Use of Fish Scales as Biosorbent for the Removal of Copper in Water

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Abstract Industrial effluents are the major sources of copper in the environment. It is important to regulate copper levels in water because excess amounts of copper in the human body is associated with adverse health effect such as liver and kidney damage, anemia, schizophrenia, and Alzheimer's disease. Conventional copper removal methods are not environmentally friendly due to the increased level of chemical sludge, energy input, and nutrient requirements from the removal processes, and expensive due to the implementation of large infrastructure. Biosorption is a better alternative to conventional copper removal methods. Studies have shown that using fish scales as a biosorbent was effective in absorbing lead, chromium and arsenic in water. In this study, fish scales of *Tilapia nilotica* Linnaeus were used as a biosorbent in the investigation of copper sorption capabilities under different biosorbent to copper mass ratios and contact times. With the use of a biosorbent to copper mass ratio of 13.5, 33.988% (SE=1.008%) of the copper was removed after two hours. After eight days, 97.2% (SE=0.235%) of the copper was removed with the application of a biosorbent to copper ratio of 10.0. Larger amounts of biosorbent added and longer contact times yielded higher sorption percentage

Introduction

Copper is one of the inorganic contaminants that the Environmental Protection Agency regulates in ground water and drinking water. It is listed under the National Primary Drinking Water Regulations for inorganic chemicals along with common contaminants such as chromium, lead, mercury, and cadmium (EPA, 2006). Copper is also one of the metals that the World Health Organization most immediately concerned about on its threats to public health (WHO, 1984).

Copper is naturally deposited in rocks as mineral form, which is mostly associated with sulfur (USGS, 2007). Examples of common minerals that contain copper are azurite, malachite, tennantite, chalcopyrite and bornite (MII, 2007). Anthropogenic sources of copper include the production of plastic material, copper and other nonferrous smelting, and steel blast furnaces (EPA, 2006). The major source of copper contamination in drinking water is from plumbing material, due to the corrosion of copper pipes from passing water through these pipes (EPA, 2006). Copper released into the environment indefinitely persist, circulate and accumulate in the food chain (Vijayaraghavan et al., 2004). Higher plants and animals are exposed to copper by consuming contaminated food and water.

Although copper is an essential nutrient to humans, excess intake of copper would cause acute and chronic adverse health effects such as stomach and intestinal distress, liver and kidney damage, and anemia (EPA, 2006). Not only does toxicity of copper cause physical damages to the human body, it can also worsen the symptoms of mental diseases. High levels of copper have been associated with people with mental illness, such as paranoia, obsessive-compulsive schizophrenia (CDA, 2006), and Alzheimer’s disease (Pfeiffer and Iliev, 1972).

Examples of conventional copper removal methods are ion exchange, chemical precipitation, ultra filtration, and electrochemical deposition (Aslam et al., 2004). These removal methods are expensive due to implementation of new infrastructures and are not environmentally friendly because they increase the volume of chemical and biological sludge due to the additional chemicals in the treatment (Kratochvil and Volesky, 1998). Compared with conventional methods for copper reduction, biosorption is a better alternative.

Biosorption is defined as the ability of biological materials to accumulate heavy metals from wastewater through metabolically mediated or physical-chemical pathways of uptake (Ahalya et al., 2003). The biological materials used in the process are usually inexpensive dead biomass that are naturally abundant or waste biomass of algae, moss, fungi or bacteria (Kratochvil and Volesky, 1998). Advantages of biosorption are the significant amount of energy savings from a more efficient wastewater treatment system operating for fewer hours; it is economically attractive because waste biomass is inexpensive and widely available (Mustafiz et al., 2002). Biosorption also offers low operating cost, minimization of chemical and biological sludge, and no additional nutrient requirements (Kratochvil and Volesky, 1998).

A recent study done by Mustafiz et al. (2003) suggested that fish scales of Atlantic cod, *Gadus morhua* Linnaeus, be a better alternative to reduce the level of lead, arsenic and chromium in water. Following Mustafiz et al. (2003), this study will investigate the sorption capabilities of fish scales of *Tilapia nilotica* Linnaues for the uptake of copper in water. Scales of tilapia were used instead of Atlantic cods' because tilapias are less expensive and have higher availability than Atlantic cod in the Bay Area. The uptake abilities of scales from different fish species should be similar because most fish scales contain significant portions of organic protein (collagen), and the structure of collagen shows that it contains the possible functional groups, such as phosphate, carboxyl, amine and amide, that are involved in the biosorption of heavy metals (Mustafiz et al., 2003). Since the use of fish scales as a biosorbent is a recent innovation done by Mustafiz et al., there are no other relevant studies with which to compare the present study. However, other biosorption studies done on copper removal were with the use of cyanobacteria (Solisio et al., 2004), and wool (Sheffield and Doyle, 2005) as the biosorbent. Using fish scales as a biosorbent would be a better alternative than cyanobacteria or wool because fish scales are already a waste generated from households and markets. Cyanobacteria removed from the environment to use as biosorbent in waste water treatments may affect higher level organisms that depend on it, which would disrupt the food chain. Using wool as a biosorbent is not preferred because it is a valuable resource for the textile industry and not a waste product.

The objective of this study is to investigate the copper sorption capabilities of fish scales of *Tilapia nilotica* Linnaeus under two variables, ratios of the amounts of fish scales to the initial concentration of copper and contact times. Advantages of using fish scale as a biosorbent are cost-effective, minimal energy usage and is environmentally-friendly. With these advantages, wastewater treatment facilities could adopt the use of fish scales in biosorption processes.

Methods

Preparation of Fish Scales and Copper Solutions Scales of *Tilapia nilotica* Linnaeus, used as biosorbent in this study, were collected from Market 88 in San Leandro, California. The biosorbent were soaked in deionized water for 24h and rinsed three times with deionized water. The biosorbent was transferred to a laboratory tray, air dried for two days in the fume hood, and stored in a beaker at room temperature.

All copper solutions used in this study were prepared by adding 62.83g of copper(II) sulfate anhydrate ($CuSO_4$) in five liters of deionized water, which resulted in initial concentrations of roughly 5g Cu/L. The entire five liters Cu stock solution was distributed in 50 different 125mL Erlenmeyer flasks, 100mL of Cu stock solution in each flask, and stored at room temperature. Glacial acetic acid from Fisher Scientific was diluted to make a 0.5M acetate buffer with the pH=3.9, and 0.1M NaOH was used to adjust the buffer to the desired $pH=4\pm0.5$. The acetate buffer was used to maintain the acidity of the Cu solutions at the $pH=4\pm0.5$. Two milliliters of the acetate buffer was added to each sample before the biosorbent was added.

Varying Biosorbent to Copper Ratios All samples were treated at room temperature, and with $pH=4\pm0.5$. The initial and final copper concentrations ([Cu]), in units of grams of Cu per liter of solution, were determined by spectrophotometer. The spectrophotometer was calibrated with Cu stock solution. The absorbance of the samples were measured and [Cu] was calculated from the equation,

$$[Cu]=\frac{absorbance}{0.1679}$$

The initial [Cu] of each sample was determined. Different amounts of biosorbent (0.050g, 0.20g, 0.50g, 2.0g, 3.0g, 4.0g, 5.0g, and 7.0g) were added to eight separate flasks containing Cu stock solution with constant initial [Cu]. Different amounts of biosorbent represent different biosorbent to Cu ratio (BS:Cu) (Table 1). BS:Cu was calculated using the equation,

$$BS:Cu = \frac{mass\,of\ biosorbent}{mass\,of\ copper} = \frac{mass\,of\ biosorbent}{initial\,[Cu]\times volume\,of\ sample}$$

Table 1. Amount of biosorbent (g) used with corresponding BS:Cu.

amount of biosorbent	BS:Cu
0.00	0.00
0.05	0.01
0.20	0.04
0.50	1.00
2.00	4.00
3.00	6.00
4.00	8.00
5.00	10.00
7.00	14.00

The samples were shaken on an Innova 2100 platform shaker, by New Brunswick Scientific, at 250 revolutions per minute (rpm) for two hours. After the treatment, the biosorbent was filtered out using filter paper. Although no biosorbent was added to the untreated sample, it was filtered before the determination of the final [Cu] to control for any copper sorption caused by the filtering processes. The filtrate was collected, then the final [Cu] was determined. The calculated initial and final [Cu] were used to determine the specific uptake (Q_{eq}), in units of gram of Cu removed per gram of biosorbent used, and the sorption percentage (%S). Q_{eq} and %S were calculated using the equations,

$$Q_{eq} = \frac{(initial\,[Cu] - final\,[Cu])\times volume\,of\ sample}{mass\,of\ biosorbent}$$

$$\%S = \frac{(initial[Cu] - final[Cu])}{initial[Cu]} \times 100\%$$

Varying Contact Time Each sample was treated under constant amount of biosorbent (5.00g; BS:Cu = 10.00) while at different contact times (45min, 2hr, 6hr, 12hr, 2d, 4d, and 8d) with a platform shaker at 250 rpm. The biosorbent in the samples were filtered out and the final [Cu] of the filtrate was determined. The %S and the removal rate were calculated. The removal rate (kr), in units of grams of Cu removed per minute, was calculated using the equation,

$$kr = \frac{(initial[Cu] - final[Cu])}{contact\ time}$$

All treatments were done in doublets, except the ones treated under varying BS:Cu (0.20g, 0.50g, 2.0g, 3.0g, 4.0g, 5.0g, and 7.0g), were done in triplets.

Result

The sorption percentages and the specific uptake under the application of varying BS:Cu with constant contact time of two hours were averaged for each treatment (Table 2).

Table 2. Average (± standard error) with varying BS:Cu. Amount of biosorbent (g), BS:Cu, sorption percentage (%S), Qeq specific uptake (gCu removed / g biosorbent).

amount of biosorbent	BS:Cu	%S	Qeq
0.000 (± 0.000)	0.000 (± 0.000)	0.117 (± 0.117)	n/a
0.053 (± 0.002)	0.101 (± 0.004)	0.820 (± 0.117)	0.082 (± 0.015)
0.204 (± 0.005)	0.392 (± 0.010)	1.248 (± 0.341)	0.031 (± 0.008)
0.494 (± 0.000)	0.950 (± 0.003)	1.874 (± 0.946)	0.020 (± 0.010)
2.010 (± 0.005)	3.869 (± 0.016)	12.859 (± 0.577)	0.033 (± 0.002)
3.000 (± 0.000)	5.664 (± 0.009)	21.249 (± 1.361)	0.037 (± 0.002)
4.011 (± 0.006)	7.731 (± 0.011)	24.863 (± 1.896)	0.032 (± 0.002)
5.008 (± 0.005)	9.659 (± 0.008)	30.609 (± 0.802)	0.032 (± 0.001)
7.000 (± 0.000)	13.503 (± 0.038)	33.988 (± 1.008)	0.025 (± 0.001)

With the increased amount of biosorbent applied (larger BS:Cu), higher sorption occurred. With the highest application of biosorbent (7.0g), BS:Cu = 13.503 (SE=0.038) , the mean sorption percentage was 33.988 (SE=1.008). All data, instead of their averages, were plotted and a linear regression line which intersects the origin, was fitted in the graph with the equation

$$\%S = 2.9345 \times (BS : Cu)$$

R^2=0.944 (Fig. 1). The regression line intersecting the origin suggests that without the application of biosorbent, the sorption percentage was zero. The linear regression equation could be used to estimate the sorption percentage under a fixed contact time of two hours using BS:Cu as the variable.

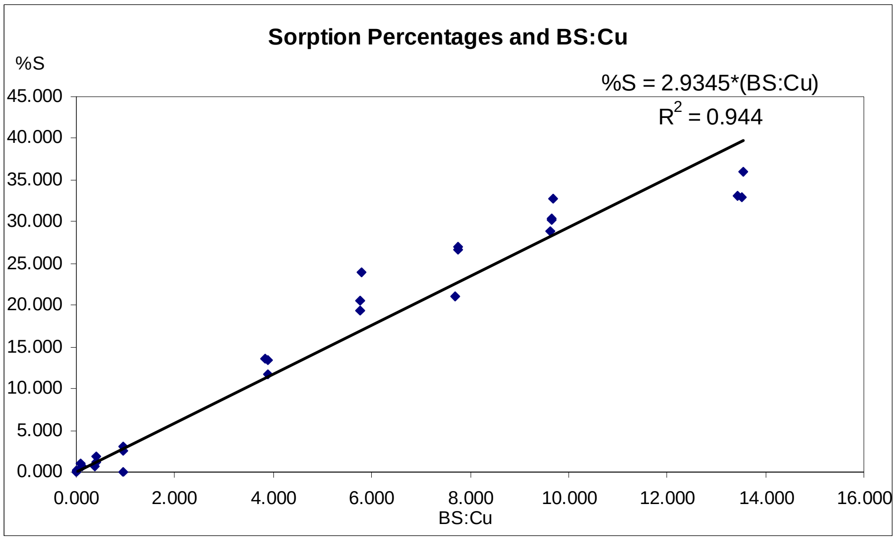

Figure 1. Graph of biosorbent to copper mass ratio and sorption percentage with constant contact time of two hours. Linear regression %S=2.9345*(BS:Cu), R^2=0.944.

The highest specific uptake of copper occurred with the smallest BS:Cu (BS:Cu=0.101 ± 0.004; Q_{eq}=0.082 ± 0.015). Treatments with BS:Cu larger than 0.101, the values of specific uptake were two time less than the highest specific uptake value (Fig. 2). With a fixed contact time of two hours, the mean specific uptake of all samples was 0.034 (SE=0.004) grams of Cu removed per gram of biosorbent used.

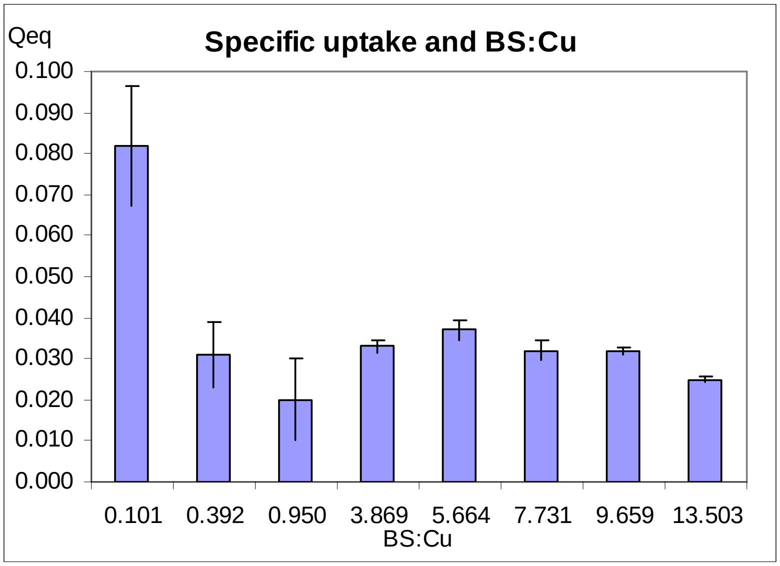

Figure 2. Mean specific uptake (g Cu removed / g biosorbent) with standard error bar at the specified BS:Cu. With a constant contact time of two hours, highest specific uptake occurred with smallest BS:Cu (BS:Cu=0.101 ± 0.004; Q_{eq}=0.082 ± 0.015)

With the application of constant amount of biosorbent (5.00g; BS:Cu=10.00), longer contact time yielded a higher sorption percentage (Table 3). For treatments with contact time of two hours and longer, the solutions were cloudy and light blue powders were observed. After the treatment, most of the powders were taken out of the solution during the filtering processes.

Table 3. Averages (± standard error) of data with constant BS:Cu and varying contact time. Contact time (hr) and the removal rate (gCu removed / hr).

contact time	%S	removal rate
0.000	0.234 (± 0.234)	n/a
0.750	1.998 (± 0.813)	0.135 (± 0.056)
2.000	29.602 (± 0.679)	0.751 (± 0.016)
5.333	53.199 (± 0.389)	0.510 (± 0.007)
15.000	71.086 (± 0.194)	0.242 (± 0.002)
47.750	69.610 (± 15.601)	0.074 (± 0.017)
96.000	72.059 (± 11.353)	0.038 (± 0.006)
190.500	97.183 (± 0.235)	0.026 (± 0.000)

Increased sorption percentages occurred with increasing contact time. A 97.2% (SE=0.235%) of Cu sorption was achieved with a contact time of eight days (190.5 hr). The relationship between the sorption percentage and the contact time was a logarithmic increase with the equation,

$$\%S = 14.548 \times Ln(contact\ time\ in\ hrs) + 18.01$$

R^2=0.8424 (Fig. 3). This equation could be used to estimate the sorption percentage with the contact time as the variable while the amount of biosorbent is fixed at BS:Cu=10.

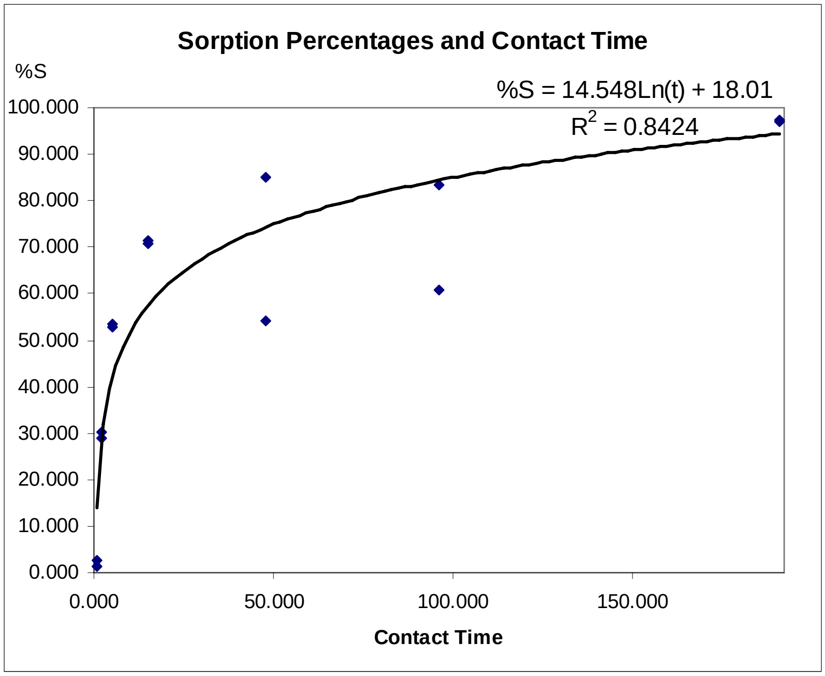

Figure 3. Graph of the contact time (hr) and sorption percentage. With constant BS:Cu, longer contact time yielded a higher sorption percentage. Logarithmic regression equation, %S = 14.548*Ln(contact time in hrs) + 18.01; R^2=0.8424.

The removal rate (grams of Cu removed per hour) peaked within the contact time of two hours, and decreased with longer contact time (Fig. 4). The mean removal rate was 0.254 grams of Cu removed per hr (SE=0.069).

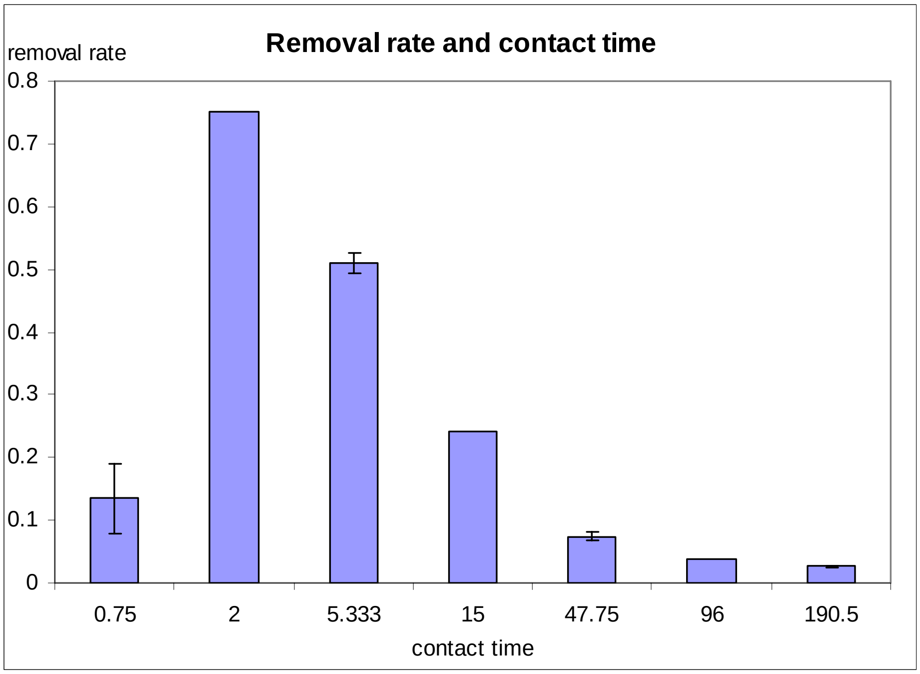

Figure 4. Bar chart of contact time (hr) and mean removal rate (gCu removed / hr) with standard error bars under specified contact time. With constant BS:Cu, peak removal rate occurred in two hours; decreasing removal rate was associated with longer contact time. Mean removal rate was 0.254 ± 0.069 gCu removed/hr.

Discussion

Both the specific uptake and removal rates were measures of removal efficiencies which did not show the amount of Cu removed in water. Highest specific uptake was achieved with the application of the smaller amount of biosorbent (Fig. 2), and the highest removal rate was achieved with the contact time of less than or equal to two hours (Fig. 4). The results implied that with the application of fish scales as a biosorbent in Cu removal, it is most efficient to use smaller amount of biosorbent and a contact time of less than two hours. Efficiency was high under these conditions, but did not give desirable level of Cu removed in the treatment. The values of specific uptake and removal rate are useful when applied to a filtering system because they suggest the rate of change of how the biosorbent is interacting with the wastewater.

With the application of fish scales as a biosorbent in Cu sorption process, higher sorption percentages occurred with increasing amount of biosorbent and contact time. Two equations were derived from this study, one could be used to estimate the sorption percentage with a fixed contact time of two hours and the BS:Cu (a mass ratio of the grams of biosorbent divided by the grams of Cu in solution) as the variable,

$$\%S = 2.9345 \times (BS : Cu)$$

Following is a sample calculation to estimate the amount of fish scales needed to remove all of Cu (%S=100) in a one liter industrial effluent with the [Cu]=2.00 mg/L. The [Cu] in one liter industrial effluent was converted into the mass of Cu = 0.00200g. The equation was rewritten to solve for BS,

$$BS = \frac{100 \times Cu}{2.9345} = \frac{100 \times 0.00200}{2.9345} = 0.0680g$$

Under the fixed contact time of two hours, 0.0680g of fish scales are needed to remove 100% of the Cu in one liter of industrial effluent with the [Cu] = 2.00 mg/L.

The other equation derived from this study could be used to estimate the sorption percentage with a fixed BS:Cu=5.00 and the contact time in hours as the variable,

$$\%S = 14.548 \times Ln(contact\ time\ in\ hrs) + 18.01$$

Removing 100% of Cu in a certain volume of industrial effluent with the application of the amount of biosorbent equal to BS:Cu=5.00, the required contact time will be,

$$Contact\ time\ in\ hrs = e^{\left[\frac{(100-18.01)}{14.548}\right]} = 280.3hrs \cong 12\ days$$

About 12 days are needed for the biosorbent to removal 100% of the Cu in solution.

The use of fish scales as a biosorbent in Cu removal is highly depended on different conditions. Depending on which one is the limiting factor, the availability of fish scales or time, conditions could be adjusted to make it suitable for different cases. If the amount of fish scales are limited, a longer contact time would give higher Cu removal; if little time is allowed for treatment, larger amount (large BS:Cu) would give higher Cu removal. Maximized sorption percentage would occur with the application of a larger amount of biosorbent and a longer contact time.

Two possible roles of the biosorbent, physical and microbial, could be used to explain the Cu removal pathways. It was observed that the solution was cloudy and a blue powder was filtered out from the samples with contact times of two hours and longer. It was possible that when fish scales were in Cu solution for a longer time, the outer layers of the scales were corroded and “washed” out, which was the blue powder observed after the treatment. Increased amount of the blue powder suggest a larger surface area of the biosorbent in contact with the Cu solution. The larger the surfaced area, the more Cu was physically adsorbed onto the biosorbent. However, if Cu is removed physically from water, further treatment should be applied to extract or retrieve the Cu from the used fish scales. Directly disposing the used fish scales as raw garbage defeats the purpose of the biosorption process, because the Cu could be re-released into the environment.

The other possible role contributing to Cu removal was microbes in the fish scales. The study done by Mustafiz et al. (2003) suggested that microbes were responsible in heavy metal removal with the application of fish scales as a biosorbent. It was possible that a longer contact time allowed the microbes to be released into the Cu solution. The microbes needed to be in solution for a longer time in order to absorb Cu. As the result, shorter contact time did not allow enough time to trigger the Cu sorption abilities of the microbes in the biosorbent.

Further investigation is needed to make distinction between the physical and microbial roles of the fish scales in Cu removal capabilities. To investigate the physical role of the fish scales in Cu removal capabilities, the scales could be pulverized to a specified size for the treatments. For the investigation of the microbial roles of Cu removal, on the other hand, the scales could be sterilized or apply different antibiotics to terminate a specific microbe in the scales. In addition to the investigation of physical and microbial roles, future studies could be done for the application of fish scales as a biosorbent in Cu removal in other conditions such as varying pH and temperature in the treatment.

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