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

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Abstract

This study deals with the biosorption capacity of the microflora as Klebsiella pneumoniae biomass as a biosorbent for the removal of copper (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, Copper (II), Klebsiella pneumoniae, 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]. Copper (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, CuCO₃ (aq) and/or organic complexes[8]. However, excess of copper (II) ions in water can cause toxic and harmful effects to the living organisms present and as well as to consumers. [9]. Copper 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 biosorption 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.

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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 copper 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 (°C), 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 °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 tests [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 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 biosorbent 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. Copper 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 equilibration by UV/VIS spectrophotometer (Jenway 6715, UK), Flame Atomic Absorption Spectrophotometer (240FS AA, Agilent Technologies) and Inductively Coupled Plasma-Optical Emission Spectrometer (JCP-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:

\[ \frac{(Co - Ce)}{Co} \times 100 \]

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 copper (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 HNO3) 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 analyte 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 copper, 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 wastewater 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 causes rotten-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.

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

3.2. Selection of most active bacterial strain 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. Five different bacterial strains 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

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

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 amorphous 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].

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].

![Figure 4](image4.png)

**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.

![Figure 5](image5.png)

**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 disorbate in the boundary layer decreases.

![Figure 6](image6.png)

**Fig. (6):** Effect of temperature (°C) 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.

![Graph](image)

**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 82.8 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].

![Graph](image)

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

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 copper 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 copper 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].

![SEM micrograph and corresponding EDX spectrum of K. pneumoniae control (A&B) before cupper (II) loading.](image)

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

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

### 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.
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$_2$SO$_4$ and CaCl$_2$ for three washing cycles. It was found that 0.1 N H$_2$SO$_4$ 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$_2$SO$_4$ compared to other desorbent agents so, it safe to be used for desorption purpose without increasing the biomass weight loss [41].

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 copper in the liquor work, colorless sodium hydroxide solution is added to the desorbed 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 copper (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):

$$\text{CuSO}_4 + 2\text{NaOH} \rightarrow \text{Cu(OH)}_2 + 2\text{Na}_2\text{SO}_4$$

Copper 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.

[#42]

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

Conclusion

In this paper, a copper 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 copper-contaminated wastewater.

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Conflicts of interest
There are no conflicts to declare.

References

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