Egypt. J. Chem. **Vol. 64,** No. 11 pp. 6623 - 6633 (2021)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Biorecovery of Cupper (II) using *Klebsiella pneumoniae* Isolated from Wastewater Effluents

Alaa S.Amin[a]; Shimaa S. Hussien[* b]; Wael A. Hafez[a] Osman A. Desouky[b]

[a] Chemistry Department, Faculty of Science, Banha University.

[b]Ore Processing Department, Nuclear Materials Authority, P.O. Box 530 El-Maadi, Cairo, Egypt.

Abstract

This study deals with the biosorption capacity of the microflora as *Klebsiella pneumoniae* biomass as a biosorbent for the removal of cupper (II) ions from polluted water sample. The biosorption of Cu(II) ions is determined at varying experimental conditions using a batch technique. The effect of pretreatment, initial metal ion concentration, initial pH, temperature, biosorbent dosage and contact time have been investigated. The maximum biosorption efficiency of Cu(II) ions was 93.0% at the optimum biosorption conditions which are: 200 mg/L of Cu(II) ions as initial concentration, pH 7, temperature 30 °C, contact time 60 minutes, 0.7 mg/50 mL (dry wt. cell) biomass dose and mixing speed 175 rpm where the bacterial biomass pretreated with sodium hydroxide. SEM and IR analyses are used for characterization of the biosorbent agent before and after the biosorption process. The present work reveals that *K. pneumoniae* biomass is a good choice as a biosorbent agent for the recovery of Cu(II) ions from the contaminated water samples.

Key words*:* Biosorption, Cupper (II), *Klebsiella pneumonia*e, Wastewater.

1. Introduction

Water is considered to be an essential element of all the life forms covers approximately 71 % of the earth's surface and makes up two thirds of our bodies. Despite the water is earth's most abundant natural resources, only about 1 % of the water is usable for human use [1,2]. The world health organization report stated that a sufficient quantity of freshwater is not available for nearly 1.1 billion people and they suffer from water shortage. About 50 % of people will sustain their living the water-stressed zones by 2025, and nearly 1.7 million people were losing their lives due to the consumption of polluted water [3]. Also, about 4 billion people showed various health issues due to water borne diseases annually [4], that is due to increasing in clean water prices, several climatic and environmental changes in addition to the exponential growing populations [2]. Heavy metals contamination is a major problem of our environment .This problem is receiving more and more attention throughout the world, in general and in developing countries in particular [5], heavy metals and other constituents leach into the soil and damage the flora and animal life on the earth [6]. Heavy metals must be removed from effluents before they are discharged to meet quality requirements [7]. Cupper (Cu) is an essential trace element for most microorganisms, its present in industrial wastes is primarily in the form of the bivalent Cu(II) ions as a hydrolysis product, $CuCO_3$ (aq) and/or organic complexes[8]. However, excess of cupper (II) ions in water can cause toxic and harmful effects to the living organisms present and as well as to consumers. [9]. Cupper is mainly employed in electric good industry and production of bras ,so it can be found in many wastewater sources as byproducts from electronics plating, plating, wire drawing, cupper polishing, paint processing, paper and wood pulp production, printing operations, printed circuit board manufacturing, air conditioning tubing and fertilizers [10, 11]. There are a lot of conventional methods for removing cupper from wastewaters include precipitation and sulfide precipitation, evaporation, ion exchange, membrane filtration and reverse osmosis. These processes may be ineffective or extremely expensive [12]. However, microbial biosorbation is most cost-effective due to its technically simple, low cost operating, minimized temperature and energy demand, feasible biomass regeneration, high efficiency, wide pH range of applicability, low levels of secondary pollution and recyclability [13]. Also, [14] in its study emphasized these advantages compared to other techniques used

*Corresponding author e-mail: mash_1997@yahoo.com.; (S.S. Hussien).

Receive Date: 01 June 2021, Revise Date: 14 June 2021, Accept Date: 14 June 2021

DOI: 10.21608/EJCHEM.2021.2016.3847

to remove heavy metals from wastewater. Biosorption is the method of non-living biological material extracting metal or metalloid species, compounds, and particulates from a solution [15]. Biosorption is especially well suited to the treatment of wastewater effluents with dilute heavy metal ion concentrations or when extremely low heavy metal concentrations are needed [16]. Microorganisms such as yeasts (*Saccharomyces sp., Candida sp., etc.*), fungi (*Aspergillus sp., Rhizopus sp*., etc.), bacteria – Gram-positive (*Bacillus sp., Corynebacterium sp., etc*.), Gram-negative (*Escherichia sp., Klebsiella sp., Pseudomonas sp., etc*.) and cyanobacteria (*Anabaena sp., Sinechoestys sp., etc*.), and algae can absorb heavy metals and radionuclides from their external environment [17]. The microflora is a macroscopic group formed from a large number of bacteria or fungi that associate with each other and work together to adapt to harsh environments through self-structural adjustment under severe conditions [18, 19]. It include all bacteria that use organic nutrients for growth. These bacteria are universally present in all types of water, food, soil, vegetation, and air. Under this broad definition, primary and secondary bacterial pathogens are included, as are coliforms (*Escherichia, klebsiella, enterobacter, citrobacter, serratia*) [20]. *Klebsiella pneumoniae* is gram negative bacteria with the ability to remove Cu(II) ions, is abundant in nature and can be found in surface water used for human use or recreation. The organism can live in water delivery systems despite chlorination. Several strains cause positive faecal coliform tests despite being the only species found in the water sample. As a result, the public health effects of *Klebsiella* in water are a major concern [21]. *Klebsiella* was once considered a major population pathogen capable of causing serious primary pneumonia, but such cases are now extremely rare, Extreme *Klebsiella* infections are now widespread in hospital patients whose resistance has been reduced by their primary infection. The epidemiology of these hospital-acquired infections does not tend to be dominated by waterborne *Klebsiella*. As a result, *Klebsiella pneumoniae*in water sources should not be considered a health risk [22]. Biosorption can take place through passive uptake or through the metabolic activity of living organisms. However, there are many benefits of using dry biomass over living organisms, including: (1) the ability to store at room temperature; (2) the ability to use as a biosorbent for a prolonged period of time without losing its biosorptive properties; (3) are unaffected by the effluent toxicity; and (4) have higher removal capacities than living microorganisms [23, 24]. In the present work, we focused on the cupper biosorption process using dried biomass of *Klebsiella pneumoniae* species as a biosorbent are investigated with various chemical methods, Fourier transformer infrared (FTIR) spectrometry and scanning electron microscope (SEM). Parameters studied include the effects of solution pH, contact time, biosorbent dosage, initial metal ion concentration and temperature by batch method.

2. Materials and Experimental

2.1. Material:

For various batch experiments, all chemicals of analytical reagent grade obtained from the standard source were used without purification.

2.1.1. Cupper (II) solution preparation

1000 mg of cupper nitrate obtained from Scharlau was dissolved in one liter of doubled deionized water to make the stock solution. The stock solution was diluted to appropriate volume to use in the experiments at various concentrations. By adding 1N HCl/1N NaOH to the solution, the pH of the solution was modified in the range of 3–9 for various experiments. Double distilled water [DDW] was used in all the experiments.

2.1.2. Sample collection

Wastewater samples were collected in screw capped sterile borosilicate glass and plastic bottles from the two pump station of 6th of October City industrial zones' Cairo, Egypt. In which all wastewater from the city is collected (part of the residential area and the industrial wastewater). Some physicochemical parameters of wastewater as., temperature (ºC), pH, turbidity, total dissolved solids (TDS), total suspended solids (TSS),biological oxygen demand (BOD), chemical oxygen demand (COD), sulfates and other heavy metals as Cu (mg/L) were measured according to [25].

2.2. Methods:

2.2.1. Isolation, purification and identification of microbial isolates

Wastewater samples were serially diluted (10^{-1}–10^{-5}). One mL of each dilution was inoculated into a 250-mL Erlenmeyer flask containing nutrient broth. Flask were incubated at room temperature on a rotary shaker at 100 rpm for 24-48 hrs. A loop of enriched sample from nutrient broth flask was streaked on nutrient agar Petri dishes. Inoculated nutrient agar Petri dishes were incubated at 35 ^{O}C for 24-48 hrs, the plating was done in triplicate. Grown individual bacterial colonies (based on shape and colour) on the surface of nutrient agar Petri dishes were picked up. Microbial isolates were streaked for several consecutive (3-5) times in nutrient agar medium until the pure single colonies were obtained. Different microbial isolates were identified using biochemical testes [26].

2.2.2. Preparation of bacterial biosorbents

Five bacterial isolates were separated from wastewater effluents of pump station II. They were grown on nutrient agar and the standard spread plate method was performed. The prepared biomasses of the growing bacterial cells were tested for initial biosorption screening of Cu(II) ions from prepared stock solution. *Klebsiella pneumoniae* isolate was chosen and grown on 250 mL nutrient broth on a rotary shaker at 175 rpm at 36 C for 3-5 days. After the incubation period, active growing microbial cultures washed several times with doubly distilled, then centrifuged at 10,000 rpm for 10 min to get wet pellets, which then transferred to a sterile Petri dish and put in a hot air oven for 24 hours at 70°C. The dried dead bacterial biomass was powdered in a mortar to the smallest particles of less than 0.5 mm (mesh size 125-250 µm) for use as abiosorbent agent; the smaller the particle size, the greater the surface area. The biosorption ability of biomass has been increased by crushing it to prevent particle aggregation [27]. The pretreatments of the biomass are achieved by alkaline, acidic and heat drying [28].

2.2.3. Factors affecting the biosorption process

The biosorption experiments were conducted with 50 mL of 25 mg/L initial concentration of Cu(II) ions solution in 250 mL Erlenmeyer flask for 60 min contact time at room temperature 26±1°C. Effect of initial Cu(II) ions concentration on biosorption was studied between 25 -300 mg/L. Effect of initial pH on biosorption was evaluated with values ranging from 1-10 using 0.1 mol/L NaOH or HCl. The effect of temperature on biosorption was performed from 10 to 70 °C. The contact time chosen for the time dependence studies were 10 to 600 minutes. The amount of *K. pneumoniae* biomass was administered between 0.1-1.30 mg.

2.2.4. Cupper measurement

The supernatants were filtered through a filter paper (Whatman No. 41) and the concentration of Cu(II) ions in the solution was measured before and after equilibrium by UV/VIS spectrophotometer (Jenway 6715, UK) , Flame Atomic Absorption Spectrophotometer (240FS AA, Agilent Technologies) and *Inductively Coupled* Plasma-Optical Emission Spectrometer (*ICP-OES*) with *Synchronous Vertical Dual View (SVDV)*(*Agilent 5100)* .The percentage rate of Cu(II) ions metal ions biosorbed by *K. pneumoniae* was calculated using the following equation:

The percent of biosorption rate (%) =

$$\frac{(Co - Ce)}{Co} \times 100$$

Where, Co and Ce are the initial and final metal concentrations in the solution (mg/L).

2.2.5. Characterization of *K. pneumoniae* biomass before and after Cu(II) ions biosorption

2.2.5.1. Fourier transform infrared analysis

Fourier transform infrared (FTIR) spectrophotometer was used to determine the functional groups presented on the surface of *K. pneumoniae* biomass before and after loading with cupper (II). The apparatus used (Model JASCO 4100 FTIR spectrophotometer, Japan) were presented in the Micro Analytical Center, Cairo University, Egypt. The samples were prepared as KBr discs

2.2.5.2. Scanning electron microscope (SEM) observation

The texture, pore structure and loaded biomass of the prepared samples are observing under high resolution Environmental Scanning Electron Microscope (ESEM),Philips XL30 vacuum at 30 KV.

2.2.6. Desorption study

The desorption process should yield the metal in a condensed form, restore the biosorbent close to the original state for successful reuse with undiminished metal uptake and physical changes or damages to the biosorbent [29]. Desorption experiments were performed by mixing *K. pneumoniae* loaded biomass (0.5 mg dry weight) with desorbing agents 50 mL of (0.1, 0.2, 0.3 N HCl and 0.1, 0.2, 0.3 N HNO_3) for 1 h on a rotary shaker (175 rpm) at 30°C for three cycles to study the elution rate.

3. Results and Discussion

3.1. Physical and chemical prosperities of the wastewater samples

6th of October City has one of the largest industrial zones in Egypt on which the entire city is established it contained six industrial zones added to Abo rawash zone and stores zone. We will study the physical and chemical properties of wastewater of the two main pump stations in the city. Samples collection, preparation and measuring were according (SM) Standard Methods for the Examination **[25]**. The two examined samples were analyzed in the National Research Center, Egypt. The analyate parameters, Reference Methods, Unit and Limits are specified as shown in Table (1). From the obtained results, it was appeared that all parameters and heavy metal concentrations in wastewater of station I was presented in permissible range. Based on this results wastewater of pump station II was chosen for further experiments Pump station II was appeared that, most of physical and chemical parameters in permissible range except total sulfids and nitrogen which exceed

to limits. Also some of these parameters as COD and BOD approach from end of permissible limits. Some of heavy metals concentration in wastewater of pump station II was exceed the permissible range as cupper, nickel, cobalt and chromium. The totally heavy metal concentration was found 20.726 mg/L. Cu (II), Ni(II), Co(II), Cd(II) and Cr(II) concentrations were 15.2 , 3.0, 1.5, 0.7 and 0.026 mg/L, respectively. Nitrogen comes to wastewater mainly from excrement and urine. It can be transmitted into groundwater by wastewaters and also by fertilizers. Excessive nitrogen (N) discharge into water sources causes ecological issues such as eutrophication and degradation of aquatic environments.

Sulfide is highly toxic to human beings. It can cause headaches, nausea and affect central nervous system even at low levels of exposure. It causes death within 30 min at concentrations of only800–1000 mg/L, and instant death at higher concentrations. It causesbrotten-egg smell ,and at concentrations above 10 ppm, the toxicological exposure limits are exceeded. The presence of sulfide in water with high concentrations can cause odour and corrosion of sewer systems under anaerobic conditions through conversion into hydrogen sulfide. Sulfide causes depletion of oxygen in water.

Table (1): Physical and chemical parameters measured in wastewater in pump station I and II.

#	Parameters	Reference Methods	Results		Unit	Limits*
			I	II		
1	Temperature	-----	25	25	^{o}C	43
2	pH	SM4500 H^{+}	7.7	7.6	---	6.0 – 9.5
3	BOD(5d,20^{0}C)	SM5210	485	565	mg/L	600
4	COD cr	SM5220B	837	973	mg/L	1100
5	Tota Suspended Solids(TSS)	SM2540D	220	124	mg/L	800
6	Oil and Grease	SM5520B	UD	UD	mg/L	100
7	Sulfids	SM4500 $S^{--}F$	10.37	21	mg/L	10
8	Total Nitrogen	SM4500NC	101	171	mg/L	100
9	Total Phosphorus	SM4500P C	2.55	4.3	mg/L	25
10	Cyanides	SM4500 $CN^{-}D$	UD	UD	mg/L	0.2
11	Phenol	SM5530 C	UD	UD	mg/L	0.05
12	Settle able Matter 10 min ,30 min	SM25400.F	12	12	mI/L	8, 15
13	Chromium	SM3111B Cr	0.15	0.7	mg/L	0.5
14	Cadmium	SM3111B Cd	0.031	0.026	mg/L	0.2
15	Lead	SM3111B Pb	0.2	0.3	mg/L	1.0
16	Mercury	SM3111B Hg	UD	UD	mg/L	0.2
17	Silver	SM3111B Ag	UD	UD	mg/L	0.5
18	Cupper	SM3111B Cu	0.1	15.2	mg/L	1.5
19	Nickel	SM3111B Ni	0.102	3.0	mg/L	1.0
20	Tin	SM3111B Sn	UD	UD	mg/L	2.0
21	Arsenic	SM3111B AS	UD	UD	mg/L	2.0
22	Cobalt	SM3111B Co	UD	1.5	mg/L	-----
23	Total heavy metals	---	1.483	20.726	mg/L	5.0

(*) Limits according to law no 93/1962 and its decree no 44/2000 concerning disharge final effluent to public sewer system. UD Under limit of detection BOD Biological oxygen demand COD Chemical oxygen demand

3.2. Selection of most active bacterial stain absorbed the maximum percentage of Cu(II) ions.

The purpose of this experiment was to study the best bacterial strain which absorbed the maximum amount of Cu (II) ions from aqueous solution. Fi**ve** different bacterial stains were isolated from wastewater samples which collected from the two pump station of 6th of October City industrial zones' which namely according to **[26]** as *Candida albicans, Pseudomonas fluroscens, Esherichia coli, Klebsiella pneumoniae and Enterobacter sp* as shown in Figure (1) **.**They were sub-cultured on nutrient agar medium, and then the isolated colonies were grown on nutrient broth at 30 °C and incubated for 1-3 days. The availability and stability for Cu(II) ions biosorption were tested. It was found that bacterial *K. pneumoniae* is the most promising one for Cu(II) ions biosorption. The results in Figure 1 appeared that *K. pneumoniae* was the best strain for maximum Cu(II) ions biosorption (39.80 %) as shown in Figure (2)**.** **[21]** mentioned that *K. pneumoniae* is a form of bacteria that can used for the removal of heavy metals or metalloids from contaminated areas, such

as copper, cadmium, mercury, and arsenic, due to its flexibility and tolerance in a variety of microenvironments, with a focus on its potential pathogenicity.

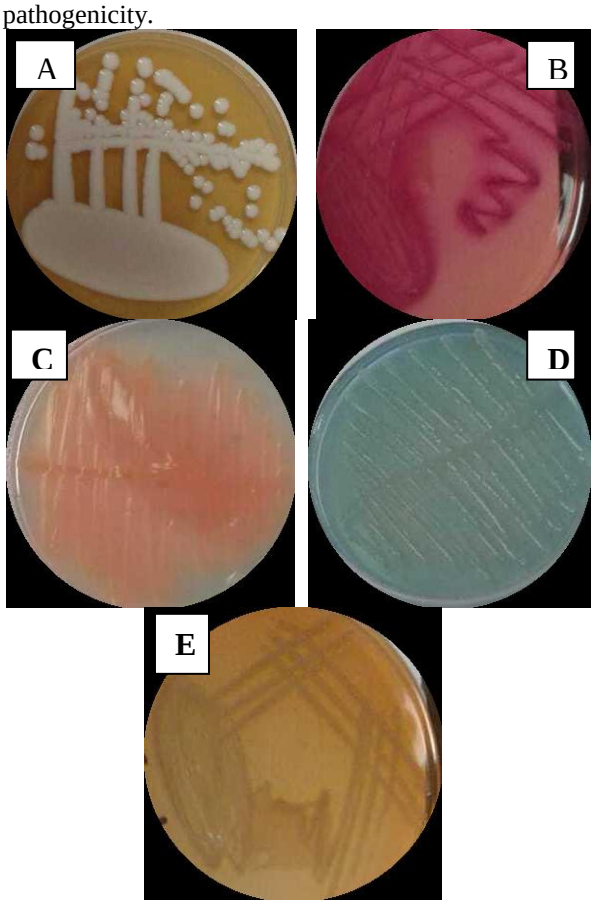


Fig. (1): Tested bacterial isolates after cultivation on specific medium for growth where (A) *C. albicans*, (B) *E. coli* , (C) *K. pneumoniae*, (D) *P. fluroscens* and (E) *Enterobacter sp.*

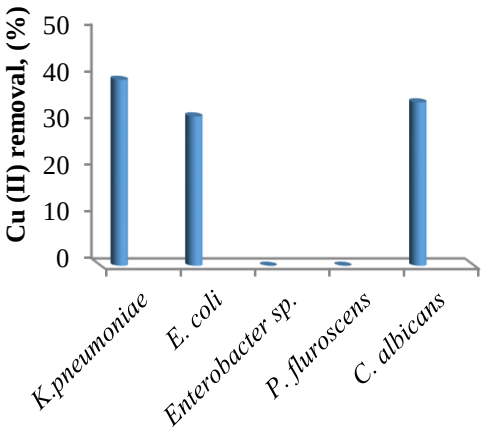


Fig. (2): Biosorption rate of Cu(II) ions by the tested bacterial strains.

3.3. Effect of different pretreatment on the biosorption of Cu(II) ions by *K. pneumoniae*

Figure (3) shows that the maximum biosorption rate (50 %) of Cu(II) ions by *K. pneumoniae* was obtained by pretreatment of bacterial biomass with 0.1 M NaOH. The comparison of Cu (II) ions biosorption rate with native and pretreated *K. pneumoniae* appears that, 0.1 M NaOH has the maximum biosorption rate. The reason may be that alkali pretreatment could remove the amorphic polysaccharide on the cell wall and change the structure of the dextran and chitin, so the biomass could be biosorbed much more Cu(II) ions on its surface. At the same time, NaOH could dissolve the inclusions in the cell which encumber biosorption, and expose much more active binding sites to improve the biosorption capacity. Furthermore NaOH makes H^+ to be dissociated from the cell wall, resulting in the increase of negative functional groups, then the improvement of the biosorption capacity may be occurred **[15]**.

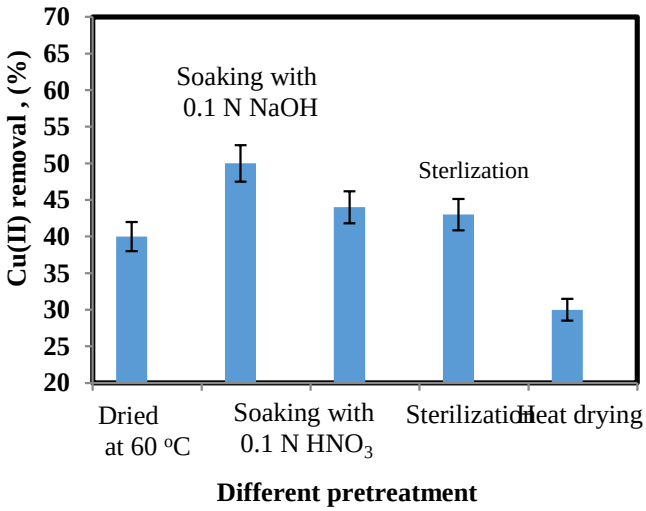


Fig. (3): Effect of different pretreatment on the biosorption of Cu(II) ions by *K. pneumoniae.*

3.4. Factors affecting the biosorption process

3. 4.1. Effect of initial Cu (II) ions concentrations

The effect of initial metal concentration on the biosorption rate *K. pneumoniae* was illustrated in Figure (4) .The biosorption percentage of Cu (II) ions shows a decreasing trend as metal ions concentration increase. The maximum removal of Cu(II) ions is 52.50% at Cu(II) ions concentration (200 mg/L).This behavior can be due to that, at higher concentrations, the increase of Cu(II) ions

concentration probably lead to the increase in the number of ions competing for the available binding sites in the biomass resulted in the limitation of vacant binding sites, which saturate beyond certain concentrations, thus decreasing Cu(II) ions biosorption **[30]**.

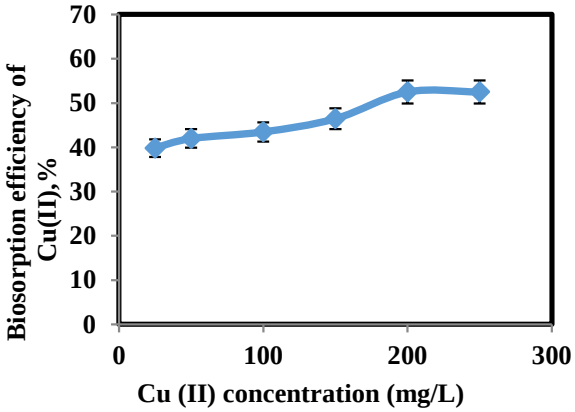


Fig.(4): Effect of initial concentrations on Cu(II) ions biosorption by *K. pneumoniae.*

3.4.2. Effect of initial pH

The percentage of Cu (II) ions removal is strongly dependent on pH as shown in Figure (5). The pH seems to be the most important parameter in the biosorptive process. It affects the metal ions chemical property, competition, the activity of its combining site, that is, the functional group of a microorganism. It has been found that the maximum removal of Cu (II) ions was found to be 62.00% at pH 7.0. At lower pH value, the cell wall of *K. pneumoniae* becomes positively charged and it is responsible for reduction in biosorption capacity. In contrary, at higher pH, i.e. more than pH 6.8, the cell wall surface becomes more negatively charged and therefore the biosorption of Cu(II) ions on to *K. pneumoniae* is more due to attraction between the biomass and the positively charged metal ions **[31]**. This agree with **[32]** who sates that the removal of most heavy metals is strongly affected by the pH of the medium. When using SMS (spent mushroom substrate) as a biosorbent, the maximum removal efficiency for the examined heavy metals (Fe(III), Ni, Co, Fe(II), Zn, Al, and Cu (II) ions were attained at pH values of approximately 7.

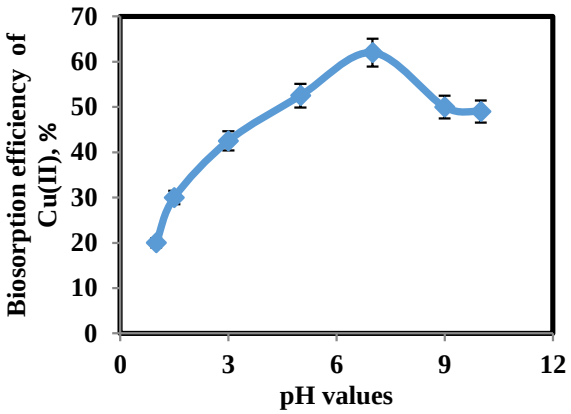


Fig. (5): Effect of initial pH on Cu(II) ions biosorption by *K. pneumoniae*.

3.4.3. Effect of temperature

Temperature affects metal biosorption onto the biosorbent; increasing the temperature improved the Cu (II) ions biosorption rate and reduced the contact time needed for heavy metal removal. The experiments were carried out at 10,20, 30, 40, 50, 60 and 70°C. The other parameters were kept constant. Cu (II) ions 200 mg/L solution was shaken 0.5 mg of *K. pneumoniae* at pH 7.0 for 30 min. It was found that the temperature 30 °C was considered the suitable degree for maximum biosorption rate of 79.82% as appeared in Figure (6). Similar observation obtained in **[33]** they reported that the increase in biosorption percentages with temperature may be attributed to either an increase in the number of active surface area binding sites available for biosorption or the decrease in the thickness of the boundary layer surrounding the biosorbent with temperature, so that the mass transfer resistance of a dsorbate in the boundary layer decreases.

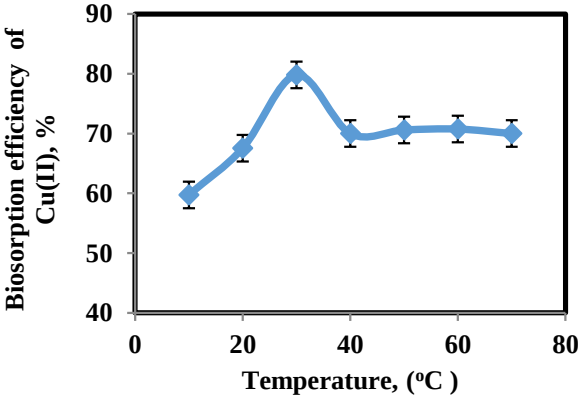


Fig. (6): Effect of temperature (ºC) on Cu(II) ions biosorption by *K. pneumoniae*.

3.4.4. Effect of contact time

The kinetics of biosorption describing the contact time in the removal of Cu (II) ions is one of the characteristics defining the efficiency of biosorption rate. The results indicate that maximum biosorption capacity occurred after 1hr where removal percentage was 82.80% . After this period, the equilibrium is reached as shown in Figure (7). As the contact time increased, more and more functional groups participated in biosorption of metal ions until it reaches equilibrium. Then when active sites on the biosorbent agent were filled, rate of biosorption became gradually constant and reached a plateau.

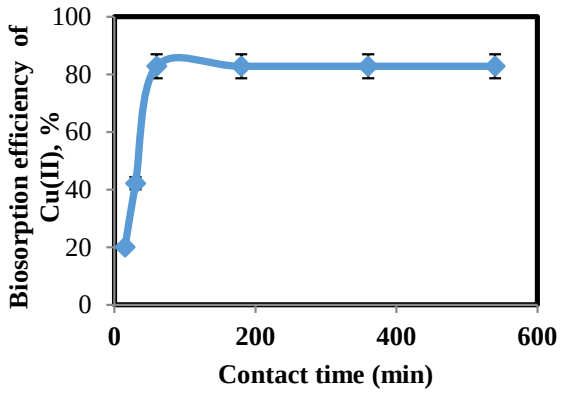


Fig. (7): Effect of contact time (minute) on Cu(II) ions biosorption by *K. pneumoniae*

3.4.5. Effect of biomass dose

Effect of biomass dosage (mg) on the biosorption of Cu (II) ions by *K. pneumoniae* is presented in Figure (8).The increase in biomass concentration from 0.1g to 1.1 (mg/50 mL) results in an extensive increase in the metal biosorption. The increase of the biosorption surface area and the availability of free biosorption binding sites help in the removal of Cu (II) ions. Maximum removal efficiency was observed at the biomass dosage of 0.7 mg, the percentage of Cu(II) ions removal increase from 82.80 to 93.0%., after this concentration equilibrium is reached. Increase in biosorption of Cu(II) ions due to the increase in biosorbent amount could be attributing to increase in surface area that leads to increase in the binding sites and chelation of metal ion with the corresponding sites **[34]**.

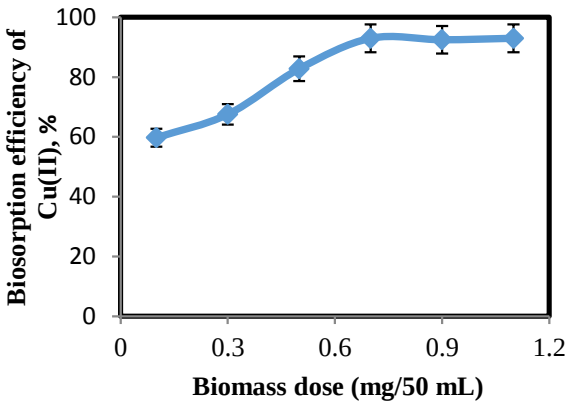


Fig. (8): Effect of biomass dosage (mg) on Cu(II) ions biosorption by *K. pneumonia*e.

3.5. Characterization of *K. pneumoniae* biomass before and after Cu(II) ions biosorption

3.5.1. Infrared spectroscopy analysis

The IR spectra were used to characterize the changes in the surface functional groups before and after the Cu(II) ions biosorption by strain of *K. pneumoniae*, and the IR spectra are shown in Figure (9). The IR absorption peaks were assigned according to the literature **[35]**. The cell surface of *K. pneumoniae* contains a large amount of carboxyl, amide, and hydroxyl groups as well as other organic functional groups, providing numerous binding sites for Cu(II) ions. The absorption peaks between 3600–3200 cm^{-1} are mainly caused by the stretching vibration of O-H arising from carbohydrates in the cells, which participate in hydrogen bonding interactions. Under the impact of Cu(II) ions, these peaks were observed to become sharper and shift towards higher frequencies, indicating that hydrogen bonding interaction involving O-H were weakened, possibly leading to the unstable structures of polypeptide or protein on the cell surface. The absorption peaks at 1650 cm^{-1} and 1402 cm^{-1} correspond to the asymmetric and symmetric stretching vibrations of the carboxylate ions, respectively. The ratios of absorption peak height at 1650 ± 10 cm^{-1} and 1400 cm^{-1} before and after addition of Cu(II) ions (i.e. COO−/COOH ratios), were 0.925 and 0.955, respectively, probably due to an increase in pH of the solution after the cell surface binding of Cu(II) ions, which represents an increase from 5.00 to 7.90 and an increase in the degree of COOH deprotonation. This obtained results indicating that cupper as a transition metal is covalently bonded to the carboxylate groups on the cell surface to form polydentate complexes **[36]**. The absorption peak at 1550 ± 10 cm^{-1} in Figure (9) was caused by the deformation of CON-H of the protein amide II, which shifts to a lower frequency after addition of Cu(II) ions, indicating that cupper can also bind with amide groups on the fungal cell

surface through covalent interaction. **[37]** has proposed that the mechanism of heavy metal Cu(II) ions immobilization on the cell surface of *Gibberella moniliformis* NT-1 is mainly achieved by the coordination binding of Cu(II) ions with hydroxyl and amide groups in chitin, as the major component of most fungal cell walls is chitin. After the interaction between the strain and Cu(II) ions, the frequency of the deformation vibration of CH in aromatic heterocyclic compounds at 827 cm^{-1} was appeared. Therefore, it is speculated that the Cu(II) ions interaction occurs during the reaction to form Cu-nitrogen-containing heterocyclic complexes. The tested bacterial stain *K. pneumoniae* under the cupper stress condition causing an increase in the amounts of produced polysaccharides and phosphate esters on the cell surface causes a high shift between the functional groups on the cell wall of bacterial strain **[38]**.

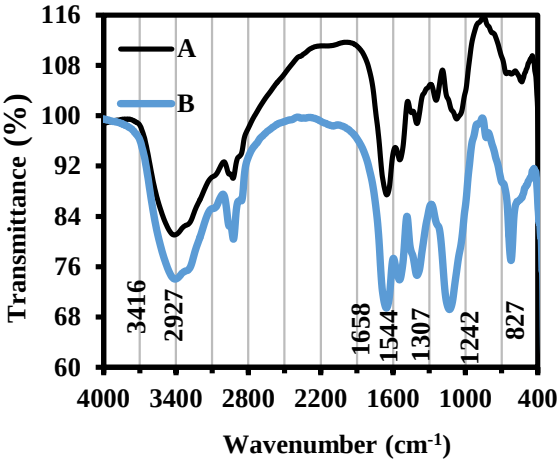


Fig. (9): FT-IR spectra of *K. pneumoniae* biomass before (A) and after Cu(II) ions biosorption (B).

3.5.2. Scanning Electron Microscope (SEM) observation

SEM images of *K. pneumoniae* without and with Cu(II) ions are shown in Figure (10A&C). Metal ions loaded cells differed in morphology from the unloaded ones. For the samples containing the unloaded cells, the majority of cells remained intact, smooth, and closely connected with one another, providing a large surface area for biosorption Figure 10A. In the case of the loaded samples Figure 10C, the matrix layers of the cell wall appeared to shrink and stick. All microstructures porosities of *K. pneumoniae* appeared with bright. The changes in cellular morphology and size may result from mechanical force and reciprocation between surface-active components and metallic ions [39]. In addition to, the EDX analysis showed that the elemental composition of *K. pneumoniae* biomass was significantly changed after Cu(II) ions biosorption Figure 10B and Figure 10D. Figure 10D shows signals of Cu(II) ions in the bacterial biomass.

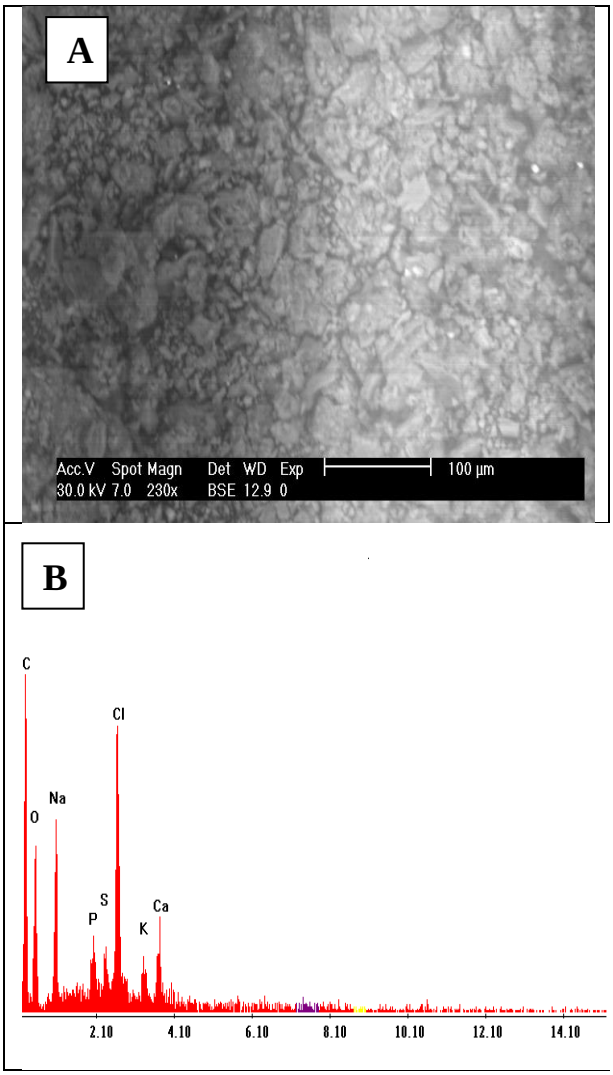
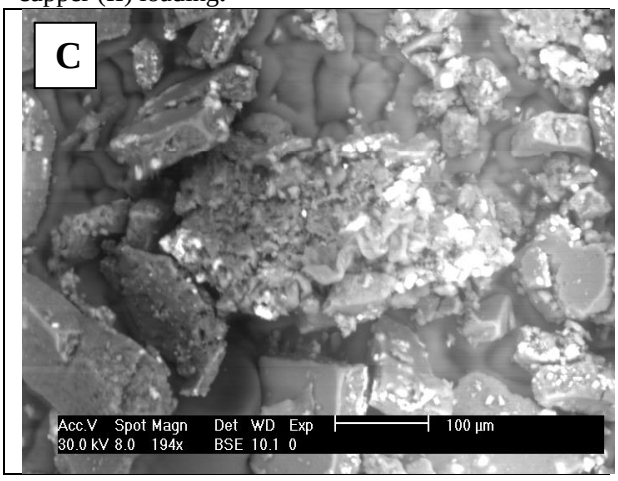


Fig. (10): SEM micrograph and corresponding EDX spectrum of *K. pneumoniae* control (A&B) before cupper (II) loading.



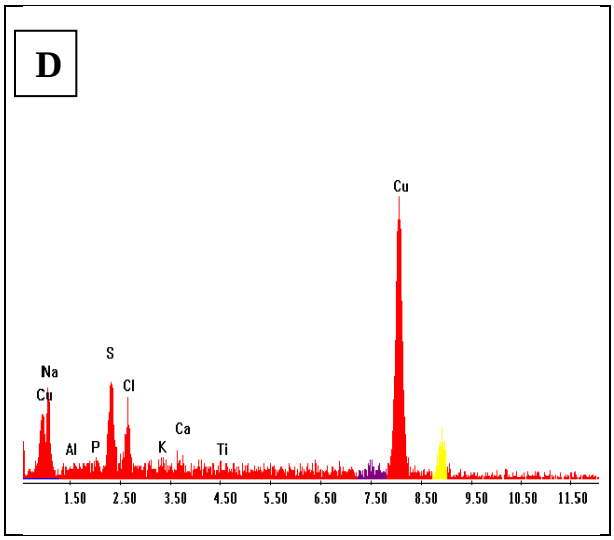


Fig. (10): SEM micrograph and corresponding EDX spectrum of *K. pneumoniae* (C&D) after Cu (II) ions loading**.**

3.6. Desorption study

Desorption/recovery of biosorbed metal is one of the most important aspects of any successful biosorption process development **[40]**. Figure (11) appeared the percentage of desorbed Cu(II) ions from the biomass using 0.1 N HCl, HNO_3, H_2SO_4 and $CaCl_2$ for three washing cycles. It was found that 0.1 N H_2SO_4 desorbent agent eluted about 90 % of the biosorbed Cu(II) ions by *K. pneumoniae* biomass

After three cycles, respectively. Higher acid concentrations could be produced higher concentration of protons which swept the metal ions away from the biosorbent. As expected, a slightly lower in weight loss could be achieved by 0.5 N H_2SO_4 compared to other desorbent agents so, it safe to be used for desorption purpose without increasing the biomass weight loss **[41]**.

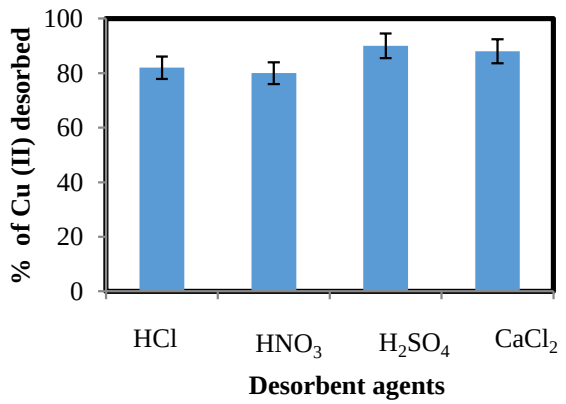


Fig.(11): Desorption of Cu(II) ions from biomass of *K. pneumoniae* by different desorbent agent.

3.7. Removal and precipitation of Cu(II) ions from wastewater of 6th of October City industrial zones' Cairo, Egypt

Using the obtained optimum biosorption conditions, the pretreated *K. pneumoniae* biomass was used for biosorption of Cu(II) ions from the wastewater of pump station II from 6th of October City industrial zones' Cairo, Egypt. Then after desorption study, to precipitate cupper in the liquor work, colorless sodium hydroxide solution is added to the desorbent solution containing Cu (II) sulphate. Approximately 2 mL of solution A (0.5 M sodium hydroxide, colorless) is added to a sample solution B of wastewater from pump station II (containing cupper (II) sulphate) with a dropping pipet. If a precipitate forms, the resulting precipitate is suspended in the mixture. The mixture is then stirred with a glass stirring rod and the precipitate is allowed to settle for about a minute **[42]**. Precipitate has a color of light blue as in eq. (1):

$$CuSO_4 + 2NaOH \longrightarrow Cu(OH)_2 + 2\,Na_2SO_4 \quad \text{.....(1)}$$

Cupper hydroxide $Cu(OH)_2$ was dried at 110°C, and then subjected to SEM and EDX analyses Figure (12). The percent of Cu(II) ions reached to (79.03%) purity.

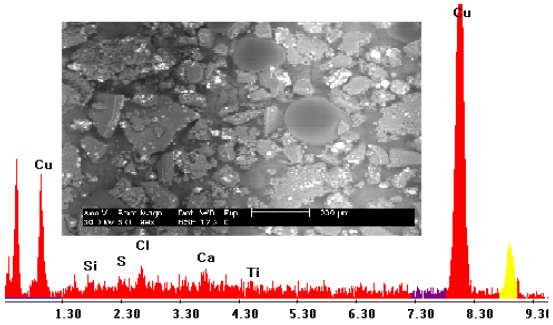


Fig. (12): SEM micrograph and EDX spectrum of cupper hydroxide ($Cu(OH)_2$).

Conclusion

In this paper, a cupper biosorbent bacterial strain of *K. pneumoniae*, which was isolated and preserved from the wastewater of 6th of October City industrial zones' Cairo, Egypt, it was identified based on the morphological and biochemical characteristics. It was found that initial Cu(II) ions concentration, pH, temperature, contact time and biomass dosage as environmental factors could substantially affect Cu(II) ions removal extent by *K. pneumoniae*. The binding of Cu(II) ions to *K. pneumoniae* was mainly achieved by forming polydentate complexes with carboxylate and amide groups and forming Cu-nitrogen-containing heterocyclic complexes through Cu(II) ions interaction. The high concentration of Cu(II) ions could be resulting in the shrinkage and sticking on the bacterial cell surface as SEM visualization. In the future, key parameters and mechanisms obtained from this study will be adopted

to guide for the experiments and field trial of bacterial bioremediation of cupper-contaminated wastewater.

Acknowledgments

The authors are grateful for financial support from the Nuclear Materials Authority, Cairo, Egypt.

Conflicts of interest

There are no conflicts to declare**.**

References

[1] Grey, D., Garrick, D., Blackmore, D., Kelman, J., Muller, M., and Sadoff, Water security in one blue planet: twenty-first century policy challenges for science. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *371*(2002), 20120406 (2013).

[2] Adeleye, A. S., Conway, J. R., Garner, K., Huang, Y., Su, Y., & Keller, A. A., Engineered nanomaterials for water treatment and remediation: Costs, benefits, and applicability. *Chemical Engineering Journal*, *286*, 640-662 (2016).

[3] World Health Organization (WHO), *World report on ageing and health*. Geneva (2015).

[4] Briggs, A. M., Cross, M. J., Hoy, D. G., Sanchez-Riera, L., Blyth, F. M., Woolf, A. D. and March, L., Musculoskeletal health conditions represent a global threat to healthy aging: a report for the 2015 World Health Organization world report on ageing and health. *The Gerontologist*, *56*(suppl_2), S243-S255(2016).

[5] Mohammed, T. Y., Elbadry, B. E. L. S. E. L. S. M., Amin, A. S., and Helmy, E. S. , Determinations of revelant impurities in copper and sulphur compounds used as pesticides. *International Journal of Academic Research*, *5*(6)127-138 (2013).

[6] Gayatri, Y., Shailaja, R. M., & Vijayalakshmi, B.,Biosorption of lead by Bacillus licheniformis isolated from e-waste landfill, Hyderabad, Telangana, India. *Journal of Bioassays*, *13*(1), 5240-5244 (2017).

[7] Witek-Krowiak, A., Szafran, R. G. and Modelski, S., Biosorption of heavy metals from aqueous solutions onto peanut shell as a low-cost biosorbent. *Desalination*, *265*(1-3), 126-134(2011).

[8] Huang, N., Mao, J., Hu, M., Wang, X., and Huo, M., Responses to copper stress in the metal - resistant bacterium Cupriavidus gilardii CR3: a whole- transcriptome analysis. *Journal of basic microbiology*, *59*(5), 446-457(2019).

[9]Macomber, L. and Imlay, J. A., The iron-sulfur clusters of dehydratases are primary intracellular targets of copper toxicity. *Proceedings of the National Academy of Sciences*, *106*(20), 8344-8349 (2009).

[10] Tong, K. S., Kassim, M. J. and Azraa, A.,Biosorption of copper ion from its aqueous solution by a novel biosorbent Uncaria gambir: Equilibrium, kinetics, and thermodynamic studies. *Chemical Engineering Journal*, *170*(1), 145-153(2011).

[11] Zhu, C. S., Wang, L. P., and Chen, W. B.,Removal of Cu (II) from aqueous solution by agricultural by-product: peanut hull. *Journal of hazardous materials*, *168*(2-3), 739-746(2009).

[12] Gabr, R. M., Hassan, S. H. A., & Shoreit, A. A. M., Biosorption of lead and nickel by living and non-living cells of *Pseudomonas aeruginosa* ASU 6a. *International Biodeterioration and Biodegradation*, *62*(2), 195-203 (2008).

[13] Zhu, Y. J., Zhou, L. B., Zhu, Y. B. and Ju, L. P., Biosorption performance investigation of heavy metal copper in acid mine drainage by the *Klebsiella oxytoca*. Legislation, *Technology and Practice of Mine Land Reclamation*, 1, *397*(2014).

[14] Xu, J., Cao, Z., Zhang, Y., Yuan, Z., Lou, Z., Xu, X. and Wang, X., A review of functionalized carbon nanotubes and graphene for heavy metal adsorption from water: Preparation, application, and mechanism. *Chemosphere*, *195*, 351-364(2018).

[15] Hussien, S. S., Biosorption of lanthanum from aqueous solution using Pleurotus ostreatus basidiocarps.bent regeneration. *Journal of environmental chemical engineering*, *2*(1), 239-259(2014).

[16] Feng, D., and Aldrich, C., Biosorption of heavy metals by biomaterials derived from the marine alga Ecklonia maxima. *Hydrometallurgy*, *73*(1-2), 1-10 (2004).

[17] Jasmidia, Z.M., Kembarenc, A., Sellyd, R. and Silalahie, A., Biosorption of manganese ions (II) by immobilized biomass on a mixture of silica gel and zeolite. *Journal of Basic and Applied Research*, *35*(2): 102-112 (2017).

[18] Qin, H., Hu, T., Zhai, Y., Lu, N. and Aliyeva, J., The improved methods of heavy metals removal by biosorbents: A review. *Environmental Pollution*, *258*, 113777 (2020).

[19] Frey-Klett P., Burlinson P., Deveau A., Barret M.,Tarkka M. and Sarniguet A., Bacterial-Fungal Interactions: Hyphens between Agricultural, Clinical, Environmental, and Food Microbiologists. *Microbiology and Molocular Biolgy Reviews*., *75*(4): 583–609 (2011).

[20]Allen, M. J., Edberg, S. C. and Reasoner, D. J., Heterotrophic plate count bacteria—what is their significance in drinking water?. *International journal of food microbiology*, *92*(3), 265-274 (2004).

[21]Tomulescu, C., Moscovici, M., Lupescu, I., Stoica, R. M. and Vamanu, A., A Review: *Klebsiella pneumoniae*, *Klebsiella oxytoca* and

Biotechnology. *Rom Biotechnol Lett*., *26*(3): 2567-2586(2012).

[22] Duncan, I.B.R., Water borne Klebsiella and human disease. Toxicity Assessment *3* (5), 581–598 (1988).

[23] Gautam, R. K., Mudhoo, A., Lofrano, G. and Chattopadhyaya, M. C., Biomass-derived biosorbents for metal ions sequestration: Adsorbent modification and activation methods and adsorbent regeneration. *Journal of environmental chemical engineering*, *2*(1), 239-259 (2014).

[24]Moreira, V. R., Lebron, Y. A. R., Freire, S. J., Santos, L. V. S., Palladino, F. and Jacob, R. S.. Biosorption of copper ions from aqueous solution using Chlorella pyrenoidosa: Optimization, equilibrium and kinetics studies. *Microchemical Journal*, *145*, 119-129 (2019).

[25] APHA (American Public Health Association), AWWA (American Water Works Association), and WEF (Water Environment Federation). Standard Methods for the Examination of Water and Wastewater, 23rd ed. (Rice, E. W., Baird, R. B., Eaton, A. D., Clesceri, L. S. eds.) Washington D.C, USA (2017).

[26] Holt, J. G., and Williams, S. T., Holt: Bergey's manual of systematic bacteriology. vol. 4 (1989).

[27] Wang, J. and Chen, C., Biosorbents for heavy metals removal and their future. *Journal of Biotechnology Advances*, *27*(2): 195-226 (2009).

[28] Shuxia, X. U., ZHANG, S., Ke, C., Jinfeng, H. A. N., Huashan, L. I. U.and Kun, W. U., Biosorption of La^{3+} and Ce^{3+} by Agrobacterium sp. HN1. *Journal of Rare Earths*, *29*(3), 265-270(2011).

[29] Tella, A.C., Owalude, S.O., Ojekanmi, C.A. and Oluwafemi, .S., Synthesis of copper-is onicotinate metal organic f frame works simply by mixing solidre actants and investigation of their adsorptive properties for the removal of the fluorescein dye. *Journal of New Chemistry*, *35*(7): 4494-4500 (2014).

[30] Hussien, S. S., Desouky, O. A. and Elkattan, M., A New Approach for Utilization of Modified Epicoccum Nigrum Biomass by Ferric Chloride Nanoparticles for Biosorption of Thorium. *Aspects Nanotechnology*, *1*(1), 40-51(2018).

[31]Hossain, M. A., Ngo, H. H., Guo, W. S. and Nguyen, T. V., Removal of copper from water by adsorption onto banana peel as bioadsorbent. *International Journal of Geomate*, *2*(2), 227-234(2012).

[32] Corral-Bobadilla, M., González-Marcos, A., Vergara-González, E. P. and Alba-Elías, F., Bioremediation of wastewater to remove heavy metals using the spent mushroom substrate of *Agaricus bisporus*. *Water*, *11*(3), 454(2019).

[33] Meena, A. K., Mishra, G. K., Rai, P. K., Rajagopal, C. and Nagar, P. N., Removal of heavy metal ions from aqueous solutions using carbon aerogel as an adsorbent. *Journal of hazardous materials*, 122(1-2), 161-170(2005).

[34] Pagnanelli, F., Petrangeli Papini, M., Toro, L., Trifoni, M. and Veglio, F. Biosorption of metal ions on Arthrobacter sp.: biomass characterization and biosorption modeling. *Environmental Science & Technology*, 34(13), 2773-2778(2000).

[35] Chen, C. and Wang, J., Uranium removal by novel graphene oxide-immobilized Saccharomyces cerevisiae gel beads. *Journal of environmental radioactivity*, *162*, 134-145(2016).

[36] Evangelou V., Marsi M. and Chappell M., Potentiometric–spectroscopic evaluation of metal-ion complexes by humic fractions extracted from corn tissue Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy *58*:2159–2175(2002). 10.1016/S1386-1425(01)00690-4

[37]Chen T., Ying L., Jing W., Lianzhen L., Kirk G. S. and Yongming L. Characterization and mechanism of copper biosorption by a highly copper resistant fungal strain isolated from copper polluted acidic orchard soil. *Environmental Science and Pollution Research International Journal*, *25*(25): 24965–24974(2018). doi:10.1007/s11356-018-2563-4.

[38]Khan Z., Rehman A., Hussain S.Z., Resistance and uptake of cadmium by yeast, Pichia hampshirensis 4Aer, isolated from industrial effluent and its potential use in decontamination of wastewater. *Chemosphere 159*:32–43(2016). 10.1016/j.chemosphere.2016.05.076 [PubMed: 27268792

[39] Huang, N., Mao, J., Hu, M., Wang, X. and Huo, M., Responses to copper stress in the metal - resistant bacterium *Cupriavidus gilardii* CR3: a whole- transcriptome analysis. *Journal of basic microbiology*, *59*(5), 446-457(2019).

[40] Kazy, S. K., Das, S. K. and Sar, P., Lanthanum biosorption by a *Pseudomonas sp*.: equilibrium studies and chemical characterization. *Journal of Industrial Microbiology and Biotechnology*, *33*(9), 773-783(2006).

[41] Diniz, V., and Volesky, B., Biosorption of La, Eu and Yb using *Sargassum* biomass. *Water Research*, *39*(1), 239-247(2005).

[42] Roberto J.C.; a Alberto E. R. and Miguel A. B., Precipitation of Copper(i1) Hydrous Oxides and Copper(ii) Basic Salts, *Journal of Materials Chemistry*, *2*(6), 657-661(1992).