Characterization and Antimicrobial Activity of Ionic Cupric Solution in Fish Processing Wastewater

(Pencirian dan Aktiviti Antimikrob bagi Pemcampuran Cupric Ionik dalam Air Sisa Pemprosesan Ikan)

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Abstract

An increase in fish product demands has an impact on the increment of fish processing wastewater. Wastewater volume and concentration depend on the raw fish composition, an additive used, and unit processing wastewater. Current employed disinfecting treatments are physical, chemical or combination of both methods such as chlorination, ozonation, ultraviolet (UV), chemical destabilization, and membrane process, which often high cost and generate harmful, toxic by-product(s) to human health. This paper studied the usage of copper particles to combat such problems. The copper particle was prepared through the chemical reduction method using ascorbic acid as a capping agent and copper sulfate pentahydrate is a precursor with assisted thermal hydrolysis method. Physical characterization of the copper product was studied through SEM analysis, while its chemical characteristics were observed via XRD, FT-IR. Its effectiveness was determined in terms of COD removal and biological properties were tested using fish processing wastewater from a nearby factory. Copper product was able to reduce COD at the highest concentration at 1000 ppm. The best disinfection efficiency of total coliform was found to be 38.60% by adding an 80uL ionic cupric copper solution of 1000ppm.

Keywords: Characterization, antimicrobial, ionic cupric, wastewater, fish processing, disinfection.

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INTRODUCTION

The fishery sector has for decades been playing an important role as a major supplier of animal protein to the Malaysian population. In 2005, fish for direct human consumption was about 1147978 tonnes while for animal feed and other purposes was 242 039 tonnes. From 2005 to 2014, average marine capture production had increased to 1387577 tonnes, the next 2 years this value had increased to 1574443 tonnes. While in 2017, total fishery production of the country amounted to 1.7 million tonnes, including close to 1.5 million tonnes from capture and 0.2 million tonnes from aquaculture (excluding seaweeds) (Of, 2018). The fish processing industry is export-oriented and comprises prawn processing, fish canning, and surimi-related production.

The increase of fisheries-related production has carried an environmental burden, as some of the fish processing factories discharge the untreated or partially treated effluents into the receiving streams and river, which then has resulted in water pollution problems. Lack of appropriate treatment technology, overutilized capacity, and poor maintenance of the treatment systems have contributed to pollution to nearby beaches and shores by releasing the effluent. This industry now facing high treatment costs and problems in the operation of conventional wastewater treatment plants where operational problems often relate to high organic loading, high salt content, and bad smell (Ching & Redzwan, 2017).

Wastewater from fish processing plant originated from water usage from activities of stunning of fish, grading, removal of slime, scaling activities (Ae, Vv, Ms, Sm, & Dave, 2013), ice preservation, cleaning, and grinding process (Ching & Redzwan, 2017), fuming, blanching, pasteurizing, cleaning of processing equipment and cooling / freezing of finished products (Sen, 2012), canning and smoking (Parvathy, Rao, Jeyakumari, & Zynudheen, 2017).

Wastewater volume and concentration depend on raw fish composition (different fish species can affect the total generation of solid wastewater), an additive used, and unit processing wastewater (Parvathy et al., 2017). The wastewater can be characterized by its chemical and physical properties. The wastewater has high loads of organic nutrients that originate primarily from carbonaceous compounds and nitrogen-containing compounds. The presence of high chemical oxygen demand (COD) and organic nitrogen concentrations characterizes the wastewater in fish processing (Ching & Redzwan, 2017).

The aerobic bacteria present in the wastewater break down the organic matter in the presence of oxygen leading to a considerable reduction of oxygen in the water. Overloads of nitrogen, phosphorous, and ammonia lead to pH variation, increase the turbidity of the water, and resulting in the decomposition of algae. The reduction in water oxygen content creates an anaerobic condition that leads to the release of foul gases such as hydrogen sulfide and ammonia, organic acids, and greenhouse gases such as carbon dioxide and methane (Ae et al., 2013), which potentially hazardous to the ecosystem, toxic to aquatic life in low concentrations, and also give rise to the development of obnoxious odors and unsightly scenes (Ching & Redzwan, 2017).

Rapid development in nanotechnology left a huge impact on environmental applications. Other than economic sectors such as consumer products, energy, transportation, cosmetics, pharmaceuticals, and agriculture (Menamo, Ayele, & Ali, 2017), its potential to evolve the old conventional wastewater treatment has been articulated lately (Li et al., n.d.). Several nanomaterials have caught the interest of many researchers to study their antimicrobial properties on cells and viruses. This including silver compound and silver ions, titanium dioxide, zinc oxide (Li et al., n.d.), and copper (Bogdanovi et al., 2014). It is well known that copper has wide potential against bacteria and fungi. A lot of researchers have been looking for new antimicrobial agents due to the development of antibiotic resistance and the surging of infectious diseases (Dealba-Montero et al., 2017). Metallic copper surfaces rapidly and efficiently kill bacteria, as the cells suffered extensive cell membrane damage and biochemical pathway disruption by chelating cellular enzymes and DNA damaged within minutes of exposure (Bogdanovi et al., 2014).

So, this research aimed to study the antimicrobial activity of copper nanoparticles in fish processing wastewater in an attempt to replace the existing method to disinfect effluent from the industry. The efficacy of copper nanoparticles as a biochemical treatment to reduce the number of microorganisms in wastewater samples was observed.

LITERATURE REVIEW

There are various processes available for the treatment of wastewater with the most common method such as chemical destabilization and membrane processes (Du et al., 2017). Compliance with the discharge limits might not be achieved completely even when some of these techniques are used. In this case, the biochemical treatment uses natural processes which rely on the help of bacteria in the decomposition of organic complex substances. The method is also more environmentally friendly comparing to physical or chemical treatments (Chowdhury et al., 2010).

Researchers conducted studies and found that the copper (II) ions were affecting the cell metabolism of the microorganisms present in wastewater (Ochoa-Herrera et al., 2011). Therefore, copper compounds are usually present in pesticides and algae-killing solutions in swimming pools or natural lakes. The ability of copper ions in interfering with the microbe's cell metabolism shows the antimicrobial effect clearly.

One of the main issues with membrane filtering application in wastewater treatment is the formation of biofilm (Du et al., 2017). It would cost a lot to change the membrane filter periodically so it is important to control bio-fouling on the membrane surfaces (Zhao et al., 2017). It is found that having copper ions on the membrane surface would show a significant anti-biofouling activity and extends the periodic changing of membrane filters by 30% (Ochoa-Herrera et al., 2011). The disinfection process of fish-processing wastewater using the ionic cupric copper solution is by destroying disease-causing organisms or rendering them inactive (Tay et al., 2006). It is found that the disinfection process will operate in the following sequences:

(a) Damaging the cell wall of the bacteria
(b) Altering the cell permeability of the pathogens
(c) Altering the pathogen's protoplasm colloidal nature
(d) Inhibiting enzyme activity in pathogens

The graphical process of disinfection can be seen in Figure 1.0 shown below. The functioning efficiency of the ionic cupric copper ions towards microorganisms is affected by the dosage of ionic cupric copper solutions. Ionic cupric copper ions will be assimilated by the microorganism which interferes with the microorganism's metabolic processes such as membrane permeability, respiration, digestion, reproduction, enzyme function, and even DNA. As a result, microorganisms will be unable to perform vital functions to survive.

According to a study conducted by European researchers, copper ion removal from biological wastewater treatment systems can be easily accomplished through adsorption and precipitation of copper ions (Crane et al., 2010). The potential environmental impact of copper ions is also much lower than that of chlorine ions. According to a review paper published in the same journal, approximately 33 to 98 percent copper ions can be removed from activated sludge treatment, which is far easier than removing E.coli, Salmonella sp., or even the toxic compounds formed from chlorine overdosing. Thus, instead of using expensive ultraviolet treatment or chlorination, which may have negative effects on the environment and make it difficult to remove excess ions, we can use copper ions to treat wastewater.

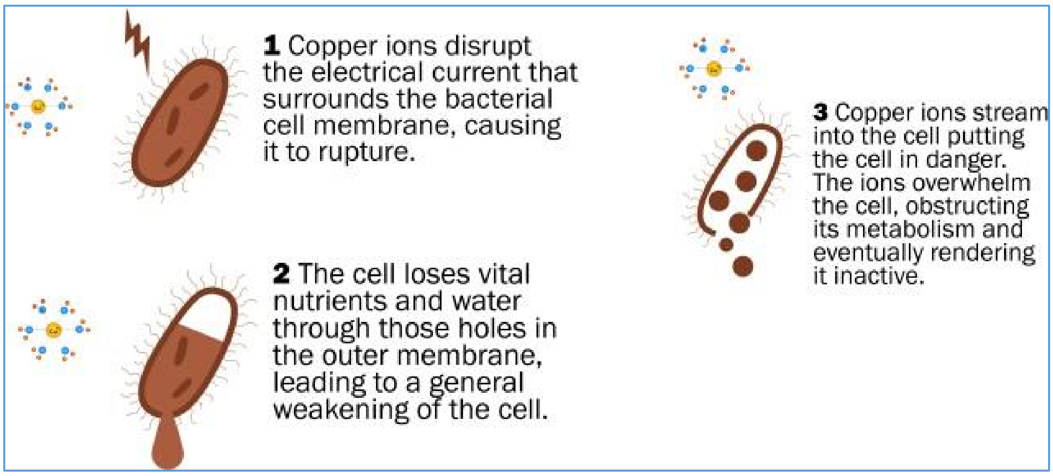

Figure 1. The mechanism of copper ions working towards bacteria.

METHODOLOGY

SYNTHESIS OF COPPER NANOPARTICLE

The copper particle was prepared by chemical reduction using ascorbic acid and copper sulfate pentahydrate with assisted thermal hydrolysis method. All chemicals were analytical grade and used as purchased without further purification. Double distilled water was employed throughout the study. The copper and ascorbic acid were prepared with the ratio of 1:2 by molarity. In this experiment, ascorbic acid acts as a capping agent that could prevent the agglomeration of small crystals (Menamo et al., 2017). A specific concentration of the ascorbic solution was added dropwise to the copper solution while heated and stirred at a slow speed to allow low agglomeration of the product. The dark green medium slowly produced the deposition of copper particles. The solution was then filtered to obtain the powder form. The product on the filter was rinsed with deionized water and absolute ethanol to remove the remaining water before being dried in the oven. It was kept in a desiccator to protect it from moisture content and oxidize (Menamo et al., 2017). It is believed that Cu2+ was reduced to Cu(OH)2 as the precursor, which then transformed to Cu2O by ascorbic acid, and finally to Cu particle (Qing-ming, Yasunami, Kuruda, & Okido, 2012).

WASTEWATER SAMPLE

Water samples were collected from the effluent treatment plant of GST Fine Food Sdn. Bhd. Located at Simpang Ampat, Pulau Pinang. The samples were aseptically collected in sterilized containers with leakproof lids, put into an icebox, and transferred to the laboratory for immediate processing within 24 hours. Sample collection and testing procedures were explained in detail at Sections 9060A and 9060B of the 18th edition of Standard Methods for the Examination of Water and Wastewater (Haller et al., 2007) to prevent any possible contamination to the water samples.

SCANNING ELECTRON MICROSCOPE (SEM), X-RAY DIFFRACTION (XRD), FOURIER TRANSFORM, INFRARED SPECTROSCOPY (FTIR), ENERGY DIFFRACTION X-RAY (EDX)

To determine the shape, size, and confirm the presence of copper was visualized using SEM, EDX, XRD, and FT-IR. XRD analysis of prepared nanoparticles was performed on a Bruker D8 Advance diffractometer operated at 40 kV and 40 mA with Cu Kα radiation wavelength of 1.54 Å as a source. A step scan mode was applied with a step width of 0.02°, a sampling time of 0.5 s, and a measurement temperature of 25 °C. The scanning range of 2θ was between 20° and 80° ((Menamo et al., 2017). FT-IR (IR Prestige-21

SHIMADZU) spectra analysis in region of 4000cm-1 -600cm-1 with maximal resolution of 0.5 cm-1 was recorded.

CHEMICAL OXYGEN DEMAND (COD)

The copper nanoparticle was tested to reduce COD of fish processing wastewater at 100, 250, 500, 750, 1000, 1250, 1500, 1750, and 2000 mg/L of copper concentration. The best concentration of copper nanoparticles that removed COD was highest then chosen for the next bacteriological testing.

ANTIMICROBIAL ACTIVITY DETERMINATION OF COPPER NANOPARTICLE

The antimicrobial activity of the product was tested using fish processing wastewater. Total coliform and E.coli were detected using series of test tube IMVIC tests according to US FDA BAM 4 and was further confirmed on Mc Conkey and EMB agar at 370C in 24 hours incubation. All broth and media were sterilized for 20 min at 1210C before any inoccupation. The dosage of the selected concentration of copper particle was varied from 10 to 100uL in wastewater to determine the antimicrobial properties of copper nanoparticles. All the experiments were repeated three times to ensure the result's accuracy.

RESULTS AND DISCUSSION

SEM analysis was used to study the surface morphology of copper particles. From Figure 2, the synthesized copper materials have sizes ranging from 4.8 to 10.7 µm and they were spherical. The stabilized copper particle formed clusters and was close to each other. However, according to Khan, Rashid, Younas, and Chong (2016), copper particles are surrounded by each other by stabilizing agents. Stabilizers playing a vital role to determine the distribution of particle size and flocculation. Ascorbic acid is strong enough to play this role. Copper is easily reduced in solution using mild reductants such as ascorbic acid. Metallic copper has been generated through the chemical reduction method using ascorbic acid as a reducing agent and copper sulphate as the precursor.

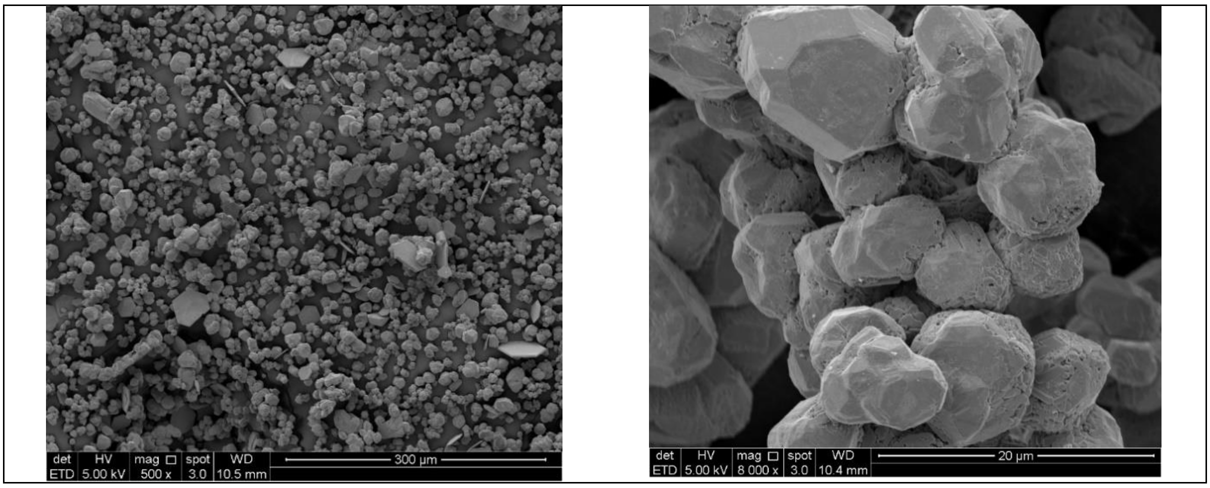

Figure 2. SEM image of synthesized Copper particle at 500x and 8000x magnification

EDX spectroscopy aided to quantify the elemental composition of the synthesized copper particle. Figure 3 showed EDX analysis has a spectrum mostly of sharp Copper peaks. The EDX spectrum of the copper particle is quite similar to that result obtained by Khan et al. (2016). The peak at 0.25 keV belongs to Cl, while the peak at 0.5 keV corresponding to the binding energy of oxygen. The appearance of peaks at 0.9, 8.1, and 8.9 keV indicated the Cu. Table 1 confirmed the copper product is almost pure copper with a little amount of chlorine impurity. This research produced higher purity of copper product compared to Khan et al.(2016) even though the procedures were almost the same with slight modification.

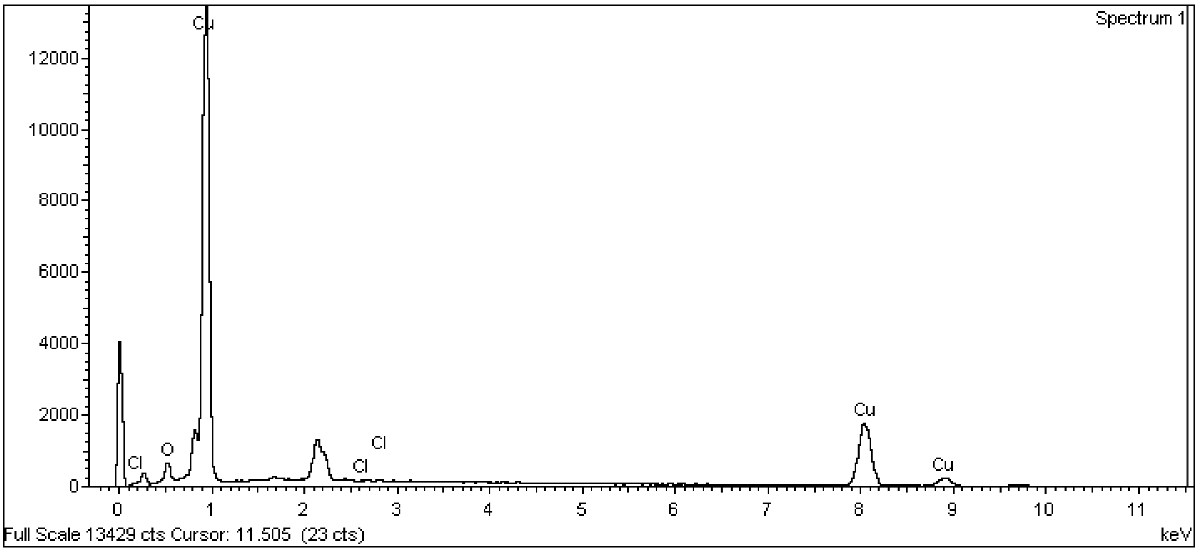

Figure 3. EDX result of synthesized Copper particle

The crystal structure of the Copper particle was verified by using XRD. From Figure 4, it peaks observed at 2θ values in between 430 and 440 and 500 to 510 correspond to plane of metallic copper. This result agrees with the result from previous research (Chandra, Kumar, & Tomar, 2014; Khan et al., 2016; Menamo et al., 2017; Ohiienko & Oh, 2018).

Table 1. Elemental analysis weight by weight percentage

Element	Percentage (%)
Copper	95.31
Oxygen	4.60
Chlorine	0.09

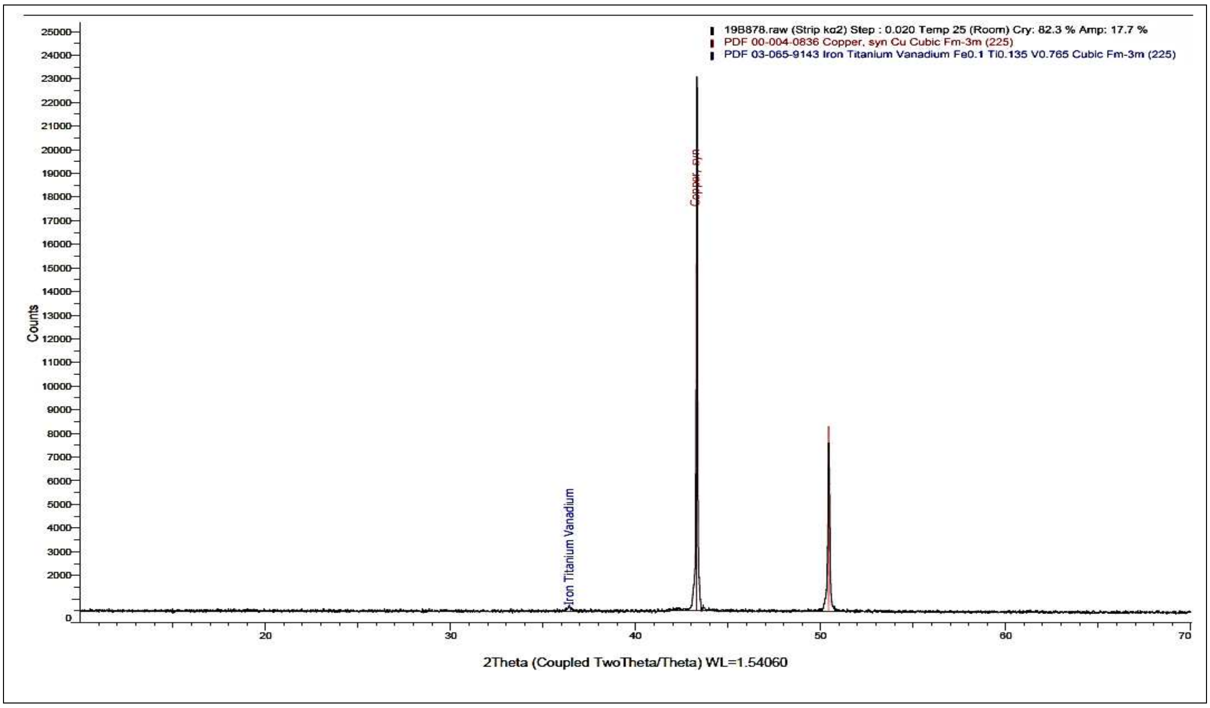

Figure 4. XRD graph of synthesized copper particle measured with the scanning 2θ ranges from 20^0 to 80^0.

From Figure 5, the IR peaks at 1743cm^{-1} is due to C=C bond (Zafar & Shamaila, 2015). Wave number at 2922 and 2856 cm^{-1} contributed by C-H stretching in ligands. The 1743cm^{-1} indicated the presence of presence of oxidated carbonyl groups (Shikha, Ankita, Kachhawah & Devra, 2015).

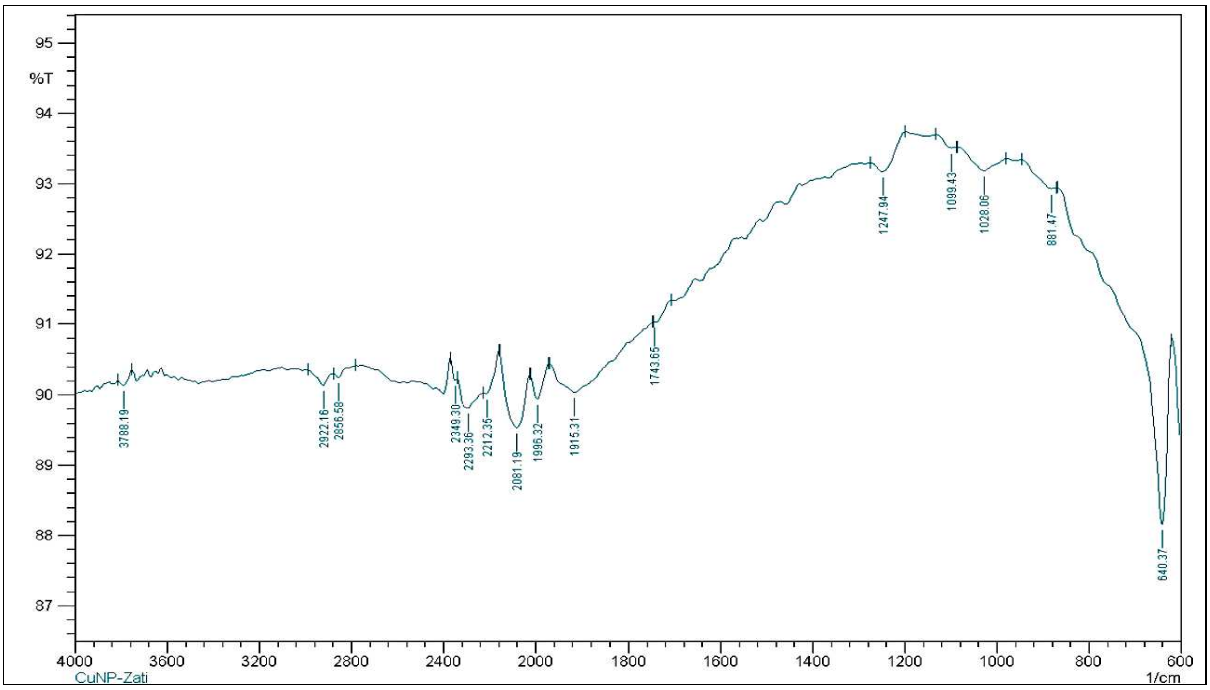

Figure 5. FTIR spectrum of synthesized copper particle

COD reading

From Table 2, the COD reading decreased as the concentration of copper nanoparticles increased until 1000mg/L copper nanoparticles. Then COD removal decreased from 1250ppm until 1750ppm and increased when 2000ppm of the copper product was applied. From COD reading, 1000ppm of copper nanoparticles was chosen for bacteriological testing.

Table 2. COD reading before and after treatment with copper nanoparticle

	Average reading (mg/L)	Average Removal (mg/L)
untreated	539.5	
100ppm	512	27.5
250ppm	476	63.5
500ppm	458.5	81
750ppm	443	96.5
1000ppm	431	108.5
1250ppm	459	80.5
1500ppm	460.5	79
1750ppm	506	33.5
2000ppm	458	81.5

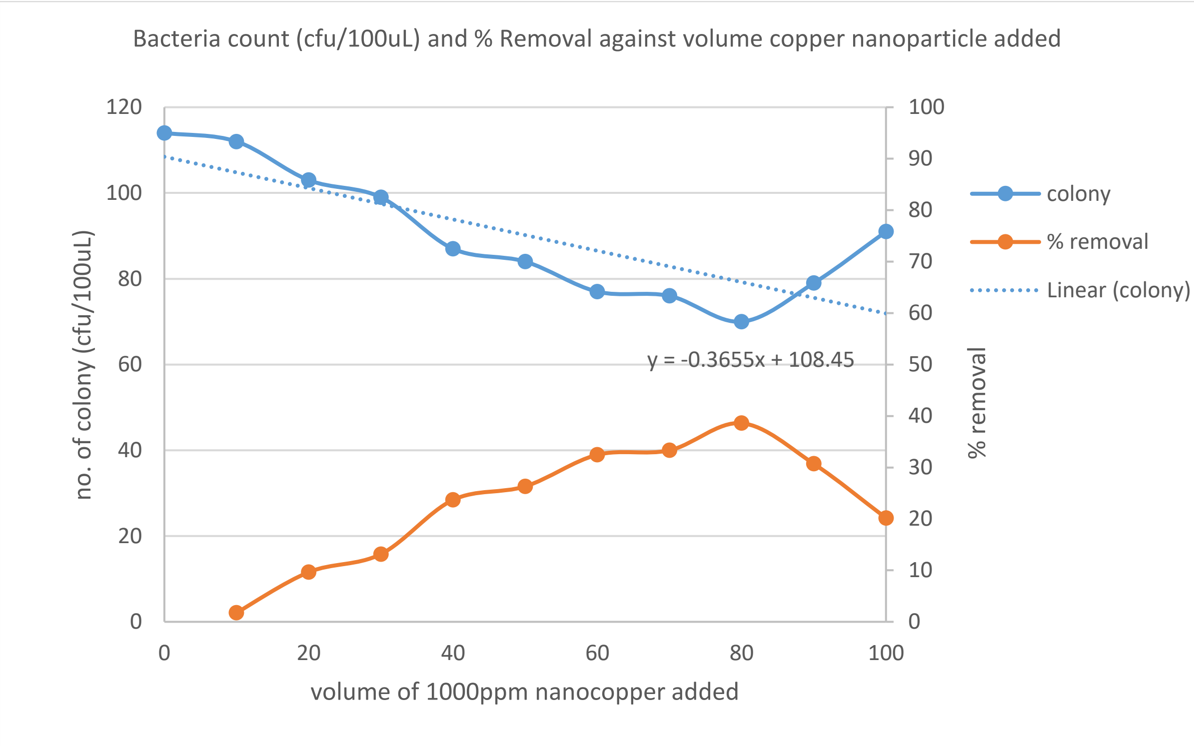

Figure 6. Graph of bacteria count of total coliform (CFU/uL) against volume of ionic cupric copper solution added (μL).

From Figure 6, the bacteria count in CFU/ml decreases as the volume of the ionic cupric copper solution increases until 90uL where the bacteria count starts to become higher again. The best dosage of the ionic cupric copper solution added can be seen at 80uL which shows the least number of bacteria count at 700000 cfu/ml, making the removal efficiency of total coliform up to 38%. Theoretically, the ionic cupric copper solution can eliminate the use of chlorine in the fish processing water as a disinfectant, but further research can be done to increase the removal efficiency percentage. By finding the best fit line of the graph which is y = -0.3655x + 108.45, a prediction can be made that as the volume of ionic cupric copper solution increases, the bacteria count of total coliform will decrease and this ultimately proves that the use of ionic cupric copper solution qualifies as a type of wastewater disinfecting in fish processing factory.

IMPLICATION AND CONCLUSION

The best disinfection efficiency of total coliform was found to be 38.60% by adding a best dosage of 80L ionic cupric copper solution of 1000ppm. Is also predicted that as the volume of ionic cupric copper solution increases, the bacteria count of total coliform will decreases accordingly. In conclusion, ionic cupric copper solution is found to be a good alternative in replacing chlorine as disinfectant used in fish processing wastewater but further research is required to ensure higher disinfection efficiency to use in industrial scale and all types of industrial wastewater.

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