Research Article
Preparation of Copper Nanoparticles/Diatomite Nanocomposite for Improvement in Water Quality of Fishponds

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Diatomite (DA) with the main component of silica (SiO2) was chemically modified by amine groups of the coupling agent 3-aminopropyl triethoxysilane (APTES) before deposition of Cu2+ ions. The mixture of DA-APTES and Cu2+ ions in the chitosan stabilizer (1%) was added with ascorbic acid and purged oxygen by N2O for antioxidation before irradiation. Cu2+ ions were reduced to Cu 0 and aggregation into copper nanoparticles (CuNPs) on DA by an electron beam (EB). Characterizations of CuNPs onto DA were determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES), UV-Vis spectra, transmission electron micrography (TEM), Fourier transform infrared (FT-IR) spectra, and BET-specific surface area. A nanocomposite of CuNPs/DA with the porous structure of DA is capable of improving the water quality by adsorption of organic pollutants and suspended solids. Therefore, the values of chemical oxygen demand (COD) and biochemical oxygen demand (BOD5) decreased for samples of fishpond water after treatment with CuNPs/DA. Antimicrobial activity of CuNPs/DA nanocomposite against pathogenous bacteria for catfish such as Aeromonas hydrophila and Edwardsiella ictaluri was estimated.

1. Introduction

Improving water quality in a fish farm is an essential key factor for maintaining the fish’s health, growth rate, and survival. Water supplies for fishponds are usually of good initial quality. However, once the water is used for culture, its quality deteriorates. Removal of organic compounds, inorganic nutrients, and bacteria from aquaculture waste-waters not only minimizes deterioration of water quality but also allows reuse of the original water in the culture of prawn, fish, and shellfish [1]. The growth of phytoplankton in fishponds caused by excess feed and fish wastes causes an increase in turbidity and suspended solids, as well as fluctuations in dissolved oxygen, carbon dioxide concentrations, and pH. The use of common chemicals for water filtration and purification of the fishponds usually leads to residue that is unsafe for human health. Nanotechnology plays an important role in water treatment to reduce organic compounds and inorganic nutrients, as well as the pathogenic bacteria for fish or shrimp in aquaculture system [2]. It may be classified based on the nanomaterials nature into three main categories: nanoadsorbents, nanocatalysts, and nanomembranes. It is well known that metal nanoparticles such as Ag and Cu have antimicrobial properties, but some problems may limit excessive use of them as an effective bactericidal agent. Therefore, metal nanoparticles can be coated on solid substances in order to deactivate microorganisms in water treatment and control nanoparticles release for the purpose of the decrease in environmental threats [3]. The used materials for the development of nanoadsorbents include activated carbon, silica, clay materials, metal oxides, and modified compounds in the form of composites [4]. These materials have innate surface properties which influence their function as adsorbent in the solution or
substrate for location of the most atoms on their surfaces. The incorporation of smaller inorganic particles into larger polymers is also desirable in order to combine the key properties of both materials [5]. Adsorbent property of polymer will be enhanced by coordination with metal nanoparticles which have a high surface area to volume ratio, thus the reactivity with other conjugates and compounds, and hence active or binding sites and strong affinity for the desired sorbate.

Diatomite (DA) is an inorganic clay mineral that does not contain harmful components; the adsorption of pollutants is fast and easy to control, so it shows superiority compared to other methods to improve water quality in aquaculture [6]. Although traditional sorbents can eliminate impurities from water, some properties, like the limitation of antibacterial activity or the sorption capacity, can decrease their efficiency in application. Combination of antibacterial nanoparticles such as Ag, Cu, and matrix as DA has been carried out to avoid the deficiencies of traditional sorbents [7]. Coupling substrates with different external functional groups such as hydrophilicity or polarity, for example, carboxyl, hydroxyl, and amino groups, will result in excellent adsorption properties [8].

In previous studies, we discussed the synthesis of nanocomposites with DA substrates such as AgNPs/DA and CuNPs/DA. In addition, their antibacterial efficacies against pathogenic bacteria in catfish by in vitro test were estimated [9, 10]. Besides the method of irradiation, others such as reduction by chemical, microwave irradiation, and thermal reduction were also reported by many authors [11–15]. CuNPs were synthesized by the polyol method showed an inhibitory activity against bacteria such as Micrococcus luteus, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa, and fungi like Aspergillus flavus, Aspergillus Niger, and Candida albicans [11]. Reduction of copper acetate by the hydrazine hydrate agent formed the CuNPs which have exhibited a very strong antibacterial activity against microbial species—Gram-positive, Gram-negative bacteria, and the Candida albicans fungus [12]. Microwave assisted synthesis of copper nanoparticles (CuNPs) by different types of copper β-diketonates complexes with glycine as a reducing agent. The results showed that the CuNPs were of various shapes and sizes and all the CuNPs exhibited good antimicrobial activity against both Gram-positive and Gram-negative bacteria [13]. Besides, copper nanoparticles were also synthesized by thermal decomposition using copper chloride, sodium oleate, and phenyl ether as solvent agents. The antibacterial activity of copper nanoparticles synthesized by thermal decomposition showed a significant inhibitory effect against highly multidrug-resistant bacterial strains, namely Staphylococcus aureus and Pseudomonas aeruginosa [14]. Antibacterial and antifungal efficacies of Cu were known globally from the previous century when French scientists invented the Bordeaux solution for the plant protector [15]. Hence, many researches on the characterizations of copper compounds and then copper nano have been investigated for applications in agriculture and many other fields.

In Vietnam, aquaculture has been identified as a key economic branch with high export turnover. Bacteria and parasites have been reported as pathogens mainly in catfish farming in Vietnam [16]. The use of antibiotics is becoming less effective because of the drug resistance of bacteria [17]. Thus, alternative products have been investigated in which nano-material is a good candidate for sustainable environment, and our research is also directed towards that target.

In this work, we used 3-aminopropyl triethoxysilane (APTES) containing amino (–NH2) group as a binder for coordination of Cu nanoparticles (CuNPs) to the interior layers of the DA matrix. Thus, the nanocomposite of CuNPs/DA is suitable for improvement of water quality by adsorption and removal of organic compounds, inorganic nutrients, and bacteria from aquaculture water. The rejection of contaminants was evaluated by the determination of the COD and BOD values of fishpond water before and after treatment with CuNPs/DA nanocomposite. Antibacterial activity of CuNPs/DA nanocomposite was tested with infectious bacteria for Tra catfish (Pangasianodon hypophthalmus) such as Aeromonas hydrophila and Edwardsiella ictaluri.

2. Materials and Methods

2.1. Materials. Natural diatomite (10–100 μm) was purchased from Phu Yen Mineral Company in Vietnam, with a main composition over 63% SiO2, median particle size of about 10–100 μm. This material was first demineralized by hydrochloric acid 3% (v/v) and washed for the formation of neutral diatomite, before being finally dried at 105°C. Chitosan with a deacetylation degree about 80% and Mw = 1.06 × 105 was prepared as reported previously [18]. Other chemicals, including copper sulfate pentahydrate (CuSO4·5H2O), (S)-lactic acid (90%), sodium hydroxide (NaOH), 3-amino propyl triethoxysilane—APTES, potassium dichromate solution (K2Cr2O7 0.1N), and ascobic acid were of analytical grade.

The pathogenous strains of Aeromonas hydrophila and Edwardsiella ictaluri were isolated from infected water samples in Vietnamese catfish farms.

2.2. Synthesis and Characterizations of CuNPs/DA Nanocomposite. Demineralized diatomite (DA) was modified with amino silane groups by stirring the specimen in a solution of 3-aminopropyl triethoxysilane—APTES/ethanol (1% or 2%, v/v) for 1 h at room temperature. The pH of the mixed solution was adjusted to 5-6 before drying at 105°C.

A mixture of CuSO4·5H2O (20 mM) in a 1% chitosan solution was prepared before adding ascobic acid 2 mM for antioxidant of colloidal copper. The heterogeneous suspension of CuSO4 solution and modified DA at the ratio 50: 20 (v/w, mL/g) was stirred vigorously and then packed in polyethylene bag with thickness about 1 cm. The mixtures were purged oxygen by N2O and then samples were irradiated at 20 kGy under EB of UELR-10-1552 linear accelerator (Russia), 10 MeV energy. Finally, the resultant powders of copper nanoparticles—CuNPs/DA in purple were rinsed with distilled water to remove all unbound
particles from the surface, and then dried at 80°C for further experiments. Figure 1 shows the change in colour of the solution of Cu$^{2+}$ ions and CuNPs colloid as well as the powders of DA and CuNPs/DA nanocomposite.

The UV-vis spectra of the AgNPs were recorded on a Jasco V-630 spectrophotometer in the range from 200–600 nm, after CuNPs were separated from irradiated CuNO$_3$/DA suspension by sedimentation and ultra-centrifugation at 30,000 RPM. The TEM images of CuNPs in nanocomposite powder of CuNPs/DA were taken on a JEOL, JEM-1400 electron microscope at an accelerated voltage of 100 kV. FT-IR spectra were performed on a Shimadzu FT-IR 8400s instrument scanning from 500–4000 cm$^{-1}$. The Brunauer–Emmett–Teller (BET) surface area analysis was done using an NOVA Station B instrument under a nitrogen atmosphere after degassing the samples at 160°C for 2 hrs.

2.3. Estimation of Influence of CuNPs/DA Nanocomposite on Water Quality (pH, COD, and BOD$_5$) from Catfish Farm. The samples of CuNPs/DA in which DA was modified by a binder reagent of APTES were used for determination of chemical oxygen demand (COD) (5220-COD) and biochemical oxygen demand for 5 days (5210-BOD$_5$) [19].

Above nanocomposites were immersed for 24 hrs in water samples which were collected from catfish farms at a ratio of 3:1000 (w/v, g/mL). Next, a volume of 50 mL treated water was introduced in a 500 mL refluxing flask, and then slowly add 5.0 mL sulfuric acid reagent, before blending with 25 mL 0.04167 M K$_2$Cr$_2$O$_7$. After that, the mixture was digested for 2 hrs. Organic matter is oxidized by a boiling mixture of chromic and sulfuric acids. The remaining unreduced K$_2$Cr$_2$O$_7$ is titrated with ferrous ammonium sulfate Fe(NH$_4$)$_2$(SO$_4$)$_2$.6H$_2$O (FAS), using ferroin indicator to determine the amount of K$_2$Cr$_2$O$_7$ consumed. The COD values were calculated as follows:

\[
\text{COD as mgO}_2/\text{L} = (A - B) \times M \times 8000/\text{mL sample},
\]

where $A$ and $B$ are volumes (mL) of FAS used for blank (without CuNPs/DA nanocomposite) and sample, respectively. $M$ is molarity of FAS and 8000 is milliequivalent weight of oxygen $\times 1000$ mL/L.
The change in pH of fish farm water was checked before and after immersing in nanocomposites by a pH Meter. These samples were also used for determination of the BOD₅ values. Testing BOD₅ was estimated from measurement of dissolved oxygen (DO) of the sample which was seeded with organisms. The values of DO at the start of the test and after 5 days were used for determination of BOD₅ as follows:

$$\text{BOD}_5, \text{mg/L} = (D_1 - D_2) - (S)V/s/P,$$

where $D_1$–DO of diluted sample immediately after preparation, $D_2$–DO of diluted sample after 5 days incubation at 20°C, mg/L, $S$ oxygen uptake of seed, Vs—volume of seed in the respective test bottle (mL), and $P$ decimal volumetric fraction of sample used; $1/P$ dilution factor.

2.4. Antibacterial Efficacy of CuNPs/DA Nanocomposite. Antibacterial activity of resultant CuNPs/DA nanocomposite was tested with Edwardsiella ictaluri and Aeromonas hydrophila using a procedure adopted from a standard testing method [20]. Bacterial strains were cultured on nutritional medium, and then bacterial suspension was adjusted at 10⁵ CFU/mL by comparing the turbidity with the on nutritional medium, and then bacterial suspension was standard testing method [20]. Bacterial strains were cultured monas hydrophila composite was tested with

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3. Results and Discussion

3.1. Preparation and Characterization of CuNPs/DA Nanocomposite by EB Irradiation. In previous articles, we reported about the mechanism of formation and deposition of Ag nanoparticles on DA by the irradiation method [9]. Most of the preparation techniques of the metal colloids are based on the reduction of precursor metal ions in solution with a stabilizing agent.

In this work, CuNPs/DA were fabricated from the suspension including CuSO₄/chitosan solution and DA, which was coordinated with amino (–NH₂) groups by APTES. Before irradiation, the mixture was stirred vigorously for the adsorption of electrophilic Cu⁺⁺ ions on the surface and embedded in amorphous silicon dioxide (SiO₂) of DA with the nucleophilic oxide groups (SiO–). Under an electron beam, species of hydrated electrons ($e^-_{\text{aq}}$) with $E^0 (H_2O/e^-_{\text{aq}}) = -2.87V_{\text{NHE}}$ and $^1H$ radicals with $E^0 (H^+/^1H) = -2.3V_{\text{NHE}}$ were generated by water radiolysis can reduce adsorbed Cu⁺⁺ ions to Cu atoms by irradiation effects in suspension solution $E^0 (Cu^{2+}/Cu) = +0.34V$, and then aggregate into CuNPs on the DA. The processes of reduction and deposition occurred simultaneously for a few seconds avoiding sedimentation. During reduction of Cu⁺⁺ to Cu⁺ and aggregation to CuNPs, the oxidation to Cu₂O or CuO easily occurs, thus ascorbic acid was added to the suspension and oxygen was purged by N₂ for prevention of oxidation.

The UV-vis absorption spectral analysis was carried out to examine the copper nanoparticles in suspension of diatomite (Figure 2). Copper nanoparticles had an absorption peak at 574.5 nm, which is attributed to the surface plasmon resonance of the metallic copper nanoparticles. The presence and morphology of CuNPs on diatomite were examined using TEM techniques for solid. The obtained results showed a homogeneous distribution of spherical-like Cu nanoparticles dispersed in the silicon dioxide of the DA matrix (Figure 3). The CuNPs average sizes were determined from a TEM image to be about 20 nm at the dose of 20 kGy for the mixture of CuSO₄ 20 mM in 1% chitosan and DA. This result confirmed that the diatomite was coated with copper nanoparticles. The FT-IR spectra of the original DA and the CuNPs/DA nanocomposite are shown in Figure 4. The FT-IR spectrum of the DA which contains silica has a broad band between 2800–3800 cm⁻¹ and the one at 1605 cm⁻¹ are respectively to the –OH stretching and bending vibrations of physically adsorbed water molecules [21]. After addition of the amino silane agent—APTES to DA, the appearance of a peak at 3000 cm⁻¹ is attributed to the amino group (–NH⁻) stretching band [22]. The small absorption peaks around 600–1000 cm⁻¹ are assigned to the Si–O–Si stretch vibrations (Figure 4(a)). After deposition of CuNPs, a sharp peak appeared at 792 cm⁻¹ due to Cu–O stretching in the presence of CuNPs in the nanocomposite (Figure 4(b)) [23]. The bands located at 1119 and 1230 cm⁻¹ might be assigned to the deformation of Cu–O–Si bonds located at the interface between Cu nanoparticles and SiO₂ in DA [5].

3.2. Influence of the BET Surface Area of CuNPs/DA Nanocomposite to the Water Quality in Fishpond. There are several water remediation techniques, but adsorption is an efficient technique. The adsorption process can eliminate all
pollutant classes (organic and inorganic) without producing by-product(s) or poisonous intermediary. Thus, it has a wider applicability in removing pollutants from a water source [24].

Diatomite has a highly porous, large surface area, strong adsorption capacity, and is chemically stable, so it acts as an adsorbent and coagulant aid for pollutants encountered in water. The use of diatomite as a template is advantageous because it improves the removal efficiency of algae, turbidity, and dissolved organic matter in raw water [25]. The essential factor for adsorption of material can relate the surface area. Nanomaterials are effective adsorbents because they possess a very large surface area and multiple sorption sites [7].

In this work, the surface area of DA and nanocomposite powders was studied by BET analysis. A specific surface area of 1.011 m²/g was obtained for a pure DA sample prepared without the addition of CuNPs. In Table 1, the signs of CuNPs/DA1 and CuNPs/DA2 were abbreviations for CuNPs/DA nanocomposites including modified DA by the APTES binder at concentrations of 1.0% (DA1) and 2% (DA2) (v/w), respectively. After loading CuNPs onto DA, the sample had a specific surface area of 1.112 m²/g. In addition, the modified samples by APTES exhibited specific surface area of 2.056 and 2.509 m²/g for CuNPs/DA1 and CuNPs/DA2, respectively, which was attributed to the uniform distribution of CuNPs on DA. The results also confirm that the amino silane agent of APTES with −NH₂ groups on DA enhances the coordination of Cu on DA, and thus the number of Cu nanoparticles on the surface of diatomite and the specific surface area increase with an increase of APTES binder. The related research also indicated the effect of nanoparticles on the BET surface area of DA. Debin et al. reported that the specific surface area of modified diatomite by SnO₂ nanoparticles was higher than...
Diatoms [26]. The selection of a suitable template as diatomite is crucial in controlling the uniform contribution of nanoparticles and improving the specific surface area of the substrate.

According to the results in Table 1, the CuNPs contents in the nanocomposite decreased insignificantly after immersion of CuNPs/DA for 24 h. The outside copper nanoparticles were possibly formed by physical adsorption so that they were removed easily by immersion in water. On the contrary, the affinity of NH2–groups in the APTES “binder” for copper as well as for other metal nanoparticles could be due to the formation of coordination bonds between the nitrogen atoms, which are considered electron-rich elements into the metal orbital [27]. The other end of the APTES aminosilane attaches to silicon atoms in the DA through a Si–O–Si bond. Thus, the CuNPs were retained on the interior channel walls of DA through the connecting bridge of APTES. This is a significant factor determining the prolonged antibacterial efficacy of nanocomposite as CuNPs/DA [10]. Counting a suitable concentration of CuNPs for the treatment of pathogenic bacteria in fishponds is based on the ecotoxicity data for aquaculture environments. The lethal concentrations (LC50) values for 48–96 hrs of NPs in fish and invertebrates are mostly in the mg or tens of mg L−1 range [28]. These values depict the low acute toxicity for many materials in NPs as Ag, Cu, an Zn, so immediate threats to aquatic ecosystems and fisheries form NPs may be very small. In this study, the decrease in the content of CuNPs in nanocomposite after immersion for 24 hrs was determined to be 67, 60, and 64 mg/kg corresponding to CuNPs/DA, nanocomposite after immersion for 24 hrs was determined to be 67, 60, and 64 mg/kg corresponding to CuNPs/DA, and CuNPs/DA2 (Table 1). Thus, the concentrations of CuNPs released in the treated water samples were under 0.2 mg/L. This value is a safety limitation comparison with the result of other authors who reported the LC50 of zebrafish for 48 h was 1.56 mg nanocopper/L [29].

The surface charge contains Cu2+ ions adsorbed and attracted, as well as generated by CuNPs, causing an increase in the electrical conductivity (EC). However, the increase was unremarkable from 5.5 μS/cm to 7.0 μS/cm for the water of catfish farms before and after treatment by CuNPs/DA, respectively. Malhotra et al. reported the toxicity of copper to crucian carp (Carassius carassius) and tested after exposing the fishes to a copper-rich medium with conductivity 25 μS/cm that is much higher compared with the EC found in this study [30].

The result in Table 1 also indicates the change in the COD parameter of untreated water from a fish farm (RAW) in comparison with samples which were treated with DA and nanocomposites of CuNPs/DA. The COD data were recorded for samples by oxidation of organic matter in a boiling mixture of chromic and sulfuric acids for 2 hrs after immersion of samples in DA and nanocomposite. This is a reflection of higher organic substances leading to a higher COD concentration in the untreated water. The COD values of other samples were lower than those of RAW and dependent on the BET surface area and content of Cu nanoparticles in the composition. The result is the lowest value of COD (59 mgO2/L) which corresponds to the sample with the highest BET and the highest content of CuNPs in the nanocomposite. This is a reflection of the higher adsorption of organic substances in nanocomposite materials which contains higher content of CuNPs. Experimental results indicate that the CuNPs reactant in DA was highly effective in the removal of organic substances, thus the COD values decreased. In related studies, authors also ascribed the decrease in values of COD to the reduction of organic matter in wastewater from aquaculture and river water [31, 32]. The pH values of all samples ranged 6.8–7.0 indicating a neutral medium in fishpond water before and after treatment with CuNPs/DA.

The various pollutants found in water today can be broadly categorized into three kinds, viz organic, inorganic, and biological pollutants. The nanoadsorbents have been found capable of adsorbing pollutants, along with biological agents equally [33]. The biochemical oxygen demand (BOD) is a measure of the amount of organic compounds that can be biologically oxidized by the oxygen naturally occurring in water. It is necessary because the degradation of organic matter by microorganisms is a major factor in dissolved oxygen, a parameter of fundamental importance to aquaculture [34]. The experimental results on BOD parameters of RAW and samples of untreated fishpond water treated with CuNPs/DA 1% and 2% APTES were of 3.9, 2.9, and 2.6 mg/L, respectively. It is proved that the presence of CuNPs has an effect on the growth of bacteria so that there is a reduction of dissolved oxygen (DO) after 5 days of seeding microorganisms.

The removal of organic contaminants from water is an important key to water purification. The initial results of the reduction in COD and BOD values have opened a new prospect in improvement in water quality in aquaculture by CuNPs/DA. Of course further studies such as toxicity and the impact of CuNPs on ecosystem by control on concentration and release the CuNPs into environment will be carried out.

Table 1: COD and BOD parameters in fishpond water samples were treated by CuNPs/DA.

<table>
<thead>
<tr>
<th>Sample</th>
<th>BET (m2/g)</th>
<th>CuNPs content (mg/kg)</th>
<th>COD (mg O2/L)</th>
<th>BOD (mg/L)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAW</td>
<td>—</td>
<td>—</td>
<td>116 ± 12</td>
<td>3.9</td>
<td>7.0</td>
</tr>
<tr>
<td>DA</td>
<td>1.011</td>
<td>—</td>
<td>97 ± 10</td>
<td>3.7</td>
<td>7.0</td>
</tr>
<tr>
<td>CuNPs/DA</td>
<td>1.112</td>
<td>1886 ± 38</td>
<td>1819 ± 40</td>
<td>68 ± 12</td>
<td>3.2</td>
</tr>
<tr>
<td>CuNPs/DA1</td>
<td>2.056</td>
<td>1949 ± 42</td>
<td>1889 ± 39</td>
<td>63 ± 11</td>
<td>2.9</td>
</tr>
<tr>
<td>CuNPs/DA2</td>
<td>2.509</td>
<td>1961 ± 40</td>
<td>1897 ± 30</td>
<td>59 ± 11</td>
<td>2.6</td>
</tr>
</tbody>
</table>

The experimental results on BOD parameters of RAW and samples of untreated fishpond water treated with CuNPs/DA 1% and 2% APTES were of 3.9, 2.9, and 2.6 mg/L, respectively. It is proved that the presence of CuNPs has an effect on the growth of bacteria so that there is a reduction of dissolved oxygen (DO) after 5 days of seeding microorganisms.

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3.3. Antibacterial Activity of CuNPs/DA. The frequent exchange of river water in order to keep the adequate conditions for the growth of fish results in the spread of diseases between fish farms [35]. Farmers have used traditional disinfectants such as hydrogen peroxide and antibiotics such as sulfonamides and tetracyclines frequently and in large amounts to combat diseases. This can result in the accumulation of antibiotic residues in water and sludge that leads to antibiotic resistance among fish pathogens. Therefore, the metallic nanoparticles have been studied as a sustainable agent to control important pathogens from fishes, mollusks, and crustaceans [36]. The use of nanoparticle-based drugs against many pathogens is a developing field in fish medicine research [28]. The particles at nanoscale are able to easily interact with bacterial membranes and penetrate inside the cell, leading to increased permeability and disturbed respiration [11, 37]. The nanoparticles-mediated increase in reactive oxygen species (ROS) level led to noticeable lipid peroxidation, protein oxidation, and DNA degradation, finally killing the cells [38]. Copper has an excellent antimicrobial activity against a wide range of microorganisms which is greatly improved when Cu is properly nano-dispersed [39]. Raffi et al. suggested that when E. coli is treated with copper nanoparticles, changes take place in its cell membrane morphology, from normal rod-shaped to an irregular form. Some bacterial cells were discovered with shrunk, the cell wall was found destroyed and uneven, and bacterial inner vacuoles were found to appear [40].

In Vietnam, DA minerals are found in abundance in several regions which were described by Phong et al. [41]. It has been used for the treatment of shrimp and fishponds, opening a new direction for the management of water quality in aquaculture. This discovery leads to benefits for the environment and economic values [6]. If DA has been modified by the deposition of an antibacterial agent such as copper nanoparticles on it, the usable value will increase manyfold. In this study, the nanocomposites of the CuNPs embedded in DA were used for pollutants adsorption and evaluation of the antimicrobial effect against two fish pathogens Aeromonas hydrophila and Edwardsiella ictaluri. The antibacterial experiment was carried out on the sample which had a mass of CuNPs dispersed on porous diatomite of about 1900 ppm. The mixture of CuNPs/DA suspension at the ratio of 0.2 mg/10 mL (w/v) and bacteria was shaken and incubated for 3 and 24 hours. The bacterial colonies on solid nutrient agar plates were reduced completely at above concentration and virtually no growth of bacteria was observed in samples containing copper nanoparticles. However, the bacteria grew densely on the disks of untreated control and blank DA, as shown in Figure 5. The result was
A nanocomposite of CuNPs/DA was synthesized by irradiation reduction of Cu$^{2+}$ ions deposited on DA which was confirmed by the presence of CuNPs on DA by reduction of Cu$^{2+}$ ions coordinated to the surface of diatomite with the negative charges, which consequently causes the bacterial inhibitory effect.

From the initial test results for decontamination and antibacterial activity of CuNPs/DA, we hope that CuNPs can be used as a "nanodrug" in water disinfection and as a nanoweapon against pathogens in aquacultural environment.

### 4. Conclusion

A nanocomposite of CuNPs/DA was synthesized by irradiation reduction of Cu$^{2+}$ ions deposited on DA which was modified by an APTES binder. Different analytical techniques such as UV Vis spectra and TEM images confirmed the presence of copper nanoparticles on DA at 20nm in diameter. The resulting nanocomposite has a higher specific surface area (BET) than diatomite. The values of COD and BOD$_5$ reduced for watersamples in fish farms treated with a nanocomposite of CuNPs/DA. The antibacterial potential was tested against two pathogens of *Aeromonas hydrophila* and *Edwardsiella ictaluri* for the catfish to be efficient. Other factors that impact ecosystems in aquaculture and human health will be investigated further. We believe that the development of simple and low-cost hybrid antimicrobial material such as CuNPs/DA as alternatives for traditional antimicrobial agents might be promising for the future.

### Data Availability

All the data related to this work are presented in Results section along with references.

### Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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