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Characterization and Investigation of Biosorption of Copper (II) from Simulated Wastewater Using Chemically Modified Cassava Leaves (*Manihot esculenta*)

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Abstract. Wastewater can contain different heavy metals that can negatively affect people and the environment. Copper (II) is one of the heavy metals that is found in both municipal and industrial wastewaters wherein it can range from 50 to 100 ppm which is above the environmental standards. This study characterizes untreated, NaOH-treated, and H_2O_2-treated cassava leaves (*Manihot esculenta*) to determine the most effective copper (II) biosorbent in terms of percentage of copper (II) from simulated electroplating waste water. The samples were analyzed through Atomic Adsorption Spectroscopy (AAS). Fourier Transform Infrared (FTIR) spectroscopy was employed to analyze functional groups and compare biosorbents before and after adsorption. Results revealed significant differences in peak intensities and frequencies, indicating chemical modifications. NaOH-treated leaves showed increased transmittance, suggesting deprotonation and removal of base-soluble materials. H_2O_2-treated leaves exhibited decreased transmittance, indicating increased functional groups and adsorption sites. The absorption bands of H_2O_2-treated leaves showed differences and fewer peaks in the fingerprint region, indicating significant modifications to the molecular structure compared to untreated and NaOH-treated leaves. Highest copper (II) removal was obtained with H_2O_2-treated leaves (38.67%) , followed by NaOH-treated leaves (18.07%) . Overall, H_2O_2-treated cassava leaves emerged as promising biosorbents for copper (II) removal from wastewater, offering efficient and environmentally friendly remediation solutions.

1 Introduction

Water, a vital resource for sustaining life and driving economies, covers about 71% of the Earth's surface, yet only about 3% is freshwater. Among various uses, agriculture accounts for nearly 70% of freshwater withdrawals, underscoring its critical role in food production. However, the availability of clean freshwater is dwindling due to pollution and overexploitation, posing significant social and economic challenges globally. As mentioned

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in the United Nations World Water Assessment Programme (2017) [1], about 80% of wastewater globally is released to the environment without treatment. Industrial and municipal activities contribute to water pollution, releasing heavy metals like copper, iron, and mercury into aquatic ecosystems. Copper, a naturally occurring transition metal, is essential in trace amounts but harmful in excess. Exposure to elevated copper levels can lead to severe health issues for humans and disrupt aquatic ecosystems' balance. Municipal wastewater, often containing suspended solids and various pollutants, exacerbates the problem, necessitating effective remediation methods. Different conventional methods have been utilized but each method has some disadvantages besides high cost [2]. Biosorption, an eco-friendly approach, has emerged as a promising technique for heavy metal removal from contaminated wastewater. By utilizing biologically derived materials, biosorption offers advantages such as low cost, minimal sludge generation, and ease of operation. Besides , it does not increase the chemical oxygen demand of water which is the major limitation for nearly all of the conventional methods [3]. Alkali and acid treatment has been used for the improvement of biosorption of natural biomasses. Alkali treatment using NaOH enhances the adsorption sites through conversion of methyl ester to carboxylic glands [4]. Acid treatment modifies the charge of adsorption for easier-binding of positively-charged pollutants [5]. The usage of hydrogen peroxide (H2O2) is seen to be a sustainable method since it does not produce secondary pollution [6]. Cassava (*Manihot esculenta*), a widely cultivated crop, has garnered attention for its biosorption potential. Cassava, primarily cultivated in tropical regions, generates significant agricultural waste, including leaves, stems, and peels. Traditionally considered as by-products, these agricultural residues possess untapped potential for various applications, including wastewater treatment. Various researches indicate that different parts of cassava have demonstrated remarkable adsorption capabilities for a range of pollutants, including heavy metals and dyes, making them attractive candidates for environmental remediation Cassava peels obtained a 61.72% chromium removal [7], modified cassava biomasses obtained a 99.85% and 86.66% lead and nickel removal, respectively [8]. Under the influence of alkaline, cassava bark residue was shown to remove methylene blue by 97.99% [9] and cassava root husk also removed direct black dye due to the multilayer adsorption behavior of the cellulose [10]. Cassava leaves, in addition to their nutrional value, contain an abundance of organic compounds such as flavonoids, polyphenols , hydroxyl and carboxylic acids [11]. These compounds, along with their structural characteristics, contribute to the leave's potential for copper (II) biosorption capacity [12]. Metal cations can be attached to the carboxyl group, while the hydroxyl group functions as chelating agent [13]. Flavonoids assist the adsorption of metal through formation of complexes [14]. By focusing on the utilization of cassava leaves for copper ion biosorption from simulated wastewater, this study aims to contribute to the growing body of research on sustainable wastewater treatment solutions. By exploring the potential of a readily available agricultural by-product, this research seeks to develop a cost-effective and eco-friendly approach to water remediation while promoting the principles of circular economy and resource efficiency.

2 Literature Review

Copper is released into the environment through natural processes like volcanic activities, decaying vegetation, forest fires, etc. or via human activities such as mining, agriculture, and different industrial and manufacturing activities. Copper in municipal wastewater is mostly caused by the elevated metal concentration on tap water and corrosion within the plumbing systems in different sectors [15] [16]. When copper is released into the atmosphere, it may enter the human body through inhalation and chronic exposure to it may irritate the nose, mouth, eyes and cause headaches, dizziness, nausea, and diarrhea [17]. Wilson's disease causes the liver to be unable to effectively eliminate excess copper [18]. Biosorption can be defined as the passive adsorption of toxic substances such as heavy metals from wastewater by biologically derived materials through binding of metals ions (biosorbate) to the surface of the biosorbent which is of biological origin. This process includes binding of ions into the functional group present on the surface of the biological materials (biosorbent) in an aqueous solution through numerous interactions more than oxidation via aerobic and anaerobic metabolism [3]. Biosorption of heavy metals and organic compounds takes place because of the physicochemical interactions of the metals and the functional group present at the surface of the biosorbent [19]. Various natural materials can be used as biosorbents where it contains chemical/functional groups such as amine, amide, imidazole, thioether, sulfonate, carbonyl, sulfhydryl, carboxyl, phosphodiester, phenolic, imine, and phosphate groups that may captivate and isolate metal ions [3]. Biomasses can be treated with different reagents for the improvement of biosorption activities such as alkali and acid treatments. Sodium hydroxide (NaOH) will improve the adsorption process of heavy metal ions by removing of base-soluble materials that can cause disruption in the adsorption process, releasing adsorption sites on the surface of biomass, converting the methyl esters present to carboxylate ligands, and increasing of internal surface area of the biosorbent. [4]. Acid can increase the adsorption of heavy metal ions through the improvement of surface area and functional groups. It increases the quantity of carboxylic acids that has metal chelating properties [5]. Hydrogen Peroxide (H_2O_2) treatment is observed to be a sustainable method in modifying the adsorbent as it does not create a secondary pollution since it only decomposes as water and oxygen [6]. Although different factors can affect biosorption, contact duration is the most important factor to consider in terms of biosorption effectiveness [20] yet other factors such as temperature, pH, nature of biosorbents, surface area to volume ratio, concentration of biomass, initial metal ion concentration and metal affinity to biosorbent greatly affects the rate of biosorption [21] Cassava is one of the most important staple food crops around the world. The solid waste from cassava production consists of cassava stems, leaves, and peels. These parts are more than 18% of the cassava crop [22]. Cassava wastes are now being analyzed for various uses such as biosorption. Cassava peels and bark has shown to have significant removal of numerous pollutants such as heavy metals and dyes The cassava peels were modified using nitric acid that has led into a 61.72% average of chromium removal [7]. Cassava peels were modified with titanium dioxide nanoparticles and used to remove lead and nickel in an aqueous solution [8]. Cassava leaves also contains the various of flavonoids [11]. The most reported flavonoids in cassava leaves are catechin, quercetin, kaempferol, rutin, and nicotiflorin [23]. Cassava leaves contain 100 mg/g to 250 mg/g of phenolic content and 155 mg/g to 165 mg/g of flavonoid content [24]. In addition, the most abundant flavonoid is found to be rutin wherein it consists at least 2.4% of dry weight [25]. It can be hypothesized that cassava leaves can have adsorption properties based on its chemical compounds. Flavonoids are found to have considerable chelating properties majority of flavonoids has a constant formation of complexes with copper and iron ions [14]. OH-groups and carbonyl moiety are some of the causes for the foundation of metal complexes [26]. Cassava leaves also contain catechins and quercetin. Quercetin has at least 3 accessible binding sites which are α-hydroxy- carbonyl, βhydroxy-carbonyl, and catechol [26]. Rutin, which is a flavanol glucosides, has shown activities of metal chelation. Metal chelating properties is due to the 5 – OH group coordination site [27]. Rutin is observed to have efficient binding to copper (II) ions that leads to 1:1 stoichiometric ratio[28]. Cassava leaves also contain carboxylic acid which can also cause adsorption of heavy metals. Carboxyl and hydroxyl groups has shown remarkable binding to metal ions wherein it can uptake at least 90% [13]. Carboxyl groups gives an accessible active and negative-charged sites wherein metal cation can be placed [14]. Thus, the metal is bonded with hydroxyl group forming a chelating ring [29]. As previously noted, there have only been a few research on cassava leaves' adsorption characteristics.

Some articles have only focused on the removal of dyes. Cassava leaf powder was analyzed for dye adsorption of methylene blue and congo red [30]. The study resulted into a 99.9% and 99.7% removal of methylene blue and congo red, respectively.

3 Materials and Methodology

12 g of shredded cassava leaves were suspended in 75mL each of 4% NaOH solution and 0.1M H_2O_2 solution for 6 hours, filtered , washed and sun-dried for 72 hours [31]. Electroplating waste water of copper (II) concentration of 108 mg/L was simulated by dissolving $CuSO_4{\cdot}5H_2O$ in distilled water. Reagents were purchased from Dalkem Corporation, Quezon City. Batch adsorption was done by suspending 0.5 g each of untreated, NaOH-treated and H2O2 – treated cassava leaves as biosorbent with 25mL of simulated electroplating waste water for 120 minutes maintaining the pH at 6 [32]. Copper (II) concentration was analyzed using Atomic Adsorption Spectroscopy (Direct Air-Acetylene Flame Method). Untreated and chemically-treated cassava leaves were analyzed using FTIR Spectroscopy with Attenuated Reflectance (ATR) Accessory to determine the functional groups before and after the biosorption experiments.

4 Results and Discussions

4.1 Characterization of Cassava Leaves

FTIR Analysis was conducted to determine the functional groups present in the untreated and chemically-treated cassava before and after the batch adsorption The spectra of the samples revealed distinctive absorption peaks indicative of various functional groups. The absorption bands of H_2O_2-treated leaves showed differences and fewer peaks in the fingerprint region, indicating significant modifications to the molecular structure compared to untreated and NaOH-treated leaves. All three biosorbents exhibited absorption peaks at around 3286.75 cm^{-1} to 3297.80 cm^{-1}, corresponding to O-H stretching bends typical of alcohol and carboxylic acid functional groups found in cellulose. A medium band in the range of 3000-2840 cm^{-1} was observed in all biosorbents, indicating C-H stretching bends. The untreated cassava leaves displayed peaks at 2850.95 cm^{-1} and 2919.35 cm^{-1}, suggesting the presence of C-H stretching bends characteristic of aldehyde compounds. NaOH-treated and H_2O_2-treated cassava leaves exhibited peaks around 2850-2919 cm^{-1}, indicating C-H stretching bends, with the H_2O_2-treated leaves showing the highest intensity. Absorption peaks at around 1730 cm^{-1} indicated C=O stretching, likely from carbonyl derivatives such as esters, lignin, and hemicellulose molecules. Peaks at approximately 1450 cm^{-1} suggested the presence of C-H bending, indicative of a methylene group. Peaks at 956.68 cm^{-1} were observed in both untreated and NaOH-treated cassava leaves but were absent in H_2O_2-treated leaves, indicating differences in O-H bending due to the treatment process. NaOH-treated cassava leaves showed absorbance deviations in the fingerprint region compared to untreated and H_2O_2-treated leaves. Peaks in the fingerprint region of H_2O_2-treated cassava leaves were fewer compared to the other two, suggesting alterations in the molecular structure due to the treatment. While the absorption bands in the frequency region were relatively close across all biosorbents, NaOH-treated leaves exhibited slight deviations in the fingerprint region compared to untreated and H_2O_2-treated leaves. The absorption bands of H_2O_2-treated leaves showed differences and fewer peaks in the fingerprint region, indicating significant modifications to the molecular structure compared to untreated and NaOH-treated leaves.

In comparing the transmittance percentage of each biosorbent, H2O2-treated cassava leaves showed decrease at different peaks. This proves that there was an increase in functional groups which are found to be carboxylic acid, amine, phenols and pyranose. With the hydrogen peroxide, it was concluded that hydroxyl radicals had improved the possible binding sites within the cassava leaves, as depicted in Figure 1. The hydroxyl radical can be formed with the presence of copper (II) and hydrogen peroxide. It follows that electrophilic substitution can occur wherein the phenol can obtain an additional hydroxyl group or will turn into a catechol. The amine groups can also be oxidized by the H_2O_2 treatment wherein it will form N- oxide. Lastly, the hydrogen peroxide can form phenols through hydroxylation of benzene with the presence of copper.

After NaOH treatment, the cassava leaves obtained an increase of transmittance percentage which are identified as carboxylic acid, phenols, and alcohol. It can be concluded that deprotonation occurred within the stated functional groups. The removal of bond from the hydroxyl group had caused the increase in transmittance percentage. It can also be concluded that the sodium hydroxide modification has removed all base- soluble materials within the biosorbent.

4.2 Analysis of Copper (II) Removal Efficiency

Table 1. Percentage Copper (II) Removal of Untreated, NaOH-Treated, and H2O2-Treated Cassava Leaves (*Manihot esculenta*)

Biosorbent	**Average % Copper (II) Removal**
Untreated cassava leaves	6.86%
NaOH-treated cassava leaves	18.07%
H_2O_2-treated cassava leaves	38.67%

Table 1 shows that H2O2- treated cassava leaves obtained the highest removal of copper (II) ions within the simulated wastewater followed by the NaOH-treated cassava leaves. To further support this finding, a one-way ANOVA was used and revealed that there is a significant difference among the three values at a significance level of 0.05. FTIR analysis of NaOH-treated cassava leaves before and after adsorption shows a decrease in percentage of transmittance indicating that there was a bond formation within the NaOH-treated Cassava leaves. The copper (II) ions have bonded with the active sites which are the -OH groups. The alcohols, at 3291.42 cm^{-1}, has 30% decrease of transmittance. There was also a decrease in transmittance in the oxime group (1634.91cm^{-1}). Oxime can act as a chelating agent. Copper can form complexes with the oxime wherein it is bonded through the deprotonated nitrogen [23]. This explains why NaOH-treated cassava leaves perform better than the untreated in copper (II) adsorption. A paired t-test was also used in determining the significant difference of concentration. The NaOH-treated cassava leaves obtained a value of 0.027 which proves that there is a significant difference between the initial and final concentration of copper (II) within the simulated wastewater. H2O2-treated cassava leaves obtained the highest percentage copper removal of 38.67%. The observed phenomenon is expected due to the acid treatment increasing the possible adsorption sites. There is a significant difference between initial and final concentration wherein the p-value is equal to 0.008. Figure 1 shows a decrease in percentage transmittance before and after the adsorption which proves that the copper (II) ions have bonded within the active sites of the biosorbent. It can be assumed that copper (II) ions had also bonded with the aromatic hydrogen due to the formation of additional phenol. The formation of phenols can be caused by the oxidation of hydrogen peroxide with the presence of copper (II) ions.

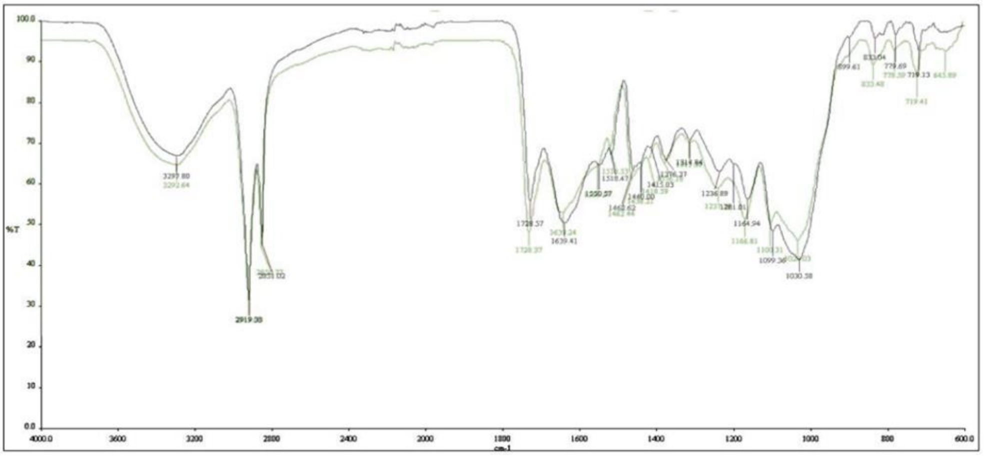

Fig 1. FTIR Spectrum of H_2O_2--treated Cassava leaves: Before and After

Copper was possibly chelated by the hydroxyl groups (-OH) for both chemically-treated cassava leaves. Metal cations can bind with hydroxyl groups due to the negative charge [13]. To further support the higher copper (II) ions adsorption capacity of chemically- treated cassava leaves, polyphenols, specifically rutin, quercetin, and hydroxyflavone, has a stoichiometry with copper of 2:1, 1:1 and 1:1, respectively [28].

5 Conclusion

FTIR analysis revealed distinct differences in the functional groups and molecular structures of untreated and chemically-treated cassava leaves. The treatment processes with NaOH and H_2O_2 resulted in noticeable changes, particularly in the intensity and presence of certain absorption peaks which indicates formation of functional groups such as hydroxyl groups. The formation of these functional groups offer more adsorption sites which yield to better copper (II) removal. H2O2-treated cassava leaves (*Manihot esculenta*) had the highest copper (II) percentage removal of 38.67% within the simulated wastewater samples. It was followed by NaOH- treated of 18.07 %. Untreated cassava leaves had the lowest which is 6.86%. Characterization of untreated and chemically-treated cassava leaves is crucial in understanding the potential mechanisms of adsorption and the effectiveness of cassava leaves as biosorbents for copper (II) removal**.**

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