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Synthesis and Application of FeCu Bimetallic Nanoparticles in Coal Mine Wastewater Treatment

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Abstract: Wastewater treatment has become a global challenge with wastewater treatment cost fast increasing. Industrial processes such as downstream processes, wastewater treatment, and several fermentation processes depend largely on the use of flocculants. Synthetic flocculants, which are conventionally used in wastewater treatment, are hazardous to the environment and are carcinogenic to human health. Therefore, bioflocculants can be used as an alternative due to their biodegradable and environmentally friendly nature. However, low efficacy hinders their industrial application. This necessitates the need for a new technology to combat wastewater treatment challenges. Nanotechnology provides the platform to explore the possible solutions to these problems. The combination of two different metals results in the formation of bimetallic nanoparticles (BNPs). Due to better properties, bimetallic nanoparticles have attracted huge attention as compared to monometallic nanoparticles from both technological and scientific views. Iron copper bimetallic nanoparticles (FeCu BNPs) were successfully stabilized by bioflocculant and used in the coal mine wastewater treatment. Infrared spectrometric analysis showed the presence of carboxyl (COO[−]), hydroxyl (−OH), and amino (−NH₂) functional groups. SEM images showed irregular and crystalline like morphology. Meanwhile, TEM analysis revealed chain like agglomerated nanoparticles. FeCu BNPs exhibited a wide pH stability range from 3, 7, and 11 with 99% flocculation activity at pH 7 and at lowest dosage of 0.2 mg/mL. After treating wastewater, the FeCu BNPs could remove pollutants such as phosphate, sulfate, calcium, chemical oxygen demand (COD), and biological oxygen demand (BOD) with phosphate having the highest removal efficacy of 99%.

Keywords: bimetallic nanoparticles; flocculation; pollutants; removal efficiency; wastewater

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1. Introduction

The most common sources of environmental pollutants among others is waste discharge and industrial effluents. Generally, where coal mine activities are common, large amounts of wastewater are generated during coal processing resulting in water resource pollution. A vast number sulfide mineral such as pyrite ore (FeS₂) is exposed to water, air and microbes during mining and generate acid mine drainage (AMD) [1] and other pollutants such as sulfur, phosphate, COD, and BOD [2]. Any water contaminated by organic pollutants, industrial effluent, bacteria, and microorganisms or any compound that deteriorate its initial quality is defined as wastewater and can be subdivided into domestic and industrial [1].

Chemical flocculants have been used extensively in pollutants removal in wastewater. This is due to their effectiveness at low dosages, long shelf life, and low cost [3]. Even though the chemical flocculants exhibit all these remarkable capabilities, in some developed countries these flocculants were banned due to the detrimental effect they possess to human, animals, and the environment [4]. The chemical flocculant shortcomings among others, include non-degradability and toxicity [5]. Recently, most emphasis is given to naturally

occurring flocculants called “bioflocculants” as alternative agents to remove pollutants in wastewater, particularly biological oxygen demand, chemical oxygen demand, and sulfur. Bioflocculants are macromolecular polymers secreted by microorganisms during metabolic processes. They may be proteins, carbohydrates, nucleic acids or lipids [6]. In the removal of heavy metals, microalgae, and flocculating inorganic solid suspensions, bioflocculants have been effectively used [7,8].

Due to their environmental friendliness and mostly nontoxic nature these bioflocculants have gained the attention of the researchers [6]. However, the natural flocculants are not as effective as the chemical flocculants; they are expensive to produce, have low efficacy, and low yield [9]. To improve the effectiveness of bioflocculants in wastewater treatment nanotechnology is viewed as a possible solution.

Most researchers have drawn their attention in recent years towards the nanotechnology. This new field of interest plays a vital role in many technological fields due to the predefined superstructures [10], these predefined structures come in different forms, such as monometallic, bimetallic, and trimetallic [11]. Trimetallic and bimetallic materials are of enormous interest than that of monometallic because of their properties can be tailored better and offer more surface area than that of single metallic [12]. Despite a weaker reduction ability, iron possesses many prominent advantages in water treatment application, including oxidation and precipitation (in the presence of dissolved oxygen), low cost, including excellent adsorption properties [13,14], while copper has been known to possess antimicrobial effects and has been used in wastewater treatment [15].

There are various techniques that can be used in the synthesis of bimetallic nanoparticle. El-Shall et al. [16] reported on a method which involves chemical reduction precursors under microwave irradiation. The objective of this study was to use a greener approach to synthesize FeCu bimetallic nanoparticles by a polysaccharide bioflocculant and evaluate its potential application on coal mine wastewater.

2. Materials and Methods

2.1. Bioflocculant Production Media

Sigma-Aldrich (St Louis, MO, USA) was used to procure all chemicals reagents and media used. The standard production medium prepared according to Zhang et al. [7] composed of glucose (20.0 g), KH_2PO_4 (2.0 g), K_2HPO_4 (5.0 g), $(\text{NH}_4)_2\text{SO}_4$ (0.2 g), NaCl (0.1 g), $\text{CH}_4\text{N}_2\text{O}$ (0.5 g), MgSO_4 (0.2 g), and yeast extract (0.5 g) in a liter of the filtered seawater. The media were autoclaved at 121 °C for 15 min.

2.2. Source of Bacterium

The organisms used for bioflocculant production was previously isolated from Sodwana Bay in KwaZulu Natal Province, South Africa. The organism was identified as *Alcaligenes faecalis* HCB2 through 16s rRNA and has been stored at 20% glycerol at 80 °C at the University of Zululand, Department of Biochemistry and Microbiology. Nutrient agar (NA) was used to revive the organism prior being used.

2.3. Bioflocculant Extraction and Purification

Prior to extraction, the organism was incubated at 30 °C in a shaker at 165 rpm for 72 h using the above-described production media. Extraction and purification was conducted using the procedures described by Zhao et al. [17] with slight modification. One liter of production medium was prepared and sterilized using an autoclave. After 72 h of fermentation, the fermented broth was centrifuged at 8000 rpm, 4 °C for 30 min to remove bacterial cells. The supernatant was mixed with one volume of distilled water and centrifuged again at 8000 rpm, 4 °C for 15 min to remove insoluble substances. Two volumes of ethanol were added to the supernatant, stirred and left to stand at 4 °C overnight. The supernatant was discarded and the precipitate was vacuum-dried to obtain the crude biopolymer. The crude product was then dissolved in distilled water and mixed with one volume of chloroform/n-butyl alcohol (5:2 v/v). After stirring, the mixture was left

to stand at room temperature for 12 h. The upper phase was separated, centrifuged at 8000 rpm for 15 min at 4 °C the supernatant was dialyzed against distilled water overnight. The dialysate was then vacuum-dried to obtain a purified biofloculant.

2.4. Synthesis of FeCu Bimetallic Nanoparticles

To synthesize bimetallic nanoparticles, firstly monometallic nanoparticles were synthesized separately in accordance to a method described by Dlamini et al. [18] where 3.0 mM iron sulfate and copper sulfate solutions were prepared using distilled water separately. Followed by 0.5 g of pure biofloculant addition in both the solutions, agitated for 5–10 min in a shaking incubator at room temperature and left to stand for 24 h at room temperature. Formed precipitate was collected by centrifugation at 8000 rpm, 4 °C, for 15 min. Solutions of copper sulfate and iron sulfate were used as a control without the addition of biofloculant. Physical observation and various characterization techniques were used to ascertain the formation of nanoparticles. After which, different volumes of FeNPs, 10, 20, and 30 mL was mixed successively with a solution of CuSO₄ (0.003 M) in glucose (6.0 mL). Precipitates were collected through centrifugation at 15,000 rpm at 4 °C for 30 min after the reaction was allowed to continue for 20 min.

2.5. Characterization of FeCu Nanoparticles

A scanning electron microscope equipped with an elemental detector (SEM-Sipma-VP-03-67) was used to detect the morphology and the elemental composition. While the functional groups present in the sample were verified and confirmed by Fourier Transform-Infrared (FT-IR) spectroscopy analysis (Tensor 27, Bruker FT-IR spectrophotometer, Gauteng, South Africa). A transmission electron microscope (JEOL USA, Inc., Peabody, MA 01960, USA) was used to obtain images of the nanoparticles. Micropipettes were used to prepare specimens, in which a diluted drop of suspension was placed on a formvar coated copper grid having 150 mesh size. The samples were left to completely dry at room temperature. Samples were viewed at 100 kV as the accelerating voltage. The images were captured digitally using a Megaview III camera, stored and measured using Soft Imaging System iTEM software. A Bruker D8 Advance diffractometer (Bruker, Gauteng, South Africa) equipped with Cu-K α radiation ($\lambda = 1.5406 \text{ \AA}$) was used to obtain diffractor patterns and the patterns were recorded at room temperature by placing dry powder of the sample on the sample holder, with 40 kV and 40 mA operating conditions.

2.6. Flocculation Activity Determination

To determine the flocculating activity, a method was used in accordance with the method previously described by Kurane et al. [19] with minor modifications was used. A hundred mL of kaolin clay suspension (4 g/L), 3 mL of 1% CaCl₂ and 2.0 mL of 0.2 mg/mL of FeCu nanoparticles were added in a 250 mL flask. The mixture was shaken vigorously for 60 s and thereafter poured into a 100 mL measuring cylinder, which was then allowed to stand for 5 min at room temperature. A UV-Vis spectrophotometer was used to measure the optical density of the clarifying supernatant and control, which was, deionized water at 550 nm. The optical density (OD) of the clarifying solution was measured in a spectrophotometer at 550 nm. The flocculating activity was determined by using the following formula:

$$\text{Flocculating activity (\%)} = \left[\frac{A - B}{A} \right] \times 100 \quad (1)$$

where A and B are the optical densities measured at 550 nm of the control and sample, respectively.

2.7. Determination of Physico-Chemical Parameters in Wastewater Samples

The wastewater samples were collected aseptically using autoclaved scotch bottles, preserved in ice, and were assessed promptly once they got into the lab. The sampling site

is Tendele coal mine, which is located in the province of KwaZulu-Natal, South Africa, and the physico-chemical parameters are shown in Table 1.

Table 1. Summary of physicochemical characteristics (mg/L) in the coal mine wastewater.

Sample Site	pH	Absorbance (680 nm)	PO ₄ ^{3−} (mg/L)	SO ₄ ^{2−} (mg/L)	Ca ²⁺ (mg/L)	COD (mg/L)	BOD (mg/L)
Coal mine	8.2	2.9	2.0	55	132	1557	123.2

2.8. Removal of Pollutants in Wastewater by FeCu Nanoparticles

Tendele coal mine wastewater samples were used during experimental modelling. The mine is located in KwaZulu Natal province, South Africa. Different pollutants, such as COD, BOD, phosphate, sulfate, and calcium were evaluated using test kits. To measure COD, Supelco COD Cell Test, Merck was used following the manufacturer's protocol, and BOD was evaluated using Supelco BOD Cell Test, Merck. While the Supelco Phosphate Cell Test, Merck was used for phosphate in accordance with the protocol of the manufacturer. Lastly, sulfate and calcium were evaluated using Supelco Sulfate Cell Test, Merck, and Supelco Calcium Cell Test, Merck, respectively. Removal efficacy of pollutants was measured by UV-Vis Pharo 300 Spectroquant®, Merck) at 680 nm. The following equation was used to calculate removal efficiency (RE) of the pollutants:

$$RE (\%) = \frac{C_i - C_f}{C_i} \times 100 \quad (2)$$

where C_i is the initial value and C_f is the value after the flocculation treatment.

2.9. Statistical Analysis

All experiments were done in triplicate and the standard deviation of the mean values was taken. The data were then subjected to a one-way analysis of variance (ANOVA) using Graph pad prism 6.1. A significant level of $p < 0.05$ was used. Different letters show that measurements are statistically different whilst the same letter shows no significance.

3. Results

3.1. Fourier Transform-Infrared Spectroscopy Analysis of FeCu Nanoparticles

Functional groups such as hydroxyl and amine play a significant role in the flocculation capabilities of the synthesized nanoparticles (Figure 1).

3.2. Transmission Electron Microscopy Analysis of FeCu Nanoparticles

The TEM images are shown in Figure 2a biofloculant and Figure 2b FeCu nanoparticles. The nanoparticles are agglomerated and are smaller in size compared to a biofloculant.

3.3. Scanning Electron Microscopy Analysis of FeCu Nanoparticles

Irregular and crystalline like morphology is shown in Figure 3 and elements such as oxygen and carbon were observed from the EDX spectra.

3.4. X-Ray Diffraction Analysis of FeCu BPNs Nanoparticles

X-ray diffraction patterns of FeCu BPNs nanoparticles are shown in Figure 4. Strong and characteristic crystalline peaks are observed between 30° and 65° at 2θ.

Figure 1. Fourier transform-infrared (FT-IR) spectrum of FeCu nanoparticles.

(a)

(b)

Figure 2. TEM images of (a) biofloculant and (b) FeCu bimetallic nanoparticles (BNPs).

Figure 3. SEM images of (a) FeCu BNPs (b) corresponding Energy-Dispersive (EDS) spectrum (c) biofloculant and (d) corresponding EDS spectrum.

Figure 4. X-ray diffractogram of FeCu nanoparticles.

3.5. Effect of Nanoparticles Dosage on Flocculation of Kaolin and Coal Mine Wastewater

For an optimal flocculation efficiency, 0.2 mg/mL dosage is required by the bimetallic iron-copper nanoparticles. The adequate dosage is required for the efficient flocculation process. The results showed that there is no statistical significance between (0.2 and 0.4 mg/mL) dosages for flocculation activity. The least dosage (0.2 mg/mL) was chosen for all subsequent experiment (Figure 5).

Figure 5. Effect of nanoparticles on flocculation activity. Different letters (a and b) denote statistical significance at $p < 0.05$.

3.6. Effect of Nanoparticles pH on Flocculation of Kaolin and Coal Mine Wastewater

The effect of pH on FeCu nanoparticles was investigated at (3, 7, and 11) is shown in Figure 6. The results showed that the nanoparticles flocculate effectively on all three pH values investigated.

Figure 6. Effect of nanoparticle pH on flocculation activity. Letter (a) denotes statistical significance at $p < 0.05$.

3.7. Flocculation Activity of Different Water Samples by FeCu Nanoparticles

The FeCu nanoparticles have the highest flocculation activity on kaolin clay with 99% flocculation activity followed by the coal mine wastewater sample with 85%.

3.8. Physicochemical Parameters in Wastewater Samples

The physicochemical properties of coal mine wastewater such as pH, absorbance, phosphate, sulfate, calcium, COD, and BOD are summarized in Table 1.

3.9. Pollutants Removal in Wastewater by Fe/Cu Nanoparticles

Table 2 shows the removal efficiency of pollutants from coal mine wastewater. FeCu BNPs showed high affinity for all pollutants with phosphate being the highest.

Table 2. Removal efficiency for pollutants from coal mine wastewater by FeCu BNPs at room temperature.

Flocculant	Parameter (mg/L)	Water Quality before (mg/L)	Water Quality after (mg/L)	Removal Efficiency (%)
FeCu BNPs	Phosphate	2.0 ± 0.0	0.04 ± 0.0	98
	Calcium	132 ± 0.0	8.16 ± 0.0	87
	Sulfate	55 ± 0.1	11.55 ± 0.1	79
	COD	1557 ± 0.0	124.56 ± 0.0	92
	BOD	123.2 ± 0.0	3.41 ± 0.0	97

4. Discussion

Flocculant binding capability depends on the number of functional groups in their molecular chains [6]. FT-IR characterization was conducted for the possible binding sites for stabilization of FeCu by the bioflocculant. As shown in Figure 1, the strong bend appeared at 3250 cm^{-1} is characteristics of hydroxyl group ($-\text{OH}$) and amine group ($-\text{NH}_2$). In addition, bands included asymmetrical stretching peak at 1743 cm^{-1} and symmetrical medium stretching peak at 1380 cm^{-1} indicating carboxyl group ($\text{C}-\text{O}-\text{H}$). Meanwhile the methoxyl group is observed by of stretching $\text{C}-\text{O}$ as indicated by a peak at 1022 cm^{-1} . The peaks at $734\text{--}513\text{ cm}^{-1}$ suggest the presence of saccharide derivatives. The presence of $-\text{COOH}$, $-\text{COO}^-$, and $-\text{OH}$ groups on the as-synthesized nanoparticles and H^+ and OH^- groups on the surface of colloidal particles may interact with the nanoparticle chains to form hydrogen bonds, which permit larger floc formation. The $-\text{OH}$ group plays the significant role in stabilizing the synthesis material [20]. Compared to the bioflocculant, the IR characterization spectrum of the bioflocculant showed that several noticeable vibrational changes occurred in FeCu. The results showed that FeCu nanoparticles were successfully stabilized by the bioflocculant, and N and O atoms are reactive sites responsible for the bonding [21].

The transmission electron micrographs of the bioflocculant and FeCu nanoparticles are shown in Figure 2. Aggregated bioflocculant particles can be found in Figure 2a. The FeCu BNPs formed a chain-like aggregation attributed to the magnetic attractive force as shown in Figure 2b. The TEM images revealed that the nanoparticles are highly aggregated, which could be due to the magnetic property of those compounds [22]. Agglomeration may be attributed to the interaction of the electrons of the interconnected particles [23].

Morphological changes were observed using SEM analysis as shown in Figure 3a,c. As indicated in Figure 3a, bimetallic FeCu was composed of irregular particles, while Figure 3c showed a crystalline bioflocculant. This suggested that the bimetallic nanoparticles were formed as the bioflocculant was morphologically modified. Zhang et al. [11] stated that bioflocculant surface morphology plays a significant role in the flocculation process. Poor or effective flocculation activity may be accounted for the surface morphological structure of bioflocculants. EDX analysis is shown in Figure 3b,d, as indicated, elements such as O and C are present in abundance which is evident from both figures. This is because these elements form the structure of the bioflocculant, and a number of elements bring about the stability and flexibility of the bioflocculant. From Figure 3b, it can be noted that the

presence of Fe and Cu in the sample which could not be found in Figure 3d. This further confirms the formation of nanoparticles by the bioflocculant. The other elements, Mg, Na, Ca, and K form part of the bioflocculant production media that was used for production of the bioflocculant. X-ray diffraction patterns are shown in Figure 4. Strong peaks are observed at $2\theta \sim 36^\circ$, 45° , and 65° . Crystallinity and smaller particle size are normally represented by strong peaks.

Each flocculant flocculates maximally at the optimum dosage. Below this dosage, significant bridging cannot occur between flocculants and colloids [5]. Contrary to this, beyond optimal dosage size, flocculation activity declines due to flocs being destabilized by excess flocculant. Wang et al. [24] alluded that high viscosity may result from excessive amounts of flocculant concentrations, which in turn can lead to a reduction in the settling of flocculated colloidal particles. From Figure 5, flocculation activity efficacy of FeCu nanoparticles can be assessed in kaolin clay. The optimum flocculation activity was achieved at a 0.2 mg/mL dosage. The nanoparticles flocculate effectively at low dosage (0.2 mg/mL). However, with the increase in dosage, from 0.4 to 0.6 mg/mL the results indicate that any dosage can be used as there was no statistical significance. Although 0.2 mg/mL was the lowest concentration, it was chosen as the optimal for all subsequent experiments. Moreover, the use of relatively smaller dosages reduces wastewater treatment costs [25]. The concentration of 0.8 mg/mL may not permit flocculation to happen effectively as it can be seen from the significant drop from 99% at 0.2 mg/mL to 91% at the concentration of 0.8 mg/mL. The findings are in agreement with those of Dlamini et al. [2], where they indicated that 0.2 mg/mL was the optimal dosage for flocculation activity of single metallic copper nanoparticles. Contrarily, Maliehe et al. [6] reported that 0.8 mg/mL dosage is optimum for the bioflocculant from which the nanoparticles were synthesized. This improvement is significant for cost effectiveness of the nanoparticles. Less dosage means that less material is required for a flocculation process.

According to Li et al. [21], one of the key factors affecting flocculation process is the pH of the flocculation mixture. Charge status of the flocculant and surface characteristics of colloidal particles in suspension may be altered by the pH and consequently affecting their flocculation capabilities [26]. The FeCu nanoparticles effectively flocculate in acidic, neutral, and alkaline conditions as shown in Figure 6. The highest flocculation activity was achieved at neutral pH (7) with 99% flocculation activity; however, at acidic pH (3) and alkaline pH (11), the flocculation activity was 95% and there was no statistical significance between the highest flocculation activities suggesting that the nanoparticles are effective in any environment. Both at acidic and alkaline pH the nanoparticles may have absorbed (H^+) and (OH^-) ions respectively, depending on the functional groups responsible for binding colloids in the nanoparticles. This may have slightly reduced the formation of flocs. Hence, the slightly decreased flocculation activity in this pH. However, the decrease was insignificant [27]. In another study by Jiang et al. [16] indicated that pH variation is an important factor in the removal efficacy of chromium wastewater by FeCu bimetallic nanoparticles.

Water is the crucial non-renewable resource on planet earth and is a building block of life in which all biotic components are sustained. Without water, there can be no life. Water should contain no chemicals or radioactive substances that may be harmful to human life and detrimental to the environment. South Africa is an emerging country, developing at fast rates leading to high rates of urbanization and development. High socio-economic development means that there is an increase for water supply and demand with industrial and agricultural sectors using the largest amounts of water from the three sectors, i.e., domestic, agricultural, and industrial sectors. From Figure 7, the effectiveness of FeCu BNPs was evaluated against three water samples, namely, kaolin, river water, and coal mine. Highest flocculation activity was observed in kaolin clay with 99% flocculation activity with 85%. Meanwhile, FeCu BNPs flocculated poorly against river water with just 65%. The poor flocculation activity in river water sample may be attributed to factors such as ionic strength, water pH, shear, molecular weight, slurry solids, flocculant dilution,

process conditions, and polymer type, collectively or individually. These factors have great influence on flocculation [28]. These findings suggested that the synthesized material is mostly suitable for industrial flocculation as opposed to domestic river water flocculation.

Figure 7. Effectiveness of FeCu nanoparticles in flocculating different water samples.

Sikosana et al. [29] found that in order to meet the South African regulatory discharge standard of 1 mg/L, that phosphate is one of the substances which wastewater treatment works (WWTW) have to lower. The key factor to prevent eutrophication of surface water is control of phosphorous discharged from industrial and municipal wastewater. One of the major nutrients contributing in the increased eutrophication of lakes and natural waters is phosphorus. Among other problems caused by presence of phosphorous in water is quality water problem, which in turn increases purification costs. Backer [30] found the possible disastrous effect of algal blooms on drinking water (creates dead zones in water, produces toxins that are extremely dangerous to people and animals, raises water treatment costs, and hurts industries that depend on clean water). Chemical precipitation is the current method in use for phosphate removal, which is expensive, and increases the sludge volume by up to 40% [31]. Forms of phosphorous usually found in aqueous solutions include orthophosphate, which is readily available for biological metabolism without further breakdown. The second form is polyphosphate: molecules with two or more phosphorus atoms, oxygen atoms combined in a complex molecule. Hydrolysis of polyphosphate results to orthophosphate forms and the process is usually quite slow. Usually secondary treatment can only remove 1–2 mg/L; the excess phosphorous is discharged in the final effluent, causing eutrophication [31]. From Table 2, the FeCu BPNs could remove up to 98% phosphate from coal mine wastewater.

Due to calcification of downstream processing, a high concentration of soluble calcium in industrial wastewater presents problems [32]. Apart from some bacteria and insects, calcium is a dietary requirement for all organisms. Skeletons and eye lenses of most marine organisms is built from calcium carbonate; meanwhile, terrestrial organisms require calcium phosphate for teeth and bone structure [31]. Calcium is largely responsible for water hardness and may negatively influence toxicity of other compounds. Iron shortage in limed soil may result from iron immobilizing by calcium. Water hardness influence aquatic organisms as a result of its toxicity. The results presented in Table 2 show the removal efficiency for calcium is 87%, which suggests that FeCu BPNs maybe applicable in coal mine wastewater, which in turn can solve the environmental problems associated with calcium.

Over the past two decades, sulfate concentration in water has come under increased scrutiny from regulatory authorities. Sulfate has no standard for drinking water or aquatic life in contrast to other pollutants, such as heavy metals, nitrate, and arsenic [33]. The synthesized FeCu BNPs showed some remarkable properties in terms of sulfate removal with 79% which is much higher than that of single metallic copper nanoparticles in a study conducted by Dlamini et al. [2], where the removal efficiency was 76%. The ability of FeCu BNPs to remove both suspended solids (colloids) and dissolved solids in the tested parameters may be attributed to surface morphology and functional groups present in the synthesized material [6,34].

Two simple parameters such as COD and BOD can be used to measure various compounds of organic matter containing materials. BOD is a standard test for assessing the oxygen-demand concentration of microbes to degrade organic matter over a given time period, usually five days. Meanwhile, COD is a standard test for water to consume oxygen in the form of potassium dichromate during the degradation of organic matter and inorganic chemicals such as ammonia and nitrite for a few hours [35]. Non-biodegradable organic matter in the influent that may be inefficiently degraded by the biological effluent treatment processes is indicated by high concentrations of BOD: COD ratios [36]. As demonstrated in Table 2, the FeCu BNPs was used to treat coal mine wastewater with an initial COD concentration of 1557 mg/L. After the treatment, the final COD concentration dropped to 124.56 mg/L, and the COD removal rate was 92%. Meanwhile, the initial BOD concentration was 123.2 mg/L and drop to 3.41 mg/L, which is equivalent to 97% removal efficiency. Therefore, the FeCu BNPs could be used for the removal of COD and BOD from coal mine wastewater. The results are comparable with those of Ma and Zhang [37], where it was reported that bimetallic zero-valent iron could remove up to 85% and 96.7% COD and BOD, respectively, from wastewater. Furthermore, the biofloculant from which the nanoparticles were synthesized was less active on BOD and COD removal. It could only remove up to 59% and 72% BOD and COD, respectively. These findings suggest that nanoparticles are more efficient compared to the biofloculant. Therefore, it can be deduced that the synthesized material is cost effective [6].

5. Conclusions

In this research, characterization and application of FeCu BNPs synthesized from a biofloculant were conducted. Results show hydroxyl group ($-O-H$) and amine group ($-NH_2$) were some of the functional groups revealed by FT-IR and the biofloculant formed a chain-like aggregation that are evident from TEM results, attributed to the magnetic attractive force. Meanwhile, SEM analysis revealed irregular FeCu BNPs and a crystalline biofloculant. This suggested that the bimetallic nanoparticles were formed as the biofloculant was modified morphologically. The nanoparticles flocculate effectively at low dosage (0.2 mg/mL). The highest flocculation activity was achieved at neutral pH (7) with 99% flocculation activity; however, at acidic pH (3) and alkaline pH (11) conditions, the flocculation activity was 95% and there was no statistical significance between the highest flocculation activities suggesting that the nanoparticles are effective in acidic, neutral, and alkaline pH. The synthesized material flocculated effectively in kaolin clay and coal mine wastewater with 99% and 85% flocculation activity, respectively. Furthermore, the synthesized material proved to be effective on removing COD and BOD with 92 and 97%, respectively, and has high phosphate removal with 98% efficacy. Lastly, the mechanism of action in pollutant removal should be further investigated for the synthesized material.

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