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Removal of Organic Constituents and Bacteria Count Disinfection from Fish Processing Wastewater by Using Ionic Cupric Copper

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Abstract. The increasing consumption of fisheries product causes large amount of wastewater produced from fish processing industry. General wastewater treatment includes the use of harmful chemical such as chlorine. This research opens a new platform to treat wastewater by using ionic cupric copper which is also known as copper nanoparticles (CuNP). Copper nanoparticles are synthesized through chemical reduction method by using Copper sulphate pentahydrate ($CuSO_4.5H_2O$) as precursor and Sodium hydroxide (NaOH) as reducing agent. The physical characterizations of CuNP are carried out by using SEM and the chemical characterizations are carried out by using XRD and FTIR. The efficiency of CuNP to treat wastewater from fish processing industry are determined by the removal of COD, Total coliform and E.Coli. The optimum concentration to reduce COD is 1000mg/L which causes 59.4% percentage removal of COD. The optimum concentration for COD removal is used to determine the volume of CuNP needed to remove total coliform and E.Coli. The optimum volume for removal of total coliform and E.Coli are 40µL and 30 µL respectively.

1. Introduction
Fisheries industrial sector are one of the top industrial sectors in the entire world as consumption of fish and fisheries product is high compared to other type of food. Demand for these fish and fisheries product is expected to increase in the upcoming years [1]. As demand for fish and fisheries increase, the production by the industry must be increased to match the demand. Thus, the generation of wastewater by the industry will increase along with the increase of production.

The specificity of wastewater generated by fish processing industry is determined by the fish type and the processing technology. Wastewater produced from fish processing industry is a mixture of suspended and dissolved organic materials [2-3]. High amount of suspended and dissolved organic materials will make the value of Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) increase drastically. Another problem that is always associated with fish industry is the odor.

The general method to treat the wastewater from fish processing industry physical-chemical method, biological method or a combination of both methods. Sedimentation, air flotation, pH adjustments and chlorination are categorized under physical-chemical method whereas activated sludge is one of the biological method [4]. Multiple processes are needed to treat the fish processing wastewater. Several studies had shown the ability of nanoparticles that is inexpensive, free from secondary pollutant and is able to simultaneously remove multiple pollutant from wastewater [5–7]. Application of nanotechnology in wastewater treatment can reduce the use of disinfectants and chemicals used in wastewater treatment [8]. These studies had proved the ability of nanoparticle to treat wastewater in a single-step biochemical process.

Nanoparticles are nano-sized particles that can be prepared with multiple synthesis method [9]. Distinctive and enhanced characteristics like particle size distribution and shape does not apply to bigger atom or particles. Instead, it exist in particles that are reduced to nano-size [10]. Copper are metal that is cheap, very responsive towards microorganisms and have insignificant effect towards human tissues [11]. A study by [6] has shown that copper do exhibit antimicrobial properties towards microorganisms. Antimicrobial activity against *Escherichia Coli* and *Staphylococcus* species are proved by [7, 12].

2. Methodology

2.1 Synthesis of Copper Nanoparticles

A solution of 0.1M Copper Sulphate Pentahydrate ($CuSO_4.5H_2O$) was prepared by dissolving 12.5g of $CuSO_4.5H_2O$ in 500mL distilled water. 500mL of 0.2M Sodium Hydroxide (NaOH) was added dropwise to the solution of $CuSO_4.5H_2O$. The solution was heated to 50°C and stirred for one hour until dark brown-black precipitate is formed. Precipitate was centrifuged at 3500 rpm for 30 minutes. It was then filtered and washed with distilled water (three times) and ethanol (one time). The obtained precipitate was dried in the oven at 80°C for three hours.

2.2 Physical and Chemical Characterization of Copper Nanoparticles

Scanning Electron Microscope (SEM) measurements was carried out by using Field Emission-Scanning Electron Microscope (FE-SEM) with FEI Quanta FEG 650 model at 5kV and 10kV to determine the shape and diameter of synthesized copper oxide nanoparticles. X-ray Diffraction (XRD) measurement to identify the phase materials of copper oxide nanoparticles synthesized through recognition of d-spacing values contained in any copper oxide nanoparticles synthesized was carried out by using X-Ray Diffraction (XRD) model D8 Advance Bruker AXS (Germany). Fourier Transform Infrared Spectroscopy (FTIR) patterns that show the surface characterization and purity of copper oxide nanoparticles synthesized are determined by using FT-IR spectrophotometer in the range of 600-4000cm^{-1}.

2.3 Test for COD Removal

Procedures to determine the Chemical Oxygen Demand (COD) value of fish processing wastewater before and after adding the copper nanoparticles was done by following the Standard Method for Examination of Water and Wastewater. A series of 0.02g-0.20g of copper oxide nanoparticles was added to 100mL fish processing wastewater and stirred for two hours to determine the efficiency of copper oxide nanoparticles for removal of COD. The value of COD of fish processing wastewater before and after addition of copper oxide nanoparticles was determined and the percentage removal for COD was calculated. The dosage of copper oxide nanoparticles that show significant removal of COD was used for the bacteriological test.

2.4 Bacteriological Test

5 serial dilutions (1:10ml, 1:100ml, 1:1000ml, 1:10000ml and 1:100000ml) for the fish processing wastewater were done using distilled water to find out which serial dilution produce cultured agar plates with countable colony forming units (between 30 and 300 colonies). Distilled water acts as a diluent for the wastewater in the serial dilution procedure without contaminating the wastewater

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sample. Serial dilution to determine countable colony for total coliform (TC) was done on Plate Count Agar (PCA) while serial dilution for *Escherichia Coli* (E.Coli) was done on Eosine Methylene Blue (EMB) agar. 0.1mL of each dilution of fish processing wastewater was pipetted on the petri dish and spread evenly by using a sterilized hockey stick. The petri dishes are incubated at 37°C for 24 hours. Serial dilution with countable colony forming units was used to test for removal of TC and E.Coli. Dosage of the selected concentration of copper oxide nanoparticle was varied from 10uL until 100uL on new PCA and EMB agar together with 0.1mL of diluted fish processing wastewater.

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3. Results and Discussions

3.1 Physical and Chemical Characterization of Copper Nanoparticles

The shape and size of synthesized copper oxide nanoparticles were obtained by using SEM measurements as shown in figure 1. SEM micrographs revealed that the copper nanoparticles synthesized were spindle-like in shape and having a size of 80-100nm. From figure 1, it can be seen that the copper oxide nanoparticles agglomerates and make up a big lump of particle due to no stabilizing agent added during the synthesis of copper oxide nanoparticles.

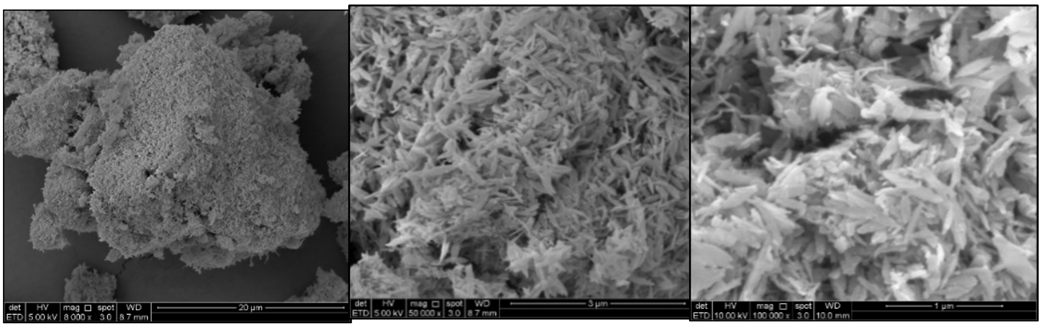

Figure 1. SEM images of copper nanoparticles synthesized at magnification (a.) 8000 (b.) 50 000 (c.) 100 000

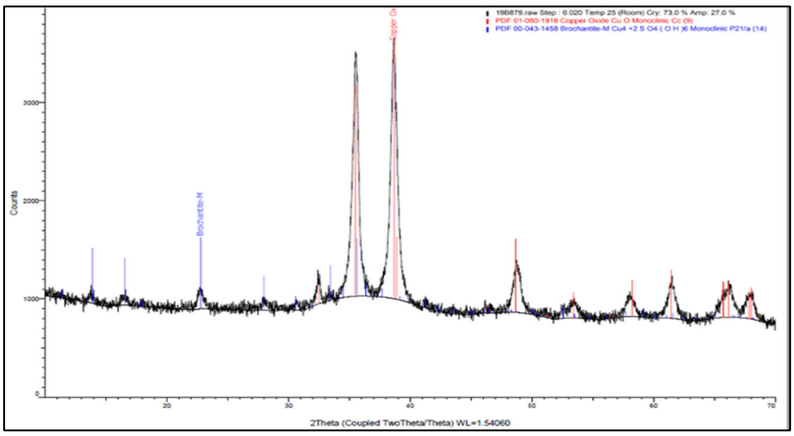

Figure 2. XRD analysis of synthesized CuO nanoparticles.

From XRD analysis in figure 2, the peak observed corresponds to monoclinic copper oxide (CuO) and monoclinic Brochantite-M Cu^{+2} S O_4 $(OH)_6$. No characteristics peak from other impurities like copper hydroxide and cuprous oxide that shows the product synthesized are made up of pure monoclinic structure of CuO phase. Sharp and narrow diffraction peaks show that the product is of high crystallinity. Occurrence of Brochantite-M Cu^{+2} S O_4 $(OH)_6$ indicates that the copper has been oxidized.

Properties and purity of copper oxide nanoparticles can be obtained by FTIR. Spectrum 490-800cm^{-1} is a zone where the basic monoclinic phase of pure Copper Oxide (CuO) is obtained. From figure 3, there is a strong absorption peak at 599cm^{-1}. Thus, the monoclinic phase of pure CuO can be proved. Metal-oxygen stretching can also be seen at 628cm^{-1} that can also prove the Cu-O stretching in monoclinic phase of CuO. Absorption peak at 1085cm^{-1} and 1118cm^{-1} shows that there is a carbonyl C=O bonds. Lastly, absorption peak at 3387cm^{-1} shows the presence of C-H bond stretching.

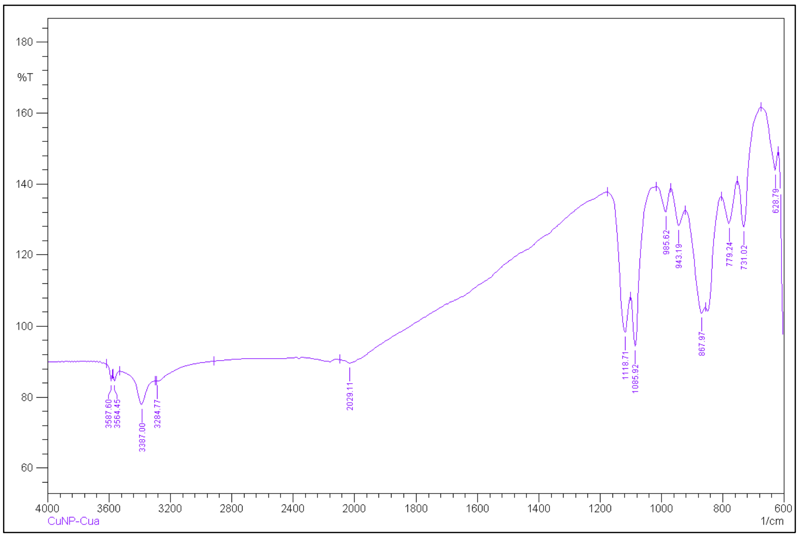

Figure 3. FTIR analysis of synthesized copper oxide nanoparticles.

3.2 COD Removal

The optimum dosage of copper oxide nanoparticles added was determined by adding various concentration of copper oxide nanoparticles into the fish processing wastewater and let it react for two hours.

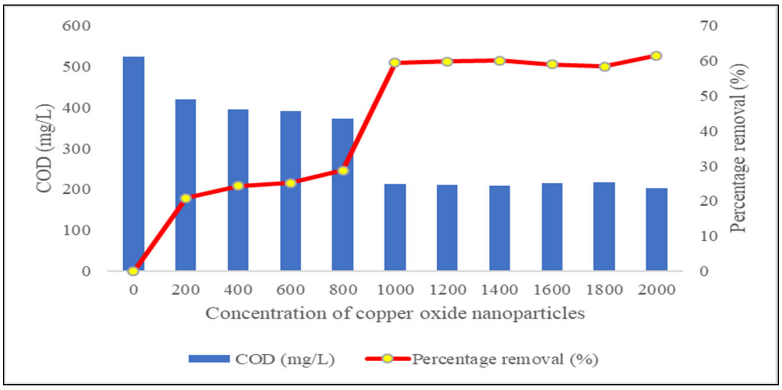

Figure 4. COD value of fish processing wastewater and COD (%) removal when CuO nanoparticles were added.

Based on the result obtained in figure 4, the percentage removal of COD increases steadily until the treatment with 1000mmg/L copper oxide nanoparticles where the percentage removal of COD increases rapidly to 59.4%. The trend then shows a slight increment in percentage removal of COD.

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The highest percentage removal of COD was at 61.4% with the addition of 2000mg/L of copper oxide nanoparticles. 1000mg/L concentration of copper oxide nanoparticles was chosen to be used for the bacteriological test as there is only a small increment in percentage removal of COD when more copper oxide nanoparticles was added.

3.3 Bacteriological Test

Serial dilution of the wastewater samples was compulsory to obtain the optimum dilution that can give the viable plate count 30-300. From table 1, it can be seen that dilution 10^{-2} gives a plate count number of 296 and 288 for TC and E.Coli respectively which was in the range of viable plate count thus this dilution was then used in the next step of the experiment.

Table 1. Number of colony forming unit per mL (CFU/mL) obtained from serial dilution.

Dilution of wastewater	**PCA agar (Total coliform) (CFU/mL)**	**EMB agar (*E.Coli*) (CFU/mL)**
Undiluted	TNTC	TNTC
10^{-1} dilution	TNTC	TNTC
10^{-2} dilution	296	288
10^{-3} dilution	88	106
10^{-4} dilution	26	22
10^{-5} dilution	1	2

TNTC: Too numerous to count

Based on figure 5, the bacteria count of total coliform is the lowest when 40µL of copper oxide nanoparticles was added. It can be concluded that the best dosage for total coliform removal is 40µL where the bacteria count of total coliform is 9600 CFU/mL, making the removal efficiency up to 67.6% based on figure 5. This shows that the copper oxide nanoparticles can be used to disinfect and were able to reduce the total coliform count in fish processing wastewater effluent.

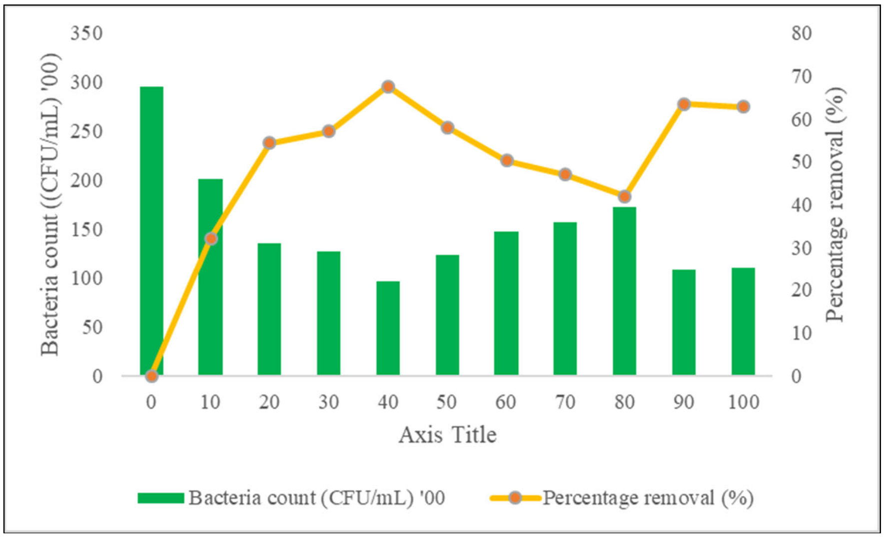

Figure 5. Bacteria count and percentage removal of total coliform when adding CuO nanoparticles

From table 2, the optimum volume of copper oxide nanoparticles for removal of E.Coli was observed at 30µL with 91.7% removal. Addition of excess copper oxide nanoparticles causes the bacteria count of E.Coli to increase again. This proves that copper oxide nanoparticles synthesized can be used to disinfect the fish processing wastewater and remove a large amount of E.Coli.

Table 2. Bacteria count of E.Coli per mL and percentage removal of E.Coli when copper oxide nanoparticles were added.

Volume of copper oxide nanoparticles added (µL)	Bacteria count of E.Coli ((CFU/mL) '00)	Percentage removal (%)
0	288	0
10	55	80.9
20	37	87.2
30	24	91.7
40	48	83.3
50	52	81.9
60	51	82.3
70	42	85.4
80	29	89.9
90	40	86.1
100	45	84.4

4. Conclusions

From this research, the ability of copper oxide nanoparticles to treat fish processing wastewater is proven. Characterization of copper oxide nanoparticles prove that the size of copper oxide nanoparticles synthesized is between 80-100nm and the structure of copper oxide nanoparticles is spindle-like structure. Analysis from XRD and FTIR also prove the formation of pure single phase CuO with monoclinic structure. The optimum concentration of 1000mg/L for copper oxide nanoparticles was able to remove 59.4% of COD. Only a small amount of copper oxide nanoparticles is needed to disinfect the fish processing wastewater which will disintegrate total coliform and E.Coli. Adding 40µL from 1000mg/L copper oxide nanoparticles would be able to yield 67.6% percentage removal of total coliform. Moreover, 91.7% percentage removal of E.Coli can be achieved by addition of 30µL copper oxide nanoparticles. copper oxide nanoparticles is found to be a good substitute for treatment of fish processing wastewater but further research need to be done in order to apply it into industrial scale and all types of industrial wastewater.

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