ASEAN Journal for Science and Engineering in Materials

Journal homepage: https://ejournal.bumipublikasinusantara.id/index.php/ajsem

Biosorption/Precipitation of Heavy Metals by Partially Degraded Keratin/Soluble Peptides/Amino Acids By-Products of Degradation of Human Hair by Keratinase Isolated from Alcaligenes Faecalis Strain AIR10

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ABSTRACTS

This research involves a systematic process to reduce the effects of heavy metals on health and the environment through the metabolism of human hair. In this study, the by-product of the breakdown of microbial keratinase was keratin; free amino acids and partially degraded peptide/keratin were used as precipitates and adsorbents, respectively, to remove Copper (Cu), Iron (Fe), Nickel (Ni), Zinc (Zn) Cobalt (Co) and Lead (Pb) from pollution. Atomic absorption spectrophotometry is used to measure metal concentrations. Degraded human hair was an efficient but insignificant weight absorber compared to untreated human hair, achieving almost 85.81±0.0077% adsorption for chloride. Our data show that alkaline pH favors lead acetate deposited by the supernatant over neutral and acidic regions. Both treated and untreated human hair showed a significant percentage of ZnSO4 and NiCl2 biosorption, with CuSO4 showing the percentage of precipitation, the lowest percentage of biosorption. Our SEM results suggest that a technology may be required to increase the surface area of treated human hair to increase the adsorption capacity.

ARTICLE INFO

Article History:
Submitted/Received 01 Feb 2022
First revised 27 Feb 2022
Accepted 01 Mar 2022
First available online 10 Mar 2022
Publication date 01 Mar 2023

Keyword:
AIR10,
Biosorption,
Degraded keratin,
Hair.

1. INTRODUCTION

Human hair keratin, a trichophytic α-keratin protein, and a microbial keratinase biodegradable structural polymer is an intermediate filament, biochemically described as a cytoskeletal coiled-coil structural protein. These supercoiled fibrous keratin molecules are highly stable and stringency, resistant to most proteolytic enzymatic attacks, insoluble in both polar and non-polar solvents basically because of hydrophobic interactions of the coiled-coil structure, intra and intermolecular hydrogen bonding, and high degree of cross-linking by disulfide bonds (Park & Son, 2009). Disulfide bond, a functional group, formed via a reaction between the sulfhydryl (-SH) side chains of two cysteine residues catalyzed by thioredoxin or protein disulfide isomerases (Lee, 2017), provides increased stability to a protein (which is directly proportional to the number of residues within the disulfide loop), decreases further entropy that may facilitate folding progression towards the native state by limiting unfolded or improperly folded conformations. It also lowers the effective local concentration of water molecules via the formation of the nucleus of a hydrophobic core of the folded protein. The folding of a polypeptide chain to its native state is highly determined by the sequence of its amino-acid residues (Anfinsen, 1973). The loss of conformational entropy associated with folding destabilizes the native conformation. This destabilization is overcome by the hydrophobic effect, hydrogen bonds, other noncovalent interactions, and disulfide bonds (Dill, 1990).

Keratin is the major macromolecular component of human hair and the second most abundant keratinous material after feather. However, it is considered a waste material in most parts of developing countries, where it constitutes a large amount of solid waste that is either incinerated, buried, or dumped in an open space where it slowly decomposes over years, due to its resistance to attack by most common proteinase enzymes, but specifically susceptible to microbial keratinase enzymes secreted by keratinophilic microflora (fungi and bacteria). Because of the extraordinary concentration of human population in the cities, shaved human hair from barbing saloons and hairstylist shops is considered one of the major metropolitan keratin pollutants that leads to massive local pollution and environmental degradation.

As a continuation of the previous study (Obinna, 2022), to address world environmental problems posed by this solid waste material one needs to capitalize on the physiochemical properties of keratin to develop a systematic process that utilizes the waste material as a resource with an economic impact since it is completely biodegradable, renewable, and readily available. The recent increase in demand for enzymes with the industrial application has necessitated the current boom in research for keratinophilic microflora (fungi and bacteria). Keratinolytic enzymes have been studied from a vast variety of bacteria, actinomycetes, and non-pathogenic fungi. However, specialized keratinolytic enzymes produced by some microorganisms (both fungi and bacteria) can efficiently degrade keratinous waste (Sharma & Rajak, 2003; Tridico et al., 2014). Many research works have implicated the following genera of bacteria as keratinolytic bacteria capable of degrading feathers: Bacillus subtilis AMR (Mazotto et al., 2010; Nnolim et al., 2020), Strain Chryseobacterium sp. kr6 shown to be a useful microbial agent in the hydrolysis of poultry feathers and de-hairing of bovine pelts (Brandelli & Riffel, 2005), Kocuria rosea (Anand et al., 1992), Xanthomonas maltophilia Strain (De Toni et al., 2002), Pseudomonas aeruginosa SU-1 (Dhiva et al., 2020), Pseudomonas microphallus and Leuconostoc (Kani et al., 2012), Scopulariopsis brevicaulis (Sacc.) (Sharaf & Khalil, 2011), Stenotrophomonas (Jankiewicz &

DOI:
p- ISSN 2828-920X e- ISSN 2828-9951

Frąk, 2017); actinomycetes (Singh et al., 2012). Alcaligenes faecalis strain AIR10 with NCBI accession number MG835355.1.was isolated in November 2020 from soil contaminated with human hair at Toscan Barbing saloon, No 14 More House Street, Ogui, Enugu North Local Government Area, Enugu State in the South-Eastern region of Nigeria and its genome was sequenced as a part of the on-going project on biodegradation of human hair keratin and application of its by-products in both; poultry feed formulation and biosorption of toxic heavy metals from contaminated aquatic environmental.

2. MATERIALS AND METHODS

2.1. Equipment

Colony counter, Spectrophotometer, Incubator, Orbital Shaking incubator, pH-Meter, Autoclave, Centrifuge, Precision Weighing Balance, Fourier transform infrared (FTIR) spectrometer (Germany), Scanning Electron Microscopy (SEM). 12 mm Whatman filter paper, and Atomic Absorption Spectrophotometry AAS.

2.2. Reagents

Phosphate buffers, K_2HPO_4, KH_2PO_4, Sodium Chloride (NaCl), Magnesium Sulphate ($MgSO_4$) Yeast extract, Ammonium Chloride (NH_4CL), Chloroform, Methanol, Yeast extract, Peptone, Sucrose, Trichloroacetic acid TCA, $NaCO_3$, Folin Ciocalteau phenol reagent, Ninhydrin (2, 2-dihydroxyindane-1,3-dione), Cysteine, $Pb(CH_3O_2)_2$, $CuSO_4$, $CoCl_2$, $ZnCl_2$, and $FeCl_2$.

2.3. Hair substrate

Human hair substrates were obtained using the method of Mazotto et al. (2010). Human hair wastes from different male individuals were collected from local barbers' shops. The human hair samples were mixed, washed with liquid detergent, rinsed several times with distilled/deionized water, and then left to dry at room temperature (25 ^oC). The hair was cut to an approximate length of 1–3 mm using scissors. Five hundred grams of this human hair was defatted with 1000 mL of chloroform/methanol (1:1 v/v) at room temperature for 1 h with agitation (200 rev min^{-1}). The filtrate was pulled together and concentrated by evaporating the solvent using a rotary evaporator, thereby leaving behind the extracted lipid. The extracted lipid was further characterized. Afterward, the hair was washed with distilled/deionized water and dried at 70ºC for 12 hr. This was used in culture medium.

2.4. Isolation and selection of keratinolytic bacteria from a barber's shop dumping site

Human hair was collected from a temporal dumping site/waste bin from a local barbing saloon named Toscan at number 14 Moore House Street, Ogui, Enugu North, Enugu State, Nigeria. A well-calibrated 1000-mL conical flask containing 1000 mL of salt medium (K_2HPO_4 0.3g, KH_2PO_4 0.4g, NaCl 0.5g, $MgSO_4$ 0.1g, Yeast extract 0.1 g, NH_4CL 0.4 g, in 1000 mL distilled H_2O maintained within the pH range of 9 with phosphate buffer) was sterilization in an autoclave at 121 ^oC for 15 min. The salt medium was allowed to cool to room temperature after sterilization and then inoculated with 10 g of the human hair sample collected from the barber's shop contaminated with soil from the same dumping site and the hair serving as the only carbon source. This was finally incubated in an orbital shaking incubator at 37^oC for 72 h at 200 rpm. However, at the end of the incubation, aliquots of this culture were streaked on the same medium containing agar (20 gL^{-1}) to obtain different colonies within 24 h of incubation at 37^oC (Plate 1). The individual colonies were sub-cultured into different Petri

DOI:
p- ISSN 2828-920X e- ISSN 2828-9951

dishes to obtain pure cultures. The pure cultures were characterized using biochemical tests and gram staining techniques. However, the four colonies were separately transferred into different labeled 250-mL conical flasks containing 150 mL of sterilized yeast extract medium (gL^{-1}: yeast extract, 5; peptone, 5; sucrose, 20; and KCl, 20) and phosphate buffer solution with pH 9. These samples were incubated at 37^{o}C in an orbital shaking incubator at 200 rpm for 4 days, for the multiplication of microbial cells. The isolate that showed the best growth and keratin degradation were selected and identified by biochemical, physiological, and morphological characterizations (Plate 2).

2.5. Degradation of Hair using keratinase

The isolated bacteria were propagated in a yeast extract medium at 37 ºC with shaking (200 rev min^{-1}) for 4 days to obtain masses of *Alcaligenes faecalis* cells, using a modified method by Mazotto et al. (2010). The cells were harvested by centrifugation, washed thrice (2000 rpm at 20 min) with saline solution (NaCl 9.0 gL^{-1}), and used for keratinase production. However, inocula of 10^8 cells ml^{-1} of each species were introduced into different sets of five samples each of oven-dried defatted human hair medium (dried DHHM: (100) gL^{-1} w/v human hair in 500 mL of ($Na_2HPO_4.7H_2O$ and KH_2PO_4) phosphate buffer at pH 9.0 and hair medium supplemented with 0.01% yeast extract (HMY). Moreover, 1 mL of 1% sodium sulfite was added. Control hair samples were incubated in phosphate buffers without inocula/enzyme. These samples were incubated at 37^{o}C in an orbital shaking incubator at 200 rpm for 4 days, the reactions were terminated by the addition of 5 mL of 10% TCA. Then the cultures were centrifuged at (3000 rpm for 10 min). The culture supernatants were used for keratinase and protein analyses. Soluble peptides were quantified by the Lowery method after 24, 48, and 72 h of incubation. The insoluble peptides, and partially degraded keratins samples after incubation settled at the bottom of the centrifuge tubes and were termed ''InsoluPPDK'' and the clear supernatant was carefully removed and labeled ''SoluSDHY''. The residues were left to completely dry in an oven at 60ºC for 24 hr. The final weight was measured and the percentage degradation was determined by weight loss in human hair. The biosorption analyses were done in three different phases. **Phase I** consists of biosorption of heavy metal ions by InsoluPPDK'. However, since the supernatant ''SoluSDHY''is rich in both free amino acids and soluble peptides, it serves as a potential adsorbent in **phase II.** The already used residual undigested/digested defatted human hair and precipitations from the use of 'SoluSDHY'' test samples and control samples were used for both FTIR and SEM analysis. This study used equation (1).

$$\% \text{ Degradation} = (W_0 - [W_{1+n}])/W_0 * 100 \quad (1)$$

where W_0 is the weight of human hair before degradation by keratinase, W_1 is the weight of un-degraded human hair (Weight of residual dried human hair) after degradation by keratinase

As a note, this study should put into consideration the weight of *Bacillus* (n), knowing too well that Keratinase enzymes secreted by the Bacillus will be dictated as soluble peptides/polypeptides.

2.6. Determination of Keratinase activity

2.6.1. The Lowry Method

A known quantity (1g) of a defatted human hair (with size approximately ≤ 0.5 mm) was mixed with 2 mL of phosphate buffer (pH 12.5) and 10 µL of enzyme solution in test tubes.

DOI:
p- ISSN 2828-920X e- ISSN 2828-9951

The solutions were incubated for 30 min at 37 ^{o}C. After incubation, the reaction was terminated by the addition of 2 mL of 10% of TCA. The undigested keratin was removed by centrifugation at 3000 rpm for 10 min. 1 mL of the supernatant was mixed with 5 mL of 4.2% $NaCO_3$ and 0.5 mL of Folin Ciocalteau phenol reagent. The reaction mixture was precipitated by standing in ice for 15 min and the insoluble precipitate was removed by centrifugation at 1000 rpm for 10 min. The absorbance of the supernatant was read at 660 nm. A control assay without the enzyme in the reaction mixture was prepared and used as the blank in all the spectrophotometric measurements. One unit of keratinase enzyme activity was defined as the amount of enzyme that released one µg of Tyrosine per minute under standard assay conditions (Lowry*, et al*., 1951).

2.6.2. The Quantitative Estimation of Amino Acids by Ninhydrin Method

This assay is based on the fact that two molecules of ninhydrin (2, 2-dihydroxyindane-1,3-dione) react with a free alpha-amino acid to produce a deep purple or blue color known as Ruhemann's purple (Moore and Stein, 1957; Vlab.amrita.edu,.2011). A known volume (1ml) each of the clear supernatant ''SoluSDHY'' and various concentrations (2000, 1000, 500, and 250 µg mL^{-1}**)** of amino acid (Cysteine) solutions were separately mixed with 1 mL of ninhydrin reagent (8% w/v of Ninhydrin in acetone) and 4 mL of diluent solvent in separate test tubes. The pH of the reactions was maintained at 5.5 by phosphate buffer solution. These were properly mixed using a vortex machine, after which the test tubes were covered with aluminum foils, followed by incubation at 90 ^{o}C for 20 min. However, after cooling to room temperature, one milliliter (1 mL) of absolute ethanol was added and properly mixed, followed by a measurement of absorbance at 570 nm. The concentrations of amino acids in the samples were determined via extrapolation from the standard graph of Cysteine.

2.7. Phase-I

2.7.1. Biosorption of Heavy Metal Ions by InsoluPPDK'

The uptake of toxic heavy metal ions onto the hair, peptides, and partially degraded keratin was carried out by batch experiments on a rotary mixer at 200 rpm. In all sets of experiments, 2.0 g of oven dried-untreated human hair and treated (peptides and partially degraded keratin) were weighed individually into 150 mL plastic extraction tubes. Moreover, 50 mL of 0.5 mmol/L of the heavy metal ion aqueous solutions were added and the system was shaken for 24 h at 37^{o}C in an orbital shaking incubator at 500 rpm. The control sample for each element contains everything except oven-dried untreated human hair and treated (peptides and partially degraded keratin). However, after agitation, the samples were centrifuged at 3000 rpm for 10 min. The supernatant was separated by decantation and the resultant mixture at the bottom was filtered via 12 mm Whatman filter paper. The residues on the filter paper were oven-dried at 50^{o}C for 24 hours. However, dried residues on the filter papers were weighed and used for identification of the functional groups involved in metal loaded-treated and metal loaded-untreated human hair complexes using an FTIR (Germany). The residual heavy metal concentrations in the aqueous supernatant were determined by AAS. The initial pH of the aqueous solutions of these heavy metals was varied within the range of 2 to 8 (higher pH values were not considered to avoid precipitation of metal hydroxides). The effects of the initial metal ion concentration on biosorption isotherms were studied in a single-metal system with 2.0 g of a treated human hair (peptides and partially degraded keratin).

DOI:
p- ISSN 2828-920X e- ISSN 2828-9951

2.7.2. Protocol-1

The uptake of the metal ions by human hair was calculated using Equation (2), which quantifies the biosorption efficacy:

$$\% \text{ Biosorption} = \frac{\{\text{Ci} - \text{Cf}\} \times 100}{\text{Ci}} \quad (2)$$

where *Ci* and *Cf* are the initial and final concentrations of heavy metals in the aqueous phase solution, respectively (in mmol/L).

2.7.3. Protocol -2

The amount of metal sorbed per unit of mass of biosorbent at time t (quantity in mmol/g) was calculated using Equation (3):

$$\text{Quantity} = \frac{(\{\text{Ci} - \text{Cf}\} \times V)}{\text{W}} \ (mmol/g) \quad (3)$$

where *V* is the total volume of the solution (*in L*), *W* is the amount of biosorbent (*in g*), and *Ci* and *Cf* are the initial and the final concentrations of heavy metals in the aqueous phase solution, respectively (*in mmol/L*).

2.8. Phase II

2.8.1. Heavy Metal Ions Precipitation Experiments by ''SoluSDHY''

The precipitation of toxic heavy metal ions by soluble peptides and free amino acids was carried out by batch experiments on an orbital shaking incubator at 500 rpm. In all the sets of experiments carried out, 5 mL of adsorbent ''SoluSDHY'' were added into 100 mL capacity conical flasks, containing 20 mL of varying concentrations of the adsorbate/heavy metal ion in aqueous solutions. The mixtures system was shaken for 24 h at 37 ^{o}C in an orbital shaking incubator at 500 rpm and pH 10. Control samples for each element contain everything except ''SoluSDHY''. However, after agitation, the samples were centrifuged at (2000 rpm for 5 min) for the precipitates to settle at the bottom of the centrifuge tubes. The supernatants were separated by decantation and were analyzed for residual metals. To study the effect of the pH on amino acids/soluble peptides precipitation by Lead acetate, pH was varied from 2, 4, 6, 7, 8, and 10.

2.8.2. Protocol-1

The uptake of the metal ions by human hair was calculated using Equation (4), which quantifies the biosorption efficacy:

$$\% \text{ Precipitation} = \frac{\{\text{Co} - \text{Cf}\} \times 100}{\text{Co}} \quad (4)$$

where *Co* and *Cf* are the initial and final concentrations of heavy metals in the aqueous phase solution, respectively (in mmol/L).

2.8.3. Protocol -2

The amount of metal precipitated per unit of weight of 5 mL of Supernatant used at time t (quantity precipitated in mmol/g) was calculated using Equation (5):

$$\text{Quantity Precipitated} = \frac{(\{\text{Ci} - \text{Cf}\} \times V)}{\text{W}} \ (mmol/g) \quad (5)$$

DOI:
p- ISSN 2828-920X e- ISSN 2828-9951

where *V* is the total volume of the solution (*in L*), *W* is the weight of 5 mL of Supernatant used (*in g*), and *Ci* and *Cf* are the initial and the final concentrations of heavy metals in the aqueous phase solution, respectively (*in mmol/L*).

2.9. Batch mode adsorption studies

Batch mode adsorption studies for individual metal compounds were carried out to investigate the effect of different metals/adsorbates and pH. Solutions containing adsorbate and adsorbent were taken in 250 mL capacity conical flasks and agitated at 150 rpm in a mechanical shaker at predetermined time intervals. At the end of each agitation, the mixtures were centrifuged at 2000 rpm for 5 min. The supernatants were analyzed for residual metal/adsorbate using AAS.

2.9.1. Effect of adsorbates

The effects of adsorbates i.e., the sorption capacities of the untreated and treated human hairs on different metals were studied. The equilibrium time and the pH were kept constant.

2.10. Effect of pH precipitation of Lead acetate by SoluPPDK'

To determine the effect of varied pH (2, 4, 6, 7, 8, and 10) on the quantity of Pb $(CH_3O_2)_2$ precipitated and percentage precipitation of Pb $(CH_3O_2)_2$ by SoluPPDK' from lead acetate solution. However, metal ion concentrations, absorbent mass, and agitation at pre-set equilibrium time were kept constant.

2.11. FT-IR characterization of Precipitates, Biosorbents, and Biosorbent-Metal Complexes.

To understand how metal ions, precipitate amino acids/soluble peptides or bind to the biosorbent, it is essential to identify the functional groups at its surface as these could be responsible for the metal binding. Structural characterization of the precipitates, untreated, treated, metal loaded-treated, and metal loaded-untreated human hair keratin were carried out to analyze physical and chemical changes produced in the samples before and after enzymatic digestion and after biosorption of heavy metals. The identification of the functional groups in the precipitates, untreated, treated, metal loaded-treated, and metal loaded-untreated human hair keratin was performed using an FTIR spectrometer (Germany). The spectra were recorded in the range of 600–4000 cm^{-1} with 16 scans and a resolution of 4 cm^{-1}. The surface morphology of the untreated human hair sample; (oven-dried peptides and partially degraded keratin) and treated samples-heavy metal complexes were observed by SEM.

2.12. SEM Analysis

Defatted human hair test samples and control samples from the above-cultured media were over-dried at 40^{o}C and were scanned with SEM for observation of hair degradation. Hair samples were put on stubs and gold-sputtered. Images were collected on a JEOL JSM 5310 SEM operating at 15 kV.

DOI:
p- ISSN 2828-920X e- ISSN 2828-9951

3. RESULTS

The results in the agar medium are shown in **Figures 1** and **2**. **Figure 1** shows an agar medium containing different colonies within 24 h of incubation at 37^{o}C. **Figure 2** shows the pure isolate that showed the best growth and keratin degradation.

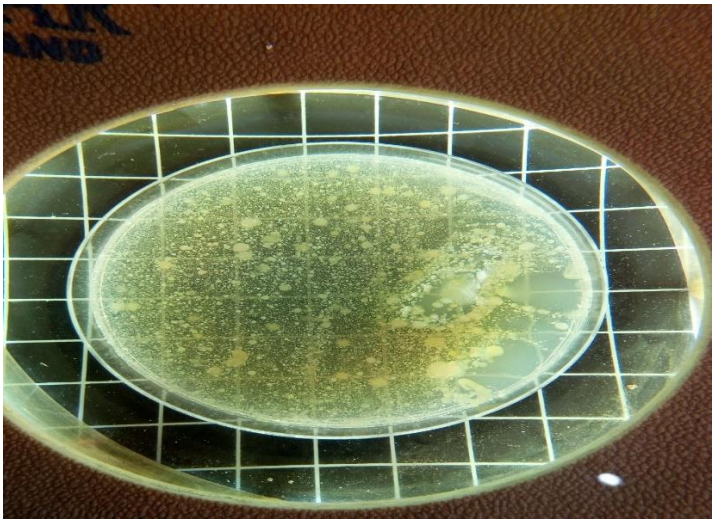

Figure 1. Agar medium containing different colonies within 24 h of incubation at 37^{o}C.

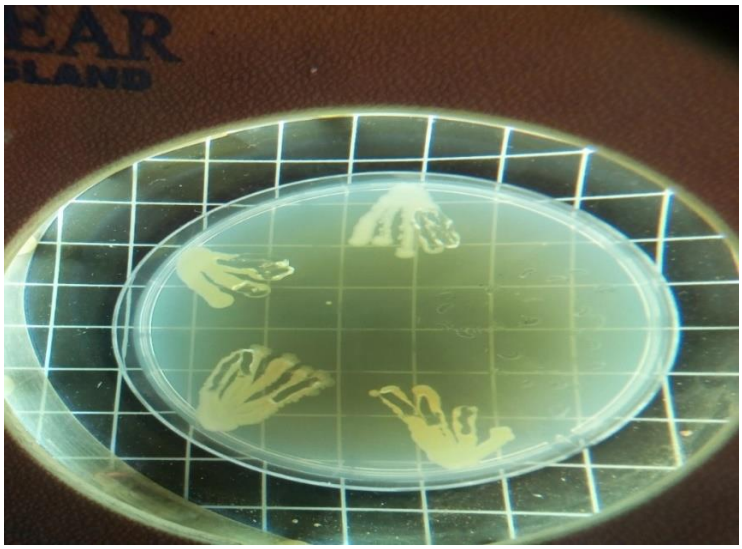

Figure 2. The pure isolate that showed the best growth and keratin degradation.

In **Table 1**, the result reveals a significant quantity of Pb $(CH_3O_2)_2$ followed by $ZnSO_4$ removed from the aqueous solution via precipitation when compared with other tested metals. Our findings further depict that both treated and untreated human hair significantly absorbed a high quantity of $ZnSO_4$ when compared with other tested metals. The result further depicts that the number of metal ions sorted by treated human hair exhibited a non-significant increase when compared with the number of metal ions sorted by untreated human hair, except $CuSO_4$ which showed a significant increase in the number of copper ions sorted by treated human hair in comparison with untreated human hair. However, $CuSO_4$ and $CoCl_2$ were the (least metal ion sorted by untreated human hair) and (least metal ion sorted by treated human hair and least precipitated by supernatant) respectively.

DOI:
p- ISSN 2828-920X e- ISSN 2828-9951

Table 1. The number of metals sorted by untreated human hair, treated human hair, and quantity of metals precipitated by supernatant.

S/N	Metals	Quantity of Metal Precipitated by Supernatant (ppm)	Quantity of Metal sorbed by Treated Human Hair (ppm)	Quantity of Metal sorbed by Untreated Human Hair (ppm)
1.	Pb $(CH_3O_2)_2$	4.926±0.0082	0.067±0.0002	0.059±0.0001
2.	$NiCl_2$	0.512±0.0001	0.035±0.0001	0.034±0.0001
3.	$ZnSO_4$	1.701±0.0002	0.114±0.0008	0.113±0.0002
4.	$CuSO_4$	0.693±0.0000	0.054±0.0000	0.004±0.0000
5.	$CoCl_2$	0.131±0.0000	0.008±0.0000	0.009±0.0000
6.	$FeCl_2$	0.313±0.0001	0.019±0.0000	0.017±0.0000

Table 2 shows that $ZnSO_4$, Pb $(CH_3O_2)_2$, and $NiCl_2$ exhibited significant percentage precipitation of the amino acid/soluble peptides in comparison with $CuSO_4$, $CoCl_2$, and $FeCl_2$. Our data indicate that both treated and untreated human hair exhibited a significant percentage biosorption of $ZnSO_4$ and $NiCl_2$ when compared with Pb $(CH_3O_2)_2$, $CuSO_4$, $CoCl_2$, and $FeCl_2$.

However, $CuSO_4$ exhibited the least percentage precipitation and percentage biosorption after treatment with untreated and treated human hair in comparison with all the tested metals.

Table 2. The percentage biosorption of metals by Untreated Human Hair, Treated Human Hair, and percentage precipitation of metals by Supernatant.

S/N	Metals	Percentage Precipitation of metals by Supernatant (%)	Percentage Biosorption of metals by Treated Human Hair (%)	Percentage Biosorption of metals by Untreated Human Hair (%)
1.	Pb $(CH_3O_2)_2$	69.69±0.1167	13.59±0.0183	12.06±0.0523
2.	$NiCl_2$	35.11±0.0089	34.55±0.0000	33.28±0.0000
3.	$ZnSO_4$	88.20±2.0611	85.81±0.0077	85.36±0.0000
4.	$CuSO_4$	7.351±0.0000	7.351±0.0000	6.246±0.1040
5.	$CoCl_2$	13.86±0.0000	12.61±0.0000	14.08±0.0520
6.	$FeCl_2$	16.59±0.0090	14.98±0.0135	12.79±0.0000

Table 3 shows that the alkaline pH of 10 exhibited a non-significant increase in both quantity of Pb $(CH_3O_2)_2$ precipitated and percentage precipitation of Pb $(CH_3O_2)_2$ by supernatant from treated human hair in comparison with other tested pH values. Our data indicate that the two alkaline pH 8 and 10 tested confirmed that percentage precipitation and quantity of lead acetate precipitated by supernatant are favored more in an alkaline region than both neutral and acidic regions.

Table 4 shows Percentage Degradation of Human Hair by Keratinase enzyme from *Alcaligenes faecalis* strain AIR10 for thirteen days.

FTIR analysis results are shown in **Figures 3-12**. **Figure 3** is the FTIR spectrum obtained for Treated Human hair fiber samples alone, in which this analysis was done in the previous study (Obinna, 2022). **Figure 4** is the FTIR spectrum obtained after biosorption of Nickel Chloride on Treated Human hair fiber samples. **Figure 5** is the FTIR spectrum obtained after biosorption of zinc sulfate on Treated Human hair fiber samples. **Figure 5** is the FTIR spectrum obtained after biosorption of copper sulfate on Treated Human hair fiber samples.

DOI:
p- ISSN 2828-920X e- ISSN 2828-9951

Table 3. the effect of varied pH on the quantity of Pb $(CH_3O_2)_2$ precipitated and percentage precipitation of Pb $(CH_3O_2)_2$ by supernatant from treated Human Hair.

S/N	Metals	Quantity of Lead acetate Precipitated by Supernatant (ppm)	Percentage Precipitation of Lead acetate by Supernatant (%)
1.	2	8.941±0.0000	99.84±0.0000
2.	4	8.941±0.0000	99.84±0.0000
3.	6	8.098±0.0002	90.43±0.0024
4.	7	8.939±0.0001	99.83±0.0001
5.	8	8.943±0.0000	99.86±0.0000
6.	10	8.945±0.0000	99.89±0.0002

Table 4. Percentage Degradation of Human Hair by Keratinase enzyme from *Alcaligenes faecalis* strain AIR10 for thirteen days.

S/N	Lab. ID	Initial Weight of Human Hair	Initial Weight of Empty Filter Paper after Oven drying	Weight of Filter Paper +Dried Degraded Human Hair	Weight of Filter paper after removal of dried Human hair	Weight of residual dried human hair	Weight of soluble peptide absorbed by Filter Paper	% Degradation	
		A	B	C	D	E = C-D	F = D-B	%D = [{A-E)/A] *100	Average
Day-1	A1	0.049	0.995	1.347	1.330	0.017	0.335	65.30612245	
	A2	0.049	0.989	1.413	1.394	0.019	0.405	61.2244898	
	A3	0.051	1.013	1.360	1.341	0.019	0.328	62.74509804	63.092±1.684
Day3	B1	0.054	0.987	1.326	1.31	0.016	0.323	70.37037037	
	B2	0.050	0.996	1.366	1.351	0.015	0.355	70.00000000	
	B3	0.049	1.032	1.387	1.372	0.015	0.340	69.38775510	69.919±0.405
Day-5	C1	0.051	1.015	1.366	1.353	0.013	0.338	74.50980392	
	C2	0.050	0.998	1.376	1.364	0.012	0.366	76.00000000	
	C3	0.051	0.989	1.373	1.359	0.014	0.370	72.54901961	74.353±1.413
Day-7	D1	0.049	1.027	1.372	1.361	0.011	0.334	77.55102041	
	D2	0.050	1.014	1.386	1.374	0.012	0.360	76.00000000	
	D3	0.051	1.009	1.392	1.381	0.011	0.372	78.43137255	77.328±1.005
Day-9	E1	0.051	1.010	1.401	1.392	0.009	0.382	82.35294118	
	E2	0.049	1.016	1.393	1.383	0.010	0.367	79.59183673	
	E3	0.05	0.984	1.402	1.394	0.008	0.410	84.00000000	81.982±1.819
Day-11	F1	0.051	0.995	1.401	1.382	0.019	0.387	62.74509804	
	F2	0.050	0.996	1.399	1.379	0.020	0.383	60.00000000	
	F3	0.050	1.017	1.395	1.374	0.021	0.357	58.00000000	60.248±1.945
Day-13	G1	0.049	1.013	1.387	1.362	0.025	0.349	48.97959184	
	G2	0.048	0.996	1.407	1.381	0.026	0.385	45.83333333	
	G3	0.050	1.014	1.421	1.395	0.026	0.381	48.00000000	47.604±1.314

DOI:
p- ISSN 2828-920X e- ISSN 2828-9951

Figure 6 is the FTIR spectrum obtained after biosorption of Colbert Chloride on Treated Human hair fiber samples. **Figure 7** is the FTIR spectrum obtained after biosorption of Iron Chloride on Treated Human hair fiber samples. **Figure 8** is the FTIR spectrum obtained after biosorption of Mercuric Chloride on Treated Human hair fiber samples. **Figure 9** is the FTIR spectrum of Precipitates obtained after precipitation of Cobalt Chloride by supernatant from treated Human Hair. **Figure 10** is the FTIR spectrum of Precipitates obtained after precipitation of lead acetate by supernatant from treated Human Hair. **Figure 11** is the FTIR spectrum obtained after biosorption of Lead Acetate on Untreated Human hair fiber samples. **Figure 12** is the FTIR spectrum obtained after biosorption of Cobalt Chloride on Untreated Human hair fiber samples.

The SEM analysis is shown in **Figures 13-17**. **Figure 13** is the SEM image of the Untreated Human hair fiber sample. This figure was adopted from the previous study (Obinna, 2022). **Figure 14** is the SEM image of the treated human hair fiber sample. **Figure 15** is the SEM image obtained after biosorption of Lead Acetate on Treated Human hair fiber samples. **Figure 16** is the SEM image obtained after biosorption of Lead Acetate on Treated Human hair fiber samples. **Figure 17** is the SEM image obtained after biosorption of Lead Acetate on Untreated Human hair fiber samples.

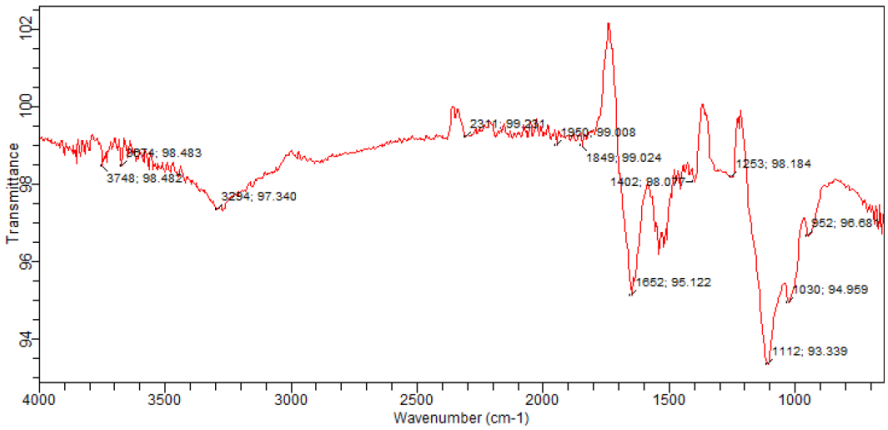

Figure 3. The FTIR spectrum obtained for Treated Human hair fiber samples alone. The figure was adopted from the previous study (Obinna, 2022).

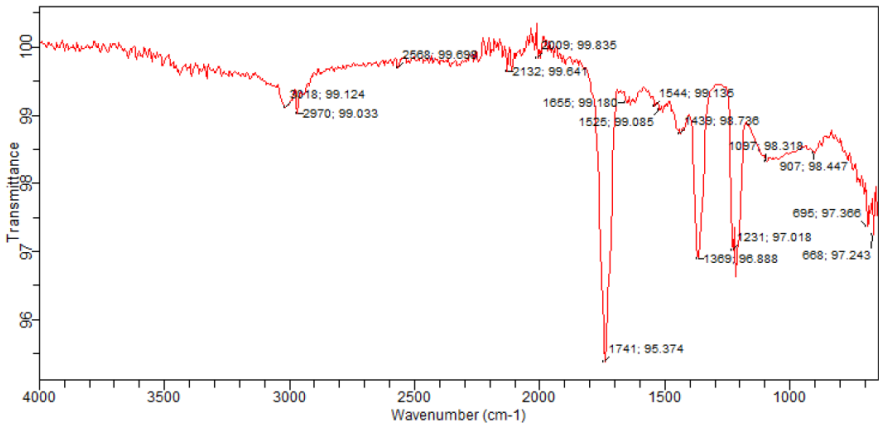

Figure 4. The FTIR spectrum obtained after biosorption of Nickel Chloride on Treated Human hair fiber samples.

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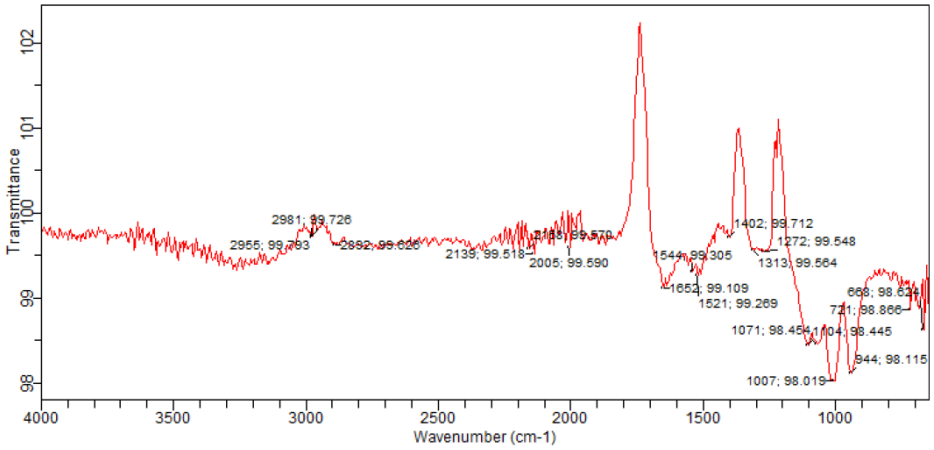

Figure 5. The FTIR spectrum obtained after biosorption of zinc sulfate on Treated Human hair fiber samples.

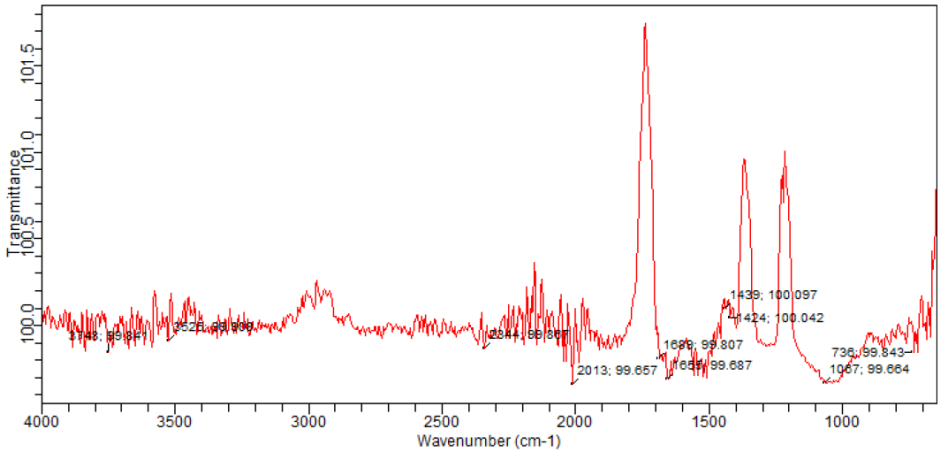

Figure 5. The FTIR spectrum obtained after biosorption of copper sulfate on Treated Human hair fiber samples.

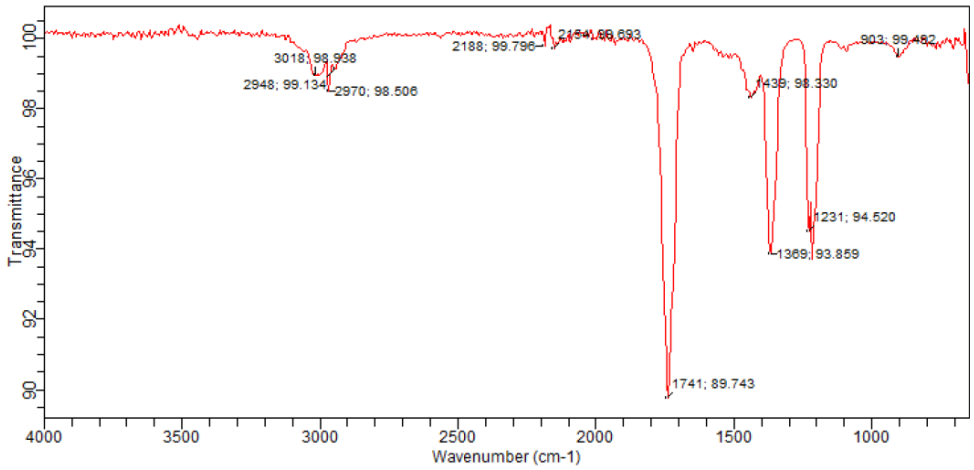

Figure 6. The FTIR spectrum obtained after biosorption of Colbert Chloride on Treated Human hair fiber samples.

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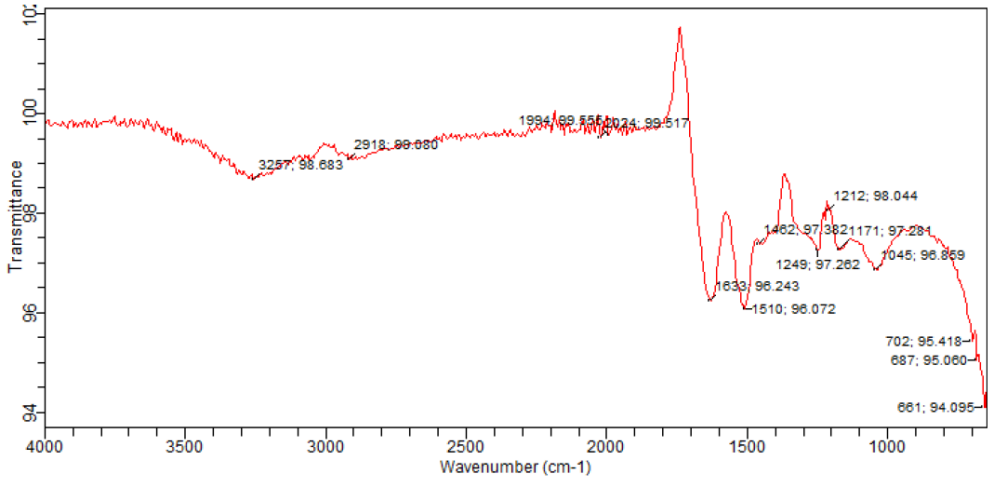

Figure 7. The FTIR spectrum obtained after biosorption of Iron Chloride on Treated Human hair fiber samples.

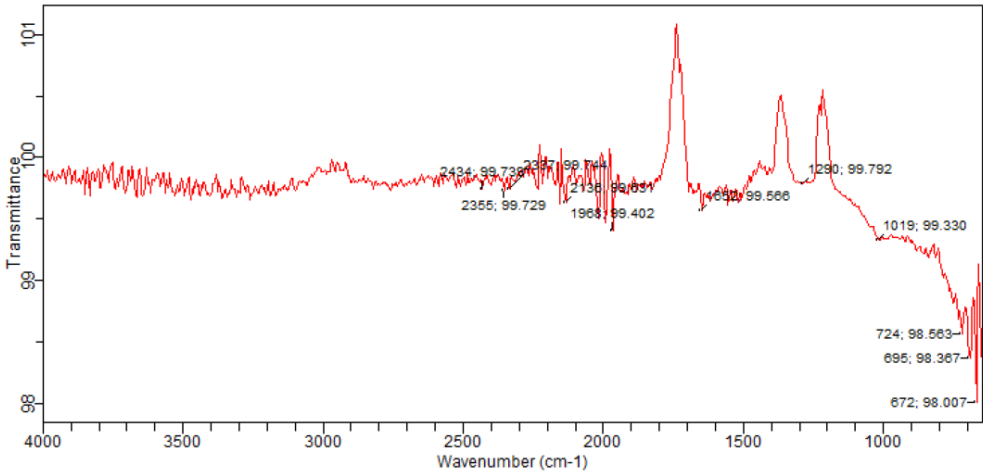

Figure 8. The FTIR spectrum obtained after biosorption of Mercuric Chloride on Treated Human hair fiber samples.

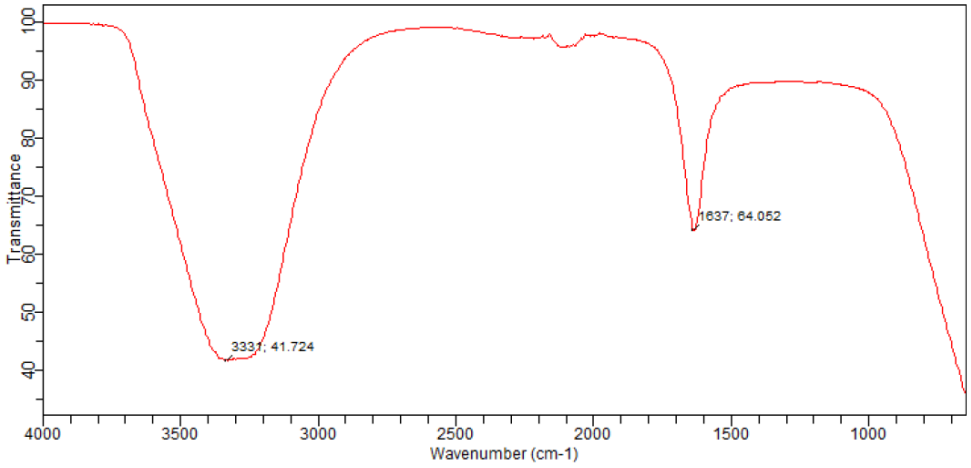

Figure 9. The FTIR spectrum of Precipitates obtained after precipitation of Cobalt Chloride by supernatant from treated Human Hair.

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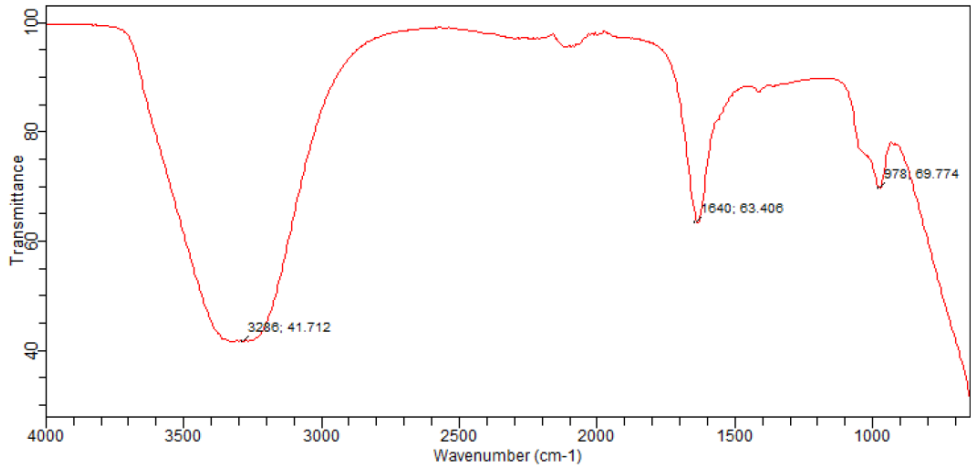

Figure 10. The FTIR spectrum of Precipitates obtained after precipitation of lead acetate by supernatant from treated Human Hair.

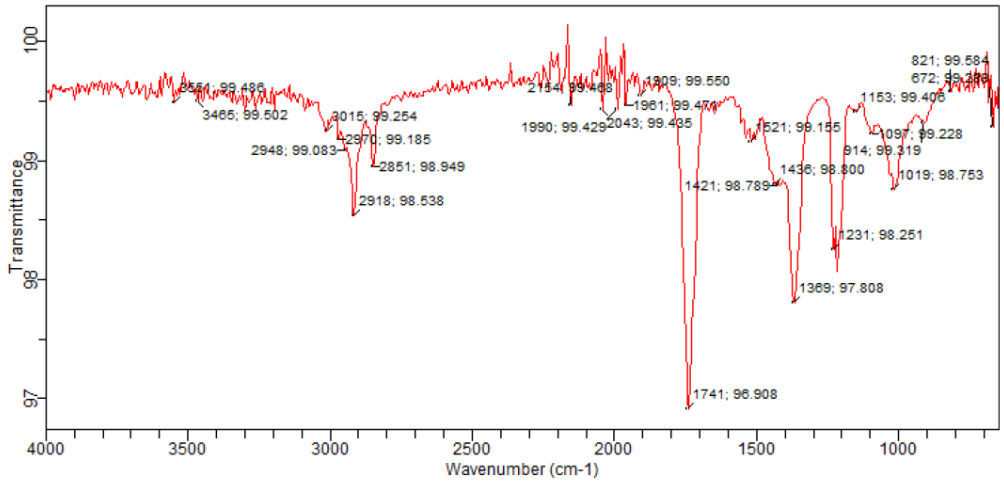

Figure 11. The FTIR spectrum obtained after biosorption of Lead Acetate on Untreated Human hair fiber samples.

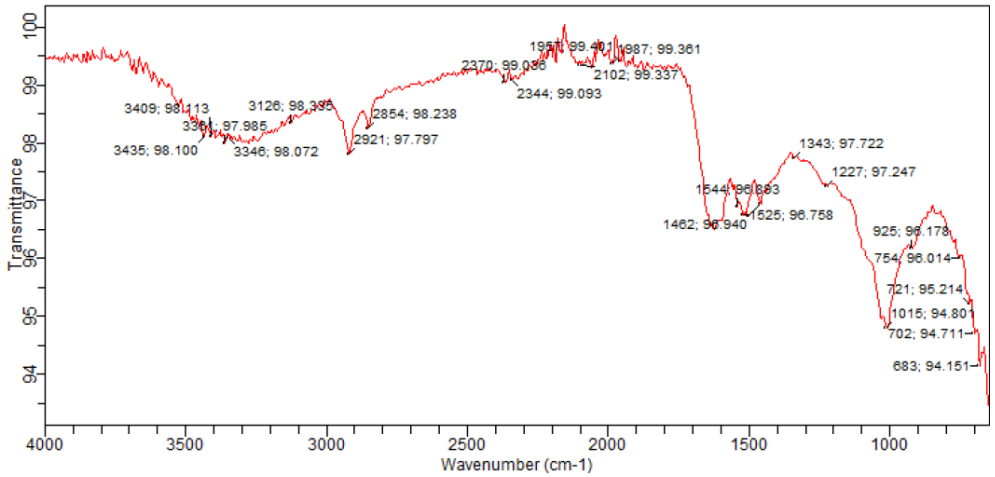

Figure 12. The FTIR spectrum obtained after biosorption of Cobalt Chloride on Untreated Human hair fiber samples.

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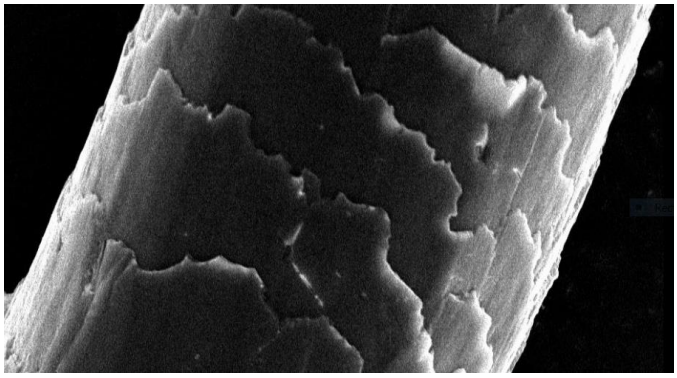

Figure 13. The SEM image of the Untreated Human hair fiber sample. The figure was adopted from the previous study (Obinna, 2022).

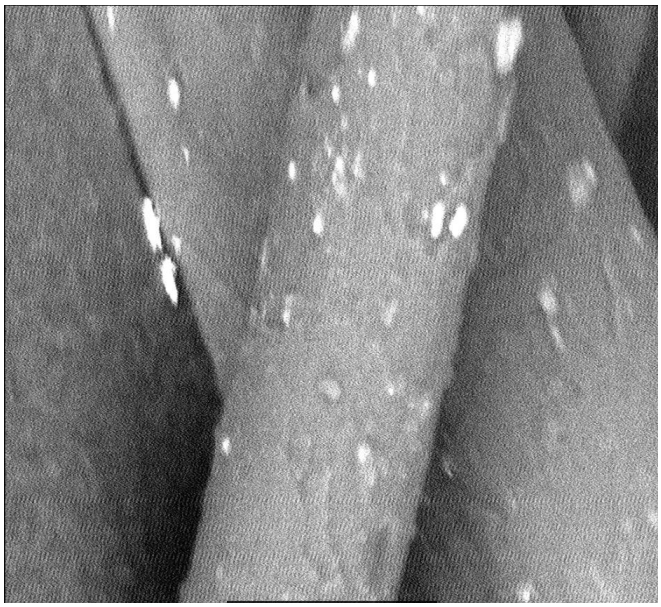

Figure 14. The SEM image of Treated Human hair fiber sample.

Figure 15. The SEM image obtained after biosorption of Lead Acetate on Treated Human hair fiber samples.

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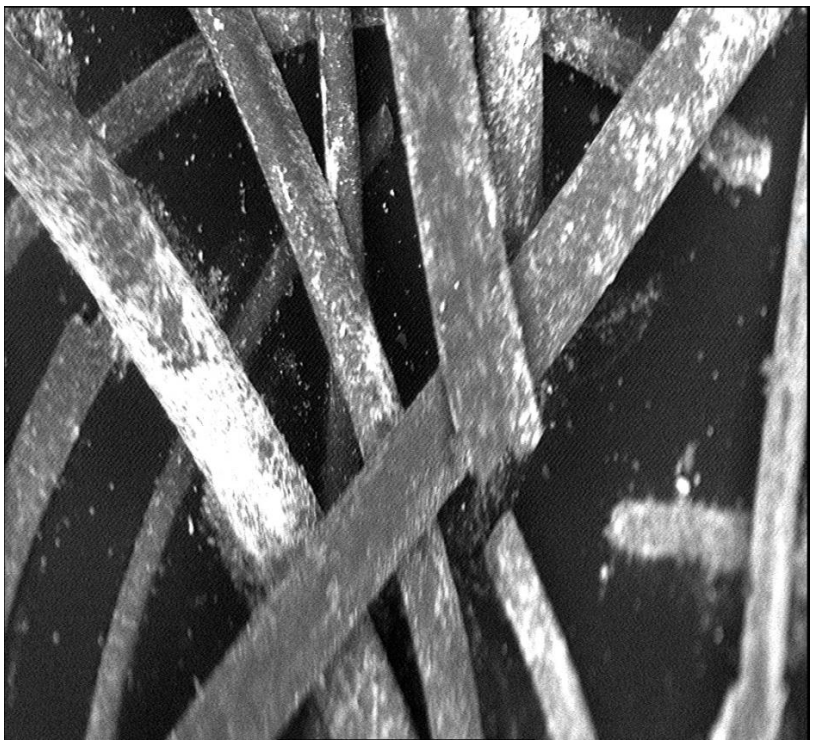

Figure 16. The SEM image obtained after biosorption of Lead Acetate on Treated Human hair fiber samples.

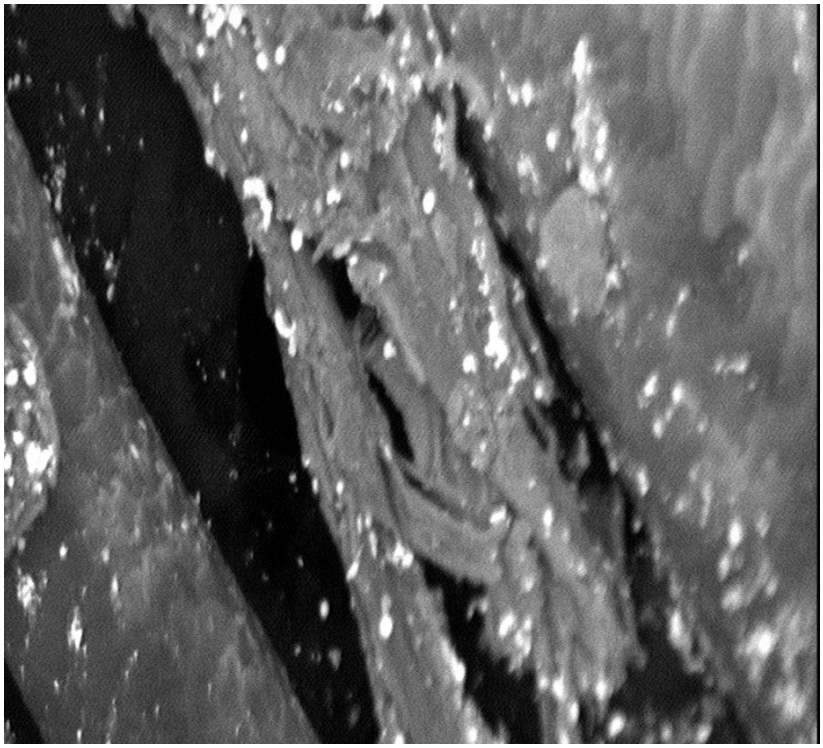

Figure 17. The SEM image obtained after biosorption of Lead Acetate on Untreated Human hair fiber samples.

4. DISCUSSION

The level of heavy metal contamination in our environment and human diets is one of the major health indices used by health regulatory bodies to monitor both water and food safety.

DOI:
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These are essential components of human health necessary for good quality of life, viewed by the World Health Organization (WHO) and International Organization for Standardization (ISO) as consumers' fundamental human rights, which should influence world economic and political decision making and should be a major concern to researchers, government, and health officers. This present research work indicates that indeed, human hair, globally regarded as a bio-waste that is composed of lipids and a major keratinous protein (Mendonça et al., 2019), is a very efficient adsorbent of heavy metal ions in wastewaters. Keratins are rich in cysteine residues, an amino acid with a thiol sulfide atom that is protonated (thiolate) when involved in metal coordination, is a good ligand for Fe, Cu, and Zn as well as for toxic metals such as Pb, Cd, and Hg. This is consistent with our results that depict that treated human hair exhibited a non-significant increase in both percentage and quantity of heavy metals sorbed concerning the untreated human air. This finding is further justified by Giuseppe, et al., who reported that the high –SH (cysteine) content of the protein (-thionin) leads to the occurrence of several divalent metal ions (Fe, Co, Ni, Cu, Zn, Ag, Cd, Hg, Pb) in the isolated metallothioneins (Genchi et al., 2020).

Disulfide bond, a functional group, provides increased stability to a protein, which is directly proportional to the number of residues within the 'disulfide loop'' and further lowers the effective local concentration of water molecules via the formation of the nucleus of a hydrophobic core of the folded protein (Klink, et al., 2000). The cleavage of disulfide bonds in biomolecules by keratinase results in the collapse of the native conformation and biological function (Wiedemann et al., 2020), leading to increase hydrophilicity and exposure of the two thiol groups for absorption of metal ions in digested keratin. This may be one of the reasons treated human hair keratin as observed in this current research work absorbs more heavy metal ions than an untreated human hair. The above facts are further supported by the findings of Giuseppe, et al., 2020 who reported that the critical targets of toxic heavy metal cadmium are the thiol groups (–SH) of cysteines present in proteins. Cysteine and methionine amino acids present in human hair fibers can interact with soft transition metal ions (e.g. Ag+, Au+, Pt2+, Pb2+, and Hg2+) through sulfur donor atoms in an aqueous solution forming metal complexes on the surface of hair fibers.

Heavy metal ions precipitate proteins from their solutions by cross-linking free amino groups, carboxylate groups, and side chains of acidic and basic amino acids. Protein precipitation occurs in a stepwise process. The addition of a precipitating agent (metal ion) and steady mixing destabilize the protein solution, thereby causing the precipitant and the target product to collide. Enough mixing time is required for molecules to diffuse across the fluid and creates an electrostatic or covalent interaction with the peptide structure. Since salts are ionic, they disrupt salt bridges in proteins. The reaction of a heavy metal salt with a protein usually leads to an insoluble metal protein salt.

The observed non-significant increase in percentage precipitation in this study indicates that the highest percentage precipitation of metabolic end-products of keratin degradation occurred at an alkaline region with a pH of 10, which is slightly above the isoelectric point of our purified keratinase. Our data indicate that the two alkaline pH 8 and 10 tested confirmed that percentage precipitation and quantity of lead acetate precipitated by supernatant are favored more in an alkaline region than both neutral and acidic regions. On the alkaline side of isoelectric pH, protein dissociates as protein anion (Pr-) which combines with a positive metal ion (cation) to form an insoluble precipitate of metal proteinate (Putnam & Neurath, 1944).

DOI:
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The surface charge of a protein, regulated by hydrolysis of metal salts, binding of multivalent ions at the protein surface, and change in pH of the protein solution, is the fundamental determining factor of functionality and stability of a protein in an aqueous environment/biological system resulting in transient protein-protein, protein-metal and protein-ligand interactions that accurately define the interactome. Protonation and deprotonation modulate the charge state of basic and acidic amino acid side chains residues as well as the protein termini (carboxy and amino terminus).

5. CONCLUSION

Our data show that alkaline pH favors lead acetate deposited by the supernatant over neutral and acidic regions. Both treated and untreated human hair showed a significant percentage of $ZnSO_4$ and $NiCl_2$ biosorption, with $CuSO_4$ showing the percentage of precipitation, the lowest percentage of biosorption. Metal ions absorbed by treated human hair showed an insignificant increase compared to untreated human hair, except for CuSO4 which showed a significant increase in the amount of Cu absorbed by treated human hair. Our SEM results suggest that a technology may be required to increase the surface area of treated human hair to increase the adsorption capacity.

6. AUTHORS' NOTE

The authors declare that there is no conflict of interest regarding the publication of this article. Authors confirmed that the paper was free of plagiarism.

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