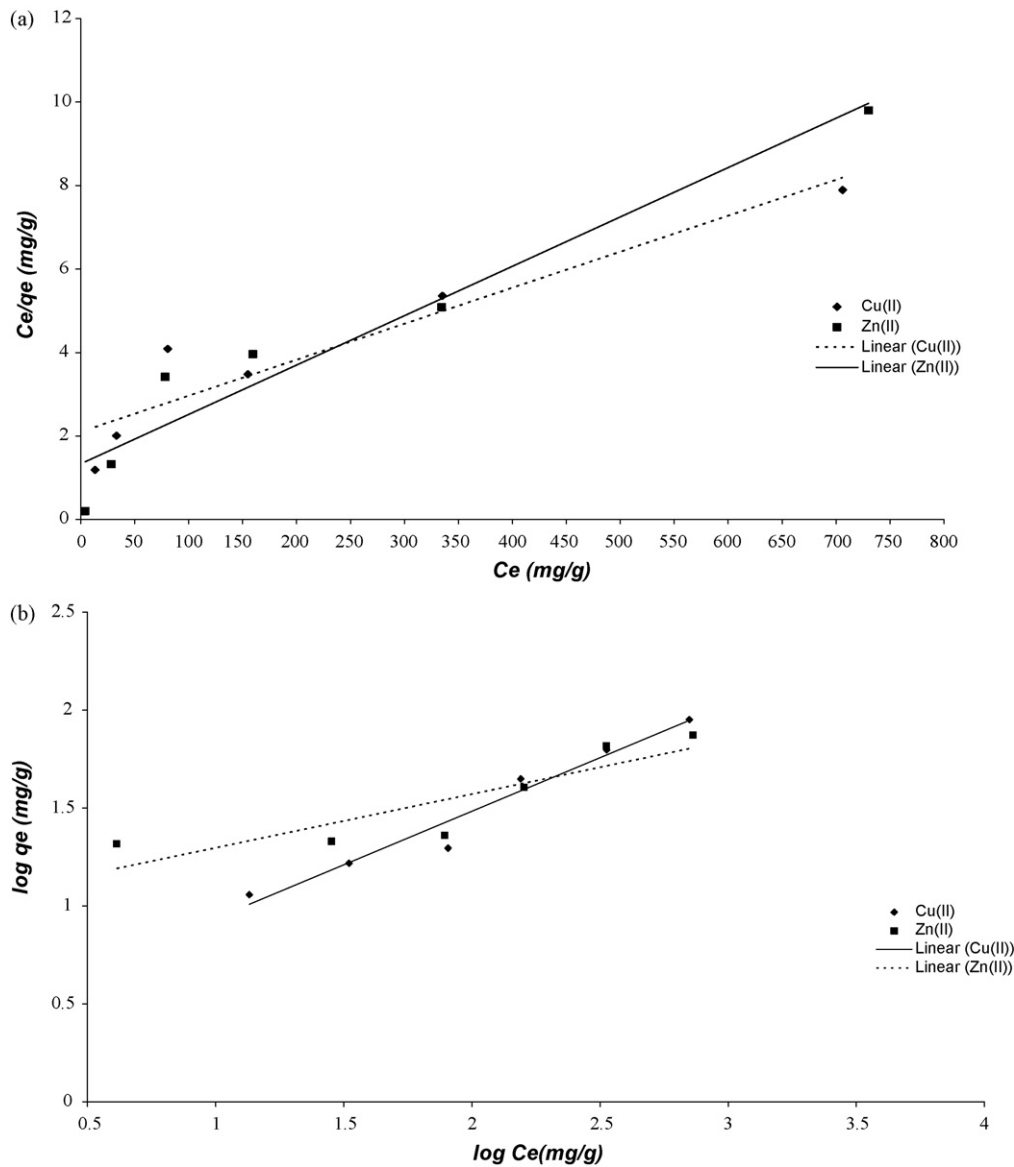


Fig. 6. (a) Langmuir model for uptake of Cu(II) and Zn(II) *R. gruss an teplitz* distillation sludge. (b) Freundlich model for uptake of Cu(II) and Zn(II) *R. gruss an teplitz* distillation sludge.

regression from the plot of $\log q_e$ against $\log C_e$. K is a measure of the degree or strength of biosorption, while $1/n$ is used as an indication of whether biosorption remains constant or decreases with increasing adsorbate concentrations. The q_{\max} value is the maximum value of q_e , which is important to identify which biosorbent has the highest metal uptake capacity and as such useful in scale-up considerations. The R^2 and q_{\max} values (Table 1) for Cu(II) suggested that the Freundlich isotherm described the sorption well which represents that multilayer of sorbate was formed on biosorbent. While for Zn(II) Langmuir isotherm described the sorption well which indicates that monolayer of sorbate was formed.

3.7. Effect of temperature

The effect of temperature on the metal biosorption experiments was investigated at five different temperatures. As seen from Fig. 7, maximum equilibrium uptakes for Cu(II) and Zn(II) were found to be at 30 °C. At higher temperatures, biosorption capacity decreased. The decrease of equilibrium uptake capacity in the temperature interval of 40–70 °C meant that the biosorption processes of these metal ions by *R. gruss an teplitz* was exothermic. This decrease at higher temperatures may be due to damage of active binding sites in the biomass [5].

Table 1
Langmuir and Freundlich isotherm parameters for Cu(II) and Zn(II) uptake by *Rosa gruss an teplitz* distillation sludge.

Metal	Langmuir isotherm parameters			Experimental value	Freundlich isotherm parameters			
	q_{\max} (mg/g)	K_L (L/mg)	R^2	q_{\max} (mg/g)	q_{\max} (mg/g)	K (mg/g)	$1/n$	R^2
Cu(II)	116.28	4.1×10^{-3}	0.8938	89.43	88.76	2.44	0.54	0.9594
Zn(II)	84.74	8.9×10^{-3}	0.9388	74.52	64.11	10.54	0.27	0.7722

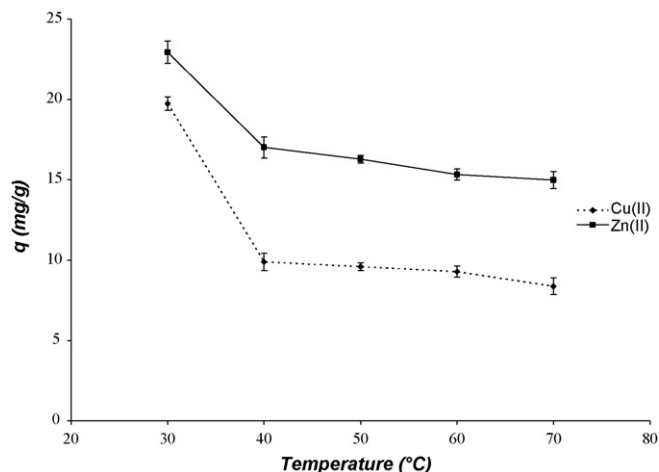


Fig. 7. Effect of temperature on uptake of Cu(II) and Zn(II) by *R. gruss an teplitz* distillation sludge. Experimental conditions: pH 5 for Cu(II) and 6 for Zn(II), biosorbent dosage = 1 g/L, initial metal conc. = 100 mg/L, contact time = 24 h, shaking speed = 120 rpm, temperature = 30 °C.

3.8. Effect of time

It was observed that the percentage uptake of Cu(II) and Zn(II) ions by *R. gruss an teplitz* (red rose) distillation sludge increased with time and at 240 min reached a constant value where no more ions were removed from the solution (Fig. 8). At this point, the amount of ions being adsorbed onto the biosorbent was in a state of dynamic equilibrium with the amount of ions desorbed from the biosorbent. The state of time required to attain this state of equilibrium was termed as the equilibrium time and the amount of ions adsorbed at this equilibrium time reflected the maximum ions biosorption capacity of the biomass under the particular condition.

3.9. Kinetic modelling

A number of models with varying degrees of complexity have been developed to describe the kinetics of metal biosorption in batch systems [17]. According to the kinetic model selection crite-

ria, several reaction-based and diffusion-based models were tested for stimulation of the obtained experimental data. In this study two different kinetic models were used to adjust the experimental data. These kinetic models included pseudo-first-order Lagergren (Fig. 9a) and pseudo-second-order Lagergren (Fig. 9b).

The pseudo-first-order Lagergren model is expressed as in the following equation:

$$\log(q_e - q) = \frac{\log q_e - K_{1,ads}}{2.303} \quad (3)$$

where q_e (mg/g) and q are the amounts of adsorbed metal ions on the biosorbent at the equilibrium and at any time t , respectively, $K_{1,ads}$ (min^{-1}) is the Lagergren rate constant of the first-order biosorption. The pseudo-second-order model is based on the assumption that biosorption follows a second-order mechanism. So, the rate of occupation of biosorption sites is proportional to the square of the number of unoccupied sites in the following equation:

$$\frac{t}{q} = \frac{1}{K_{2,ads}} q_e^2 + \frac{1}{q_e t} \quad (4)$$

where $K_{2,ads}$ is the rate constant of second-order biosorption ($\text{g}/(\text{mg min})$). The coefficient of correlation for first-order kinetic model for both Zn(II) and Cu(II) was nearly equal to 1 and the estimated value of q_e also agreed with the experimental one (Table 2). Both factors suggest that the sorption of Zn(II) and Cu(II) ions followed the pseudo-first-order kinetic model.

3.10. Effect of pretreatment

The results of pretreatment of *R. gruss an teplitz* (red rose) distillation sludge with different reagents are shown in Fig. 10a and b. The q values (mg/g) of non-treated, physically and chemically modified *R. gruss an teplitz* (red rose) distillation sludge were in the following order: PEI + glutaraldehyde (68.64) > formaldehyde (50.64) > NaOH (50.04) > κ -carragenen (49.04) > sodium alginate (44.92) > H_2SO_4 (39.44) > Triton X-100 (32.72) > $\text{Al}(\text{OH})_3$ (27.44) > PEI (27.5) > ethanol (27.2) > ammonium sulphate (25.22) > *M. olifera* seed powder (24.32) > HCl (23.3) > CaCl_2 (22.82) > boiled (22.48) > heated (21.89) > EDTA (21.05) > calcium alginate (20.7) > native (19.75) > CO_2 (18.44) > H_2S (14.12) > benzene

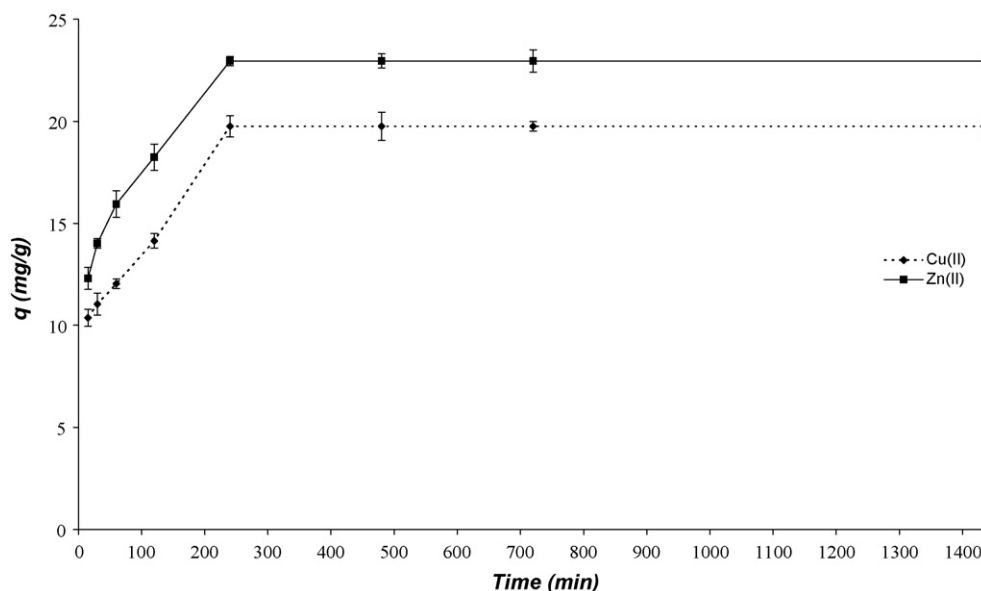


Fig. 8. Effect of time on uptake of Cu(II) and Zn(II) by *R. gruss an teplitz* distillation sludge. Experimental conditions: pH 5 for Cu(II) and 6 for Zn(II), biosorbent dosage = 1 g/L, initial metal conc. = 100 mg/L, contact time = 24 h, shaking speed = 120 rpm, temperature = 30 °C.

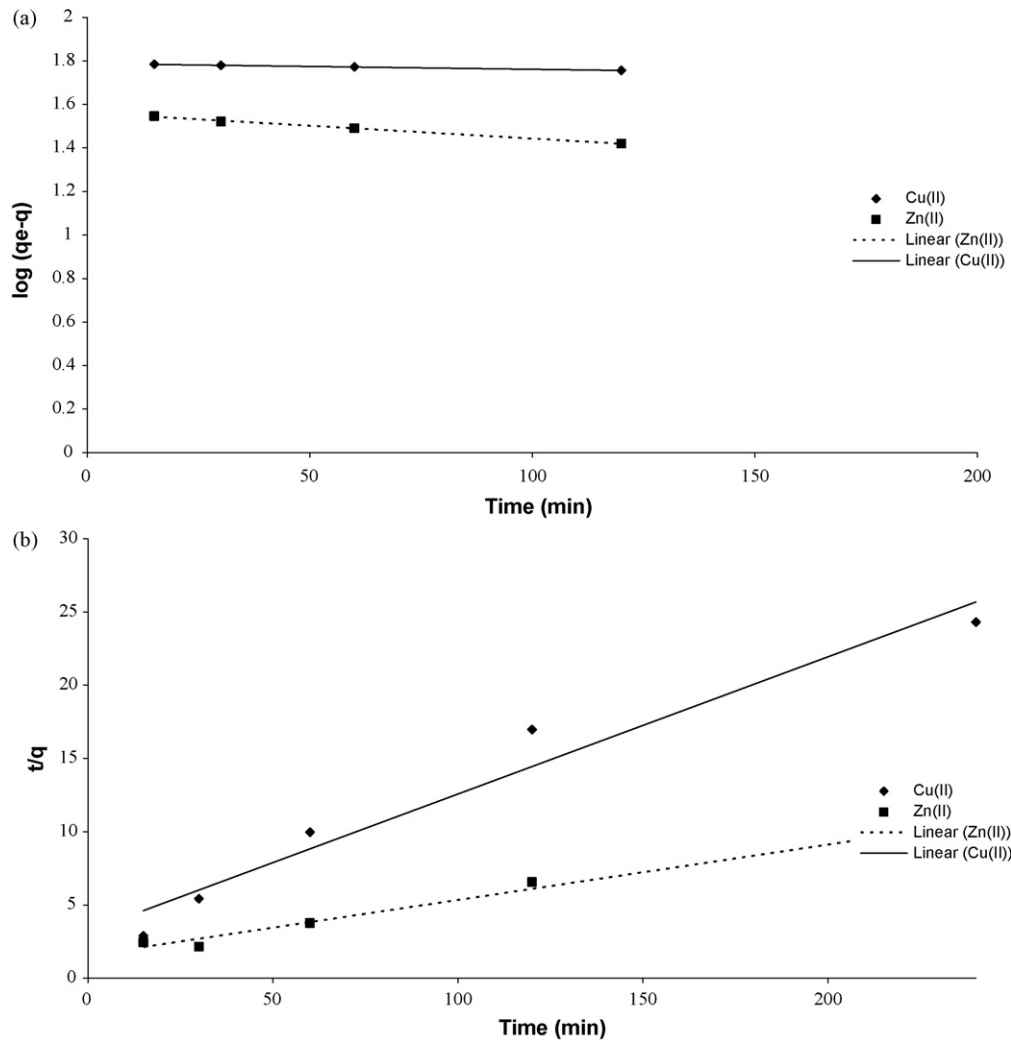


Fig. 9. (a) Pseudo-first-order Lagergren model for uptake of Cu(II) and Zn(II) by *R. gruss an teplitz* distillation sludge. (b) Pseudo-second-order Lagergren model for uptake of Cu(II) and Zn(II) by *R. gruss an teplitz* distillation sludge.

(12.14) > acetone (9.32) > methanol (9.2) > glutaraldehyde (8.9) > HNO₃ (7.76) > potash alum (3.8). Zn(II) sorption capacity (mg/g) of *R. gruss an teplitz* (red rose) distillation sludge pretreated physically and chemically was in the following order: NaOH (43.4) > glutaraldehyde (38.5) > κ -carragen (36.26) > formaldehyde (35.77) > acetone (33.18) > benzene (32.48) > CO₂ (32.06) > Na-alginate (30.46) > PEI + glutaraldehyde (27.9) > Ca-alginate (27.71) > *M. Olifera* (27.56) > PEI (26.26) > CaCl₂ (25.4) > Al(OH)₃ (24.28) > EDTA (24.13) > Native (22.95) > H₂S (22.18) > boiled (21.62) > ammonium sulphate (21.2) > HCl (20.78) > heated (19.66) > H₂SO₄ (17.42) > Triton X-100 (17.42) > potash alum (12.87) > nitric acid (11.26) > methanol (9.51) > ethanol (8.64) as indicated in Fig. 10b.

All physical modifications increased the Cu(II) and decreased the Zn(II) biosorption capacity of the biomass. Physical modifications of biomass removed minerals and organic matter from biomass. Boil-

ing removed the mineral matter by dissolving it whereas heating removed the organic and mineral matter by decomposing it, resulting in introduction of more sorption sites on biomass surface for Cu(II) uptake. The reduction in Zn(II) biosorption capacity of physical pretreated biomass may be attributed to the loss of intracellular uptake [18].

Modification of biomass with acids, apart from removal of the mineral matter also resulted in introduction of oxygen surface complexes that change the surface chemistry by increasing the surface area and porosity of original sample. H₂SO₄ presented more increase in biosorption capacity as compared to HCl and HNO₃. This might be due to solubility of more mineral matter of biomass in H₂SO₄, which introduced more porosity in biomass due to increased cellular mass and resulted in enhancement of Cu(II) uptake capacity of biosorbent [17]. The acidic treatment reduced the Zn(II) biosorption capacity. This was due to the reason that after

Table 2

Lagergren pseudo-first-order and pseudo-second-order kinetic models for uptake of Cu(II) and Zn(II) onto *R. gruss an teplitz* distillation sludge.

Metal	Pseudo-first-order kinetic model			Experimental value q_e (mg/g)	Pseudo-second-order kinetic model		
	q_e (mg/g)	$K_{1,ads}$ (min ⁻¹)	R^2		q_e (mg/g)	$K_{2,ads}$ (g/(mg min))	R^2
Cu(II)	61.36	6.9×10^{-4}	0.9991	71.24	10.68	2.7×10^{-2}	0.9579
Zn(II)	36.42	2.8×10^{-3}	0.9975	49.21	26.45	9.2×10^{-3}	0.9861

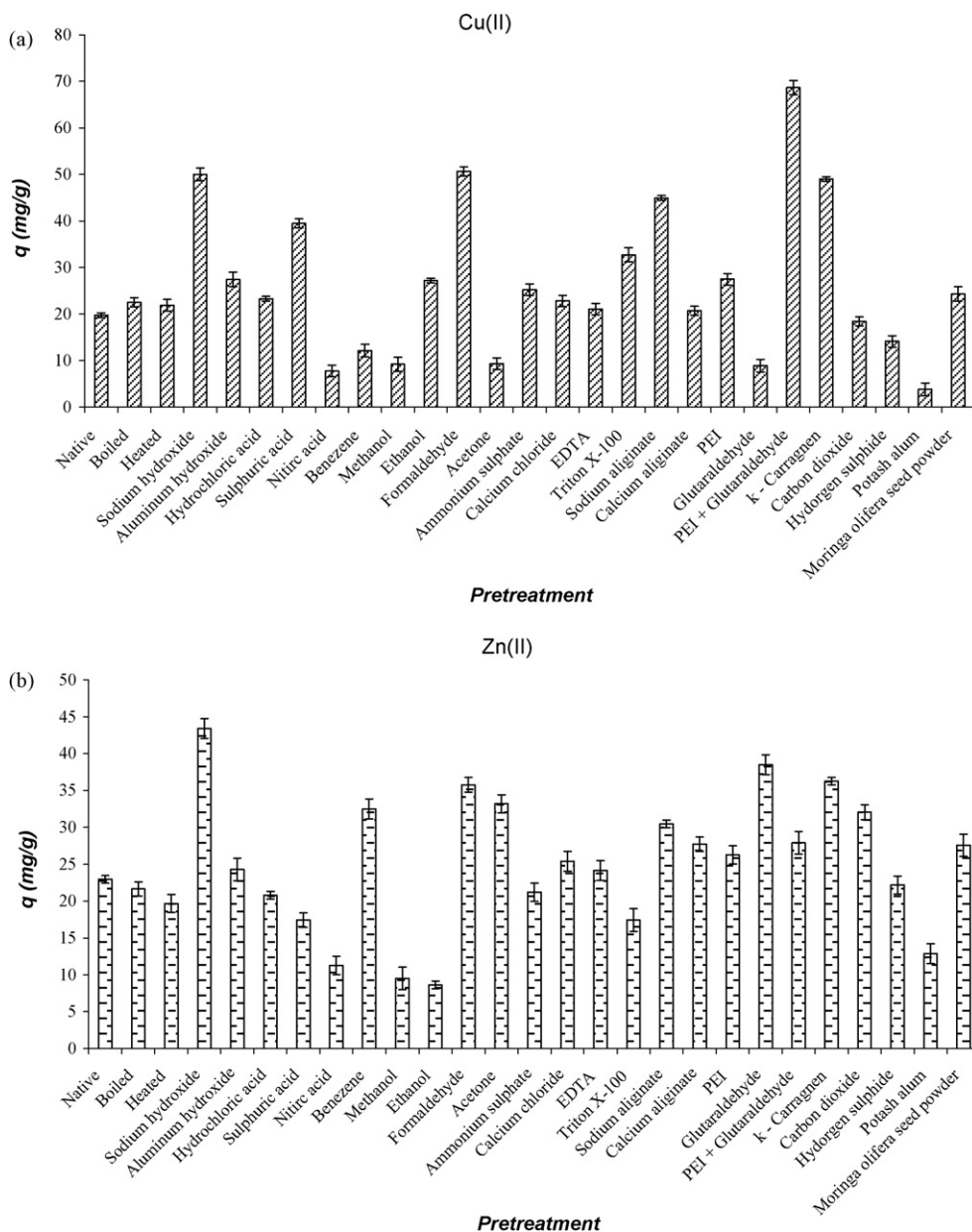


Fig. 10. (a) Effect of pretreatment on uptake of Cu(II) by *R. gruss an teplitz* distillation sludge. Experimental conditions: pH 5 for Cu(II) and 6 for Zn(II), biosorbent dosage = 1 g/L, initial metal conc. = 100 mg/L, contact time = 24 h, shaking speed = 120 rpm, temperature = 30 °C. (b) Effect of pretreatment on uptake of Zn(II) by *R. gruss an teplitz* distillation sludge. Experimental conditions: pH 5 for Cu(II) and 6 for Zn(II), biosorbent dosage = 1 g/L, initial metal conc. = 100 mg/L, contact time = 24 h, shaking speed = 120 rpm, temperature = 30 °C.

a certain concentration of an acid, the electronegativity of biomass decreased due to the remaining H^+ ions on the acidic pretreated biomass. That changed the biomass electronegativity, resulting in a reduction in biosorption capacity [19,20]. The other reason regarding the reduction in Zn(II) biosorption capacity of biomass may be the diminishing of intracellular uptake of Zn(II). The important conclusion which can be withdrawn from the results obtained after physical and acid modifications of biomass is that the most of Cu(II) biosorption occurred on surface of biomass while Zn(II) biosorption can be attributed to intracellular uptake as well as to surface attachment.

Alkali treatment of biomass may destroy autolytic enzymes that cause putrefaction of biomass and remove lipids and proteins that mask reactive sites. Removal of impurities from surface and after rupturing of cell-membrane is the reason for the increase in Cu(II)

and Zn(II) uptake capacity of biomass after basic pretreatment [11]. CO_2 form carbonic acid solution in aqueous phase. Carbonic acid thus formed during pretreatment of biosorbent may effects the metal uptake capacity of biosorbent being treated. The net increase or decrease in sorption capacity of biomass after weak acid pretreatment can be attributed to effect played by polymeric structure of biomass, strength of weak acid and oxidation state of metal. H_2S gas forms sulphonic acid in water. Sulphonic acid is a weak acid. In the present study excess of H_2S gas was passed through the biosorbent soaked in water to form saturated solution of sulphonic acid. Thus, the formed acid reduced the electronegativity of biomass due to production of H^+ ions and resulted in a reduction in biosorption capacity. $(NH_4)_2SO_4$ is a protein salting out agent. It can denature protein and cause their precipitation. $(NH_4)_2SO_4$ precipitation decreased the sorption capacity of *R. gruss an teplitz*. The

decrease in sorption capacity can be attributed to disturbance of $-NH_2$ functional groups, which might be involved in Zn(II) biosorption.

Alginate is a natural anionic polymer [21]. Alginates have been found to change the overall sorption capacity of the biomass [22–24]. Sodium alginate exhibited better overall ability to remove Cu(II) and Zn(II) in comparison to native biomass. This may be due to biosorption of sodium alginate on biomass surface. Sodium alginate has the ability to adsorb metals. Calcium alginate is a polymer having carboxylic groups responsible for binding [22]. Calcium stabilizes the biomass by binding alginates and converting it to the gel state, thus resultantly Cu(II) and Zn(II) biosorption capacity was increased. *M. olifera* is a natural coagulant. It causes the precipitation of inorganic substances present in the biomass. Due to this precipitation vacant sites are created on the biomass surface that resulted in the enhancement of Cu(II) and Zn(II) biosorption capacity. Potash alum is a strong chemical coagulant. It caused the denaturation and precipitation of proteins present in biomass cells. Proteins have $-NH_2$ functional groups which also contribute to metal biosorption in coordination with other functional groups. Thus, denaturation and precipitation of proteins cause reduction in Cu(II) and Zn(II) uptake capacity.

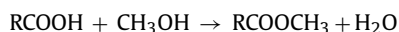
Cu(II) and Zn(II) biosorption capacity (mg/g) of biomass pretreated using PEI and PEI+glutaraldehyde increased. PEI is a well-known chelating agent. This is due to the presence of a large number of amine groups in a molecule. The biomass was modified using PEI to increase the biosorption capacity [25]. To fix the PEI onto the biomass surface glutaraldehyde was used as the cross-linking agent. As a result sorption capacity increased. Glutaraldehyde alone decreased the sorption capacity. The use of glutaraldehyde at a higher concentration resulted in more rigid biomass. The glutaraldehyde cross-linked the functional groups present on the biomass. Cross-linking has been found to decrease the biosorption capacity of biosorbents [26]. Pretreatment of biomass using formaldehyde resulted in increase in Cu(II) and Zn(II) biosorption. Kapoor and Viraraghavan [19] also reported an increase in the biosorption of Cu(II) after formaldehyde pretreatment. Pretreatment using κ -carragenin caused increase in Cu(II) and Zn(II) biosorption capacity of *R. gruss an teplitz*. κ -Carragenin causes the rupturing of the cell wall [27]. It disturbed the functional groups present on surface of biomass. The efficiency of the biomass to remove metal ions is increased when it was pretreated with EDTA. EDTA is a very strong desorbent. When biomass was pretreated with EDTA, almost all metal ions already present in the biomass (Table 3) were removed. Thus increase in the number of vacant sorption sites resulted in increase in Cu(II) and Zn(II) sorption capacity of *R. gruss an teplitz* biomass.

Cu(II) biosorption capacity (mg/g) of biomass pretreated using Triton X-100 increased. Triton X-100 is a surfactant. Surfactants are the substances with lyophilic and lyophobic groups capable of adsorbing at interfaces. The biosorption of Cu(II) on to the biomass

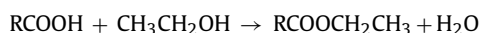
from solutions was enhanced in the presence of Triton X-100 due to reduced surface tension and increased wetting power [1]. Triton X-100 activity may also depend upon the hydrated radius of metal ion. As the hydrated radius of Zn(II) was smaller, Triton X-100 increased its diffusion rate, i.e. attachment as well as detachment increased. As a result it reduced the sorption of Zn(II) ions on biomass.

Treatment of the biosorbent with benzene extracts the lipid fraction of biosorbent [28]. Therefore, the reduction in biosorption efficiency when benzene treated biosorbent was used, revealed that lipids in the cell wall of *R. gruss an teplitz* contributed to Cu(II) biosorption. Parvathi et al. [29] observed similar results while working on the waste beer yeast by product to decontaminate effluent generated from battery manufacturing industry. Pretreatment of biomass with acetone resulted in decrease in Cu(II) biosorption capacity. Due to high polarity of acetone, cell wall structure of biomass became disturbed. It may change nature of functional groups responsible for metal biosorption. Treatment of *R. gruss an teplitz* biomass with organic solvents, such as acetone and benzene significantly enhanced Zn(II) removal efficiency of biomass. Extraction with organic solvents removed the protein and lipid fractions from the biomass surface [30]. Thus, this treatment might expose more metal binding sites and improved the adsorptive property of the biomass. $CaCl_2$ -treated biomass, compared to the raw biomass, exhibited an enhancement in total Cu(II) and Zn(II) removal in the equilibrium state. This enhancement was due to cleaning of the biomass surface by calcium ions, which might be easily replaced with Cu(II) ions during biosorption. Park et al. [31] also reported a similar increase in Cr(VI) biosorption after $CaCl_2$ treatment of *Ecklonia* sp.

Pretreatment of *R. gruss an teplitz* distillation sludge with methanol drastically reduced Cu(II) and Zn(II) biosorption capacity of biomass. Treatment of biosorbent with methanol resulted in esterification of carboxylic acids present on the cell wall of biosorbent and the reaction occurred as follows:



Results revealed that carboxylic groups on the cell wall of *R. gruss an teplitz* contributed significantly to Cu(II) and Zn(II) biosorption. A similar observation has been reported by Jianlong [32]. Ethanol is polar organic solvents. It caused polarization in *R. gruss an teplitz* distillation sludge and thus Cu(II) sorption capacity was increased. Ethanolic treatment of biosorbent drastically reduced Zn(II) biosorption capacity of biomass may due to esterification of carboxylic acids present on the cell wall of biosorbent and the reaction occurred as follows:



These results revealed that carboxylic groups on the cell wall of *R. gruss an teplitz* contributed significantly to Zn(II) biosorption a similar observation has been reported by Jianlong [32].

4. Mineral composition of biomass

Digested *R. gruss an teplitz* (red rose) distillation sludge was analyzed for metals like Na, K, Li, Cu, Zn, Mn, Pb, Cr, Co, Fe and Mg and their concentrations are shown in Table 3. All metals were found to be present in trace amount.

5. Conclusions

The present study demonstrates the removal of Zn(II) and Cu(II) from aqueous solutions using *R. gruss an teplitz* biomass. The important conclusions drawn are:

Table 3
Mineral composition of *R. gruss an teplitz* distillation sludge.

Minerals	Concentration (mg/g)
Na	4.81
K	3.14
Li	1.54
Cu	0.005
Zn	0.009
Pb	0.07
Cr	0.23
Co	0.019
Fe	0.24
Mg	0.17

- Biosorption of Zn(II) and Cu(II) by *R. gruss an teplitz* waste biomass was strongly dependent on experimental parameters.
- The change in metal ions uptake capacity (mg/g) of a specific pretreatment is strongly dependant on nature of chemicals and metals.
- Maximum biosorption capacity of Zn(II) and Cu(II) was observed for PEI + glutaraldehyde (68.64 mg/g) and NaOH (43.4 mg/g) pre-treated biomass, respectively.
- Carboxylic groups on the cell wall of *R. gruss an teplitz* contributed significantly to Zn(II) and Cu(II) biosorption.
- Cu(II) biosorption capacity was adequately described by Freundlich isotherm, whereas Zn(II) biosorption phenomenon was described by Langmuir isotherm.
- Pseudo-first-order reaction describes adequately the biosorption of Zn(II) and Cu(II) ions on *R. gruss an teplitz* waste biomass.

Acknowledgement

The authors like to thank Mr. Iftikhar Ahmad (Lecturer, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan) for his support during the present study.

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