Characterization and Performance Nanofiltration Membranes in Water Quality for Goldfish (*Carassius auratus*) Aquaculture

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ABSTRACT

Water quality is an important factor in aquaculture activities, including goldfish (*Carassius auratus*) farming. One approach to improving water quality is the use of nanofilters. This study aims to evaluate the performance of nanofilters made from Sargassum sp. with the addition of copper oxide nanoparticles. The method used involves the fermentation of bacterial cellulose from *Acetobacter xylinum* using Sargassum sp. extract as a medium, followed by homogenization and the addition of CuO-NPs at concentrations of 0.5%, 1%, and 1.5%, and subsequent oven drying. The nanofilter membrane will then be analyzed using SEM, XRD, and FTIR to characterize its properties. Performance tests will assess the quality of water used in goldfish farming, including pH, TOM, TDS, and TSS after treatment with the nanofilter. Morphological results show a rougher and denser surface with dispersed CuO-NPs. Characterization reveals cellulose I with crystallinity values ranging from 86.80% to 90.20%, with functional group peaks at 3145, 2927, and 1735 cm^{-1} indicating cellulose characteristics, and a peak at 424 cm^{-1} indicating Cu-O bonds. Statistical analysis of performance tests on the water quality of goldfish farming in the F-test shows significant differences ($p<0.05$), with pH values ranging from 8.16-8.53, TOM values <20 mg/L, TDS values <500 mg/L, and TSS values <25 mg/L, indicating that the BC/CuO-NPs 1% nanofilter is the most effective in reducing levels to more optimal values compared to the water before filtration.

Keywords: *Bacterial cellulose, goldfish, nanofilter, CuO-NPs, Sargassum sp.*

I. Introduction

In maintaining goldfish (*Carassius auratus*) farming, water quality management is crucial to ensure it meets the necessary criteria to support their livelihoods [1]. Poor water quality management can result in high acidity levels (pH) and ammonia in the water, sourced from fish feces, uneaten feed residues, and organisms within the aquarium environment, including bacteria, fungi, and infusoria [2]. The water quality management process in aquaculture often involves filtration using filters before entering fish farming areas. There

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are various types of filters, including mechanical filters, biological filters, chemical filters, and microfilters [3]. However, no filter can effectively filter out all small particles, whether organic matter or bacteria, in a single filter. In relation to this, one filter with potential that is being increasingly developed is the use of membrane nanofilters [4].

Nanofilters are filtration systems that use nanotechnology in the form of membranes with nano-sized pores to filter particles, molecules, and dissolved substances passing through the filter in order to balance water quality conditions and achieve superior results. Nanofilter membranes are often made of a variety of nanomaterials, including metal nanoparticles, metal oxides, carbon-based nanomaterials, metal-organic frameworks, and micro or organic nanoparticles. These nanofilter membranes can be constructed with a variety of nanomaterials, including bacterial cellulose and metal nanoparticles. Nanomaterial-based nanofilters have several advantages, including high selectivity, strong stability, and emphasis on anti-fouling qualities [5].

Nanomaterials, such as copper oxide nanoparticles, are utilized in nanofilter production. Copper oxide (CuO-NPs) efficiency and stability have made it popular as the most straightforward copper compound. Despite sharing some properties with noble metals like silver and gold, CuO-NPs is more cost-effective. It also readily integrates with polymers and maintains considerable chemical and physical stability [6]. In addition, CuO-NPs's antibacterial properties also allow it to release ions that bind to bacterial cell walls, inhibiting electron transport within bacterial cells in their surroundings [7], [8]. CuO-NPs nanoparticles can kill bacteria by reacting with peptidoglycan. They exhibit more effective bactericidal properties and can easily be blended with other polymers to achieve stable chemical and physical characteristics. CuO-NPs is a preferred metal material due to its relative affordability, accessibility, and ease of synthesis [7].

An eco-friendly alternative filter material is bacterial cellulose nanofibers derived from macroalgae. The selection of bacterial cellulose (BC) is due to its lightweight, unique structural and mechanical properties, high crystallinity compared to higher plants cellulose, high aspect ratio with diameters of 20-100 nm, high liquid loading capacity (highly hydrophilic properties), non-toxicity, renewability, biocompatibility, biodegradability, and high chemical purity. Unlike higher plants, BC does not require chemical treatment to remove hemicellulose and lignin [9], [10]. BC is an exopolysaccharide made of β-1,4 D-glucopyranose units, produced by aerobic microorganisms including gram-positive and gram-negative bacteria, fungi, and yeast [11]. The cellulose structure is termed cellulose I, with BC fibril diameters of 0.1 µm, 300 times smaller than wood fiber. BC fibrils possess a large surface area, high water-binding capacity, and tensile strength [12].

Sargassum sp., brown algae high in polyphenols, polysaccharides, and chromene compounds, is a widespread but underutilized marine macroalgae [13]. The goal of this study is to create a nanofilter for carp breeding constructed of Sargassum sp. and strengthened with copper oxide nanoparticles. The incorporation of copper oxide nanoparticles aims to improve the nanofilter's filtering effectiveness, particularly against biological contaminants, hence improving air quality.

II. Material and Methods

1. Materials

Research methods involve experimental procedures conducted at the Molecular Biology and Genetics Laboratory, Faculty of Science and Technology, UIN; Nano Research and

Advanced Materials, Faculty of Engineering, UM; and the Sumberpasir Research Center, Faculty of Fisheries and Marine Sciences, UB, The water samples used are from nursery pond, grow-out pond, and recirculating aquaculture systems (RAS) pond of goldfish (*Carassius auratus*) in Wajak Lor, Boyolangu District, Tulungagung.

The equipment used includes beaker glass, spatula, analytical balance, pipette dropper, oven, blender, furnace, 10-liter jars, magnetic stirrer, hotplate magnetic stirrer, High-Pressure Homogenizer (HPH) (AH-100D, Berkley Scientific), ultrasonic homogenizer (Sonicator), Scanning Electron Microscopy (SEM), X-ray diffraction (XRD), and Fourier Transform Infrared Spectrometer (FTIR) testing equipment. The materials used are *Sargassum* sp. (supplied from Madura, East Java), 2 ml tubes, distilled water, starter bacteria (*Acetobacter xylinum*, from Biotechnology Development Center, University of Muhammadiyah Malang, Indonesia), urea, sugar, NaOH, bacterial cellulose, 1% NaOH, glacial acetic acid (Merck, Germany), acetic acid (Merck, Germany), sulfuric acid (Merck, Germany), acetic anhydride (Merck, Germany), and CuO-NPs.

2. *Extraction of Sargassum sp. and BC Pellicle Synthesize*

BC production followed established procedures as detailed in a previous publication [14]. Initially, *Sargassum* sp. was blended and filtered to obtain its extract. Subsequently, 2L of water was heated on a hot plate until boiling. Suspension was prepared by adding 50 g of urea and 150 g of sugar. The boiled medium was then cooled to room temperature. A 20% (v/v) solution of *A. xylinum* was inoculated into the culture medium, and after 10 days, a pellicle formed on the surface of the medium, which was then harvested. The pellicle was treated by boiling in 1% sodium hydroxide (NaOH) at 90°C for two hours and subsequently rinsed with water to remove contaminants.

3. *Nanofilter Synthesize*

The pellicle was washed until it reached neutral pH. Subsequently, 50 grams of pellicle were mixed with 1 liter of water and blended for 5 minutes at 26,000 rpm using a blender. The mixture was further homogenized through 5 cycles at 150 bar pressure using an HPH, and the resulting solution was filtered using Whatman paper 42. The sample was then treated with acetate with 5 grams of nanocellulose sample, to which 125 ml of glacial acetic acid, 45 ml of acetic acid, and 0.4 ml of sulfuric acid (98%) were added and stirred. Then, 160 ml of acetic anhydride was added and stirred for 30 minutes, followed by incubation of the cellulose solution for 14 hours. After incubation, vacuum filtration was performed and washed with distilled water until reaching pH 7.0 (25°C) [15]. Then, CuO-NPs (0%, 0.5%, 1%, and 1.5%) was added, followed by stirring and drying using an oven.

4. *Surface Morphology*

Surface morphology testing on the nanofilter membrane was conducted using SEM (Inspect-S50 type, FEI). Prior to testing, the surface of the nanofilter membrane was coated with 10 nm of gold using a sputter coater.

5. *Crystallinity Analysis*

Crystallinity testing on the nanofilter membrane was performed using XRD. The XRD sample was cut into 1 cm^2 in size, then scanned with a 2θ angle range from 5° to 90° using CuKα radiation (λ = 1.54 Å) at 30 mA and 40 kV [16]. The crystallinity index (CI) was calculated using the Segal method:

$$CI = \frac{I_{(002)} - I_{(am)}}{I_{(002)}} x\ 100\% \quad (1)$$

6. Functional Group Analysis

The analysis of functional group changes in the nanofilter membrane was conducted using FTIR (Shimadzu IR Prestige-21). Spectra were recorded with a resolution of 4 cm^{-1} over the range of 400-4000 cm^{-1}.

7. Water Quality Analysis

The application procedure involves using a filter membrane positioned at the bottom of the syringe. Then, 200 ml of water sample is placed into the barrel and manually pressed so that it passes through the filter [17]. The analysis of water quality changes in fish farming ponds, conducted before (prefiltration) and after (postfiltration), includes pH, TOM (total organic matter), TDS (total dissolved solids), and TSS (total suspended solids).

III. Results and Discussions

1. Morphology Analysis

The use of CuO-NPs in BC causes changes in the membrane's surface structure, as shown in Figure 1.

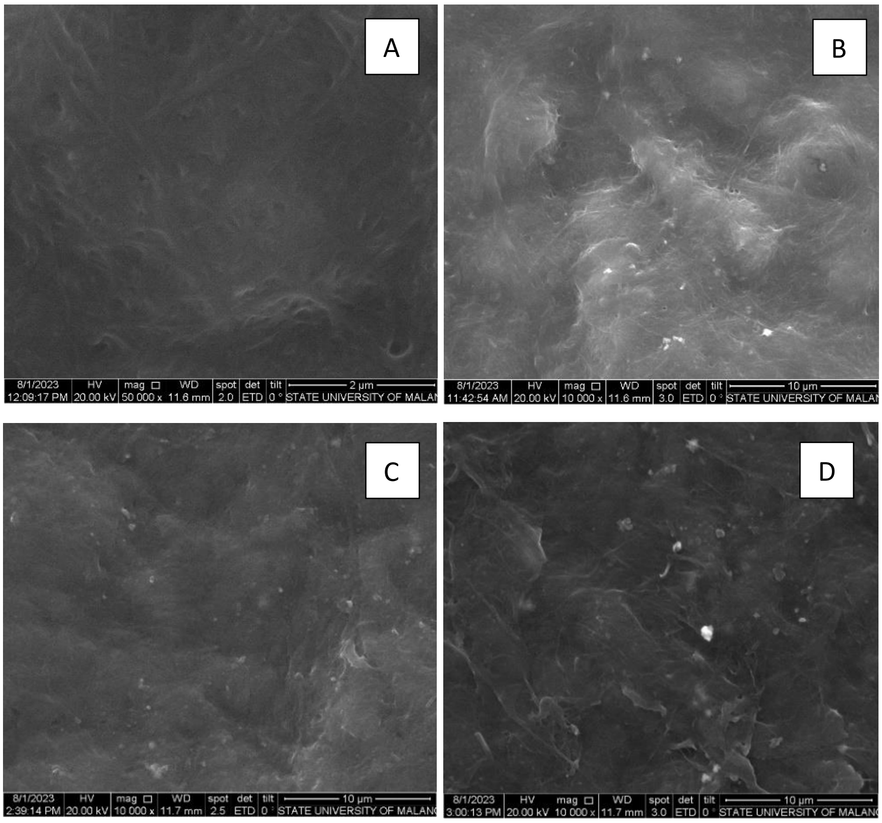

Fig. 1. Surface morphology of nanofilter BC (A), nanofilter with 0.5%wt CuO-NPs (B), 1%wt CuO-NPs (C), and 1.5%wt CuO-NPs (D)

This change reveals a smooth surface with small pores [18]. Acetate-treated BC also produces denser fibers, as shown in Figure 1A, which appear compact with small pores and no white particles. The incorporation of CuO-NPs concentrations of 0.5 %wt, 1.0 %wt, and 1.5 %wt results in cellulose fibers forming on the membrane's surface (Figure 1B, 1C, 1D). In addition to clustering on the surface, CuO-NPs are also dispersed within the BC membrane, leading to higher accumulation of CuO-NPs. The interaction between BC and CuO-NPs can be clearly observed through the presence of white particles in Figure 1. This can occur due to the ultrasonic homogenization's ability to break particle bonds, reducing agglomeration. The smooth surface morphology and small pores will be essential for aiding the filtration process of particles ranging from micro to nano sizes.

2. Crystallinity Analysis

Figure 2 shows the XRD diffraction angles obtained during characterization. In all samples, three prominent peaks are observed at 14.4°, 16.8°, and 22.5°, indicating the characteristics of pure cellulose I. Table 1 illustrates the comparative effects of incorporating CuO-NPs into the bacterial cellulose network. The crystallinity of the nanofilter is described by the crystallinity index (CI). Using the Segal equation, the crystallinity index for each sample was calculated, with cellulose crystallinity observed at an angle of 22.6° [19]. As shown in Table 1, this phenomenon occurs because cellulose polymer chains tend to form crystalline conformations due to intermolecular interactions between cellulose chains [14]. Fluctuations in crystallinity, combined with increased nanofibril crystallization, can lead to a decrease in crystallinity. The addition of CuO-NPs is indicated to lower the CI value due to the presence of foreign substances entering the BC, thereby altering its crystalline structure, as evidenced by the appearance of peaks at 35.4° and 38.6°[20] which corresponds to the crystal plane in the CuO-NPs monoclinic phase [21], [22]. Furthermore, the addition of CuO-NPs can affect the peak angle of bacterial cellulose, resulting in decreased crystallinity (Table 1) and subsequent changes in other properties.

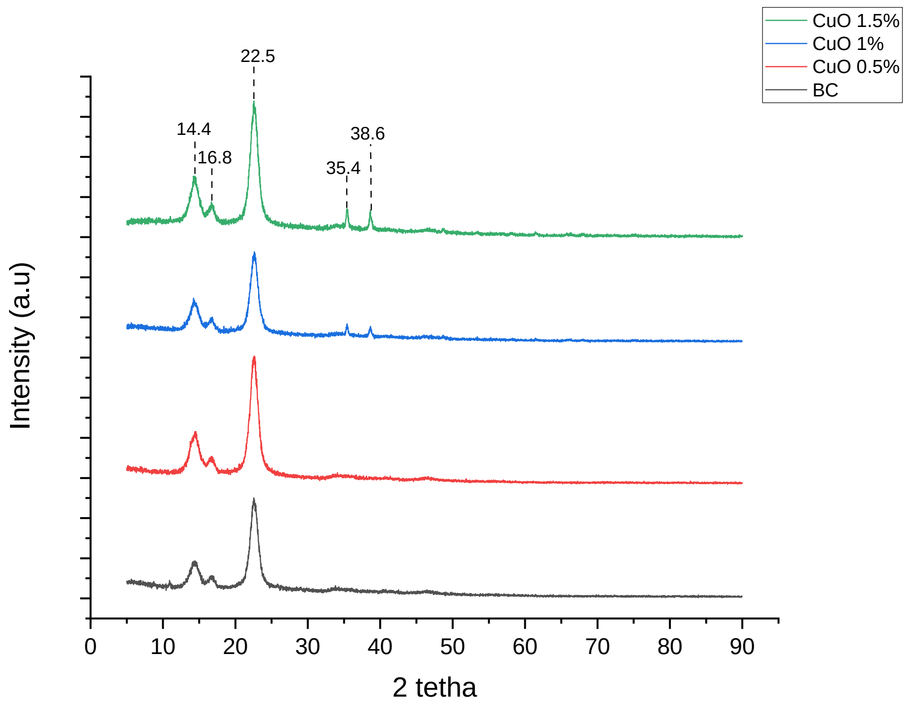

Fig. 2. X-ray diffraction of nanofilter BC/CuO-NPs

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Table 1. Crystallinity and peak of nanofilter BC/CuO-NPs

Samples	Diffraction angle (degree)					Crystallinity index (%)
BC	14.4	16.6	22.5	-	-	89.14
CuO-NPs 0.5%	14.4	16.6	22.5	-	-	89.10
CuO-NPs 1%	14.2	16.8	22.6	35.4	38.6	86.70
CuO-NPs 1.5%	14.4	16.6	22.5	35.4	38.6	86.80

3. *Functional Group Analysis*

The characteristic spectrum of bacterial cellulose, as shown in Figure 3 and Table 2, shows a broad peak at the wavenumber range of 3600 to 3400 cm^{-1}, associated with the presence of hydroxyl groups (–OH) related to the stretching of intra and intermolecular hydrogen-bonded OH groups. Meanwhile, at wavenumbers 2945 to 2927 cm^{-1}, C-H stretching vibrations occur, and at 1735 cm^{-1}, C=O stretching in carboxylate groups is observed. Additionally, in the fingerprint region with wavenumbers below 1500 cm^{-1} [23] [24], changes occur in the range of 640-400 cm^{-1} [24], indicating Cu-O bond stretching. The FTIR characterization results do not show significant peak changes, but the addition of CuO-NPs can cause differences in some functional groups. By comparing the obtained graph with similar cellulose diagrams in previous studies[25], the matching of the graph and chemical bonds indicates that the layer produced in this study is bacterial cellulose bonded with CuO-NPs. CuO-NPs bonded with BC in the nanofilter can significantly inhibit bacterial growth and therefore has the potential to become a nanofilter for fish farming.

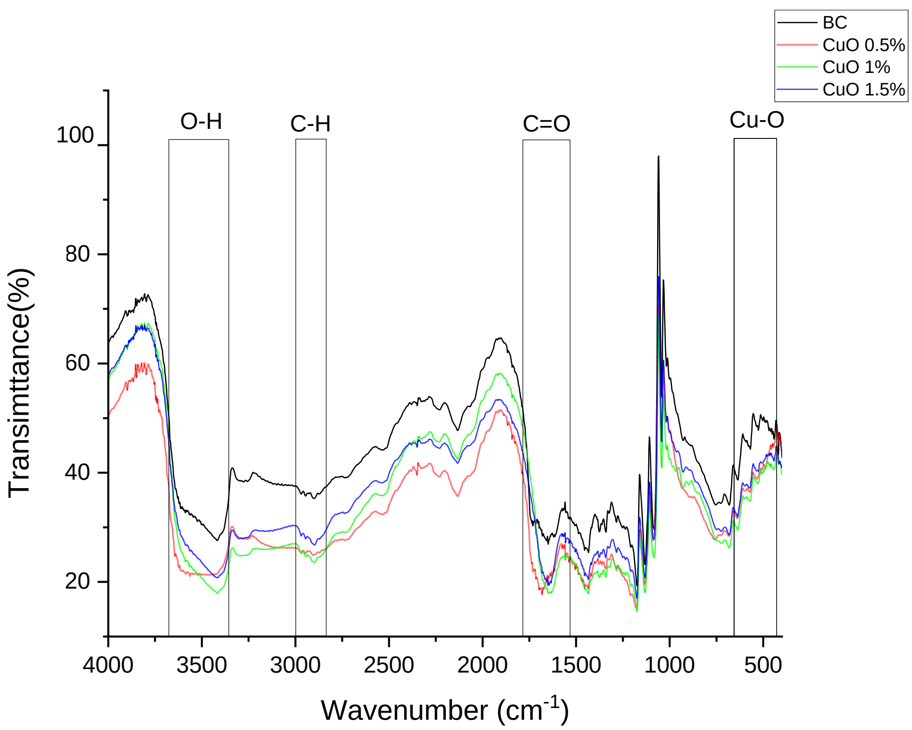

Fig. 3. FTIR spectra of nanofilter BC/CuO-NPs

Table 2. Functional groups analysis of nanofilter BC/CuO-NPs

No.	Peak of nanofilter BC/CuO-NPs				Assignment
	BC	BC/CuO-NPs 0.5%	BC/CuO-NPs 1%	BC/CuO-NPs 1.5%	
1.	3415	3415	3415	3415	O-H stretching [26]
2.	2927	2927	2927	2927	C-H stretching [27]
3.	1735	1735	-	-	C=O stretching [28]
4.	-	424	424	428	Cu-O vibration [29]

4. *Water Quality Analysis*

A. *pH*

pH measures the concentration of hydrogen ions in water, providing information about its acidity or alkalinity [30]. The pH parameter indicates the concentration of hydrogen ions released into the water, reflecting the balance between acids and bases. The pH values in nursery, grow-out, and RAS ponds in goldfish cultivation before filtration were 8.5, 9.12, and 8.28, respectively. pH measurements before and after filtration using BC nanofilters and nanofilters containing CuO-NPs at concentrations of 0.5%, 1%, and 1.5% can be seen in Figure 4. The average water pH in the study area's ponds was slightly below the recommended range of 6.5 to 8.5 [31][32]. Based on Figure 4 shows a significant difference ($p<0.05$), the water pH decreased after filtration to around 8.16-8.53, with the optimal pH value achieved by the BC/CuO-NPs 1%wt nanofilter, indicating optimal conditions in the filtration process and not affecting the growth and survival of the fish. Changes in pH can impact fish physiology, including stunted growth, increased sensitivity to bacteria and parasites, and making the water toxic for fish. High pH levels in water can increase un-ionized ammonia, affecting the toxicity of chemical compounds [4]. During the study, there were no significant fluctuations in pH range, ensuring it did not affect water quality or the survival of the goldfish.

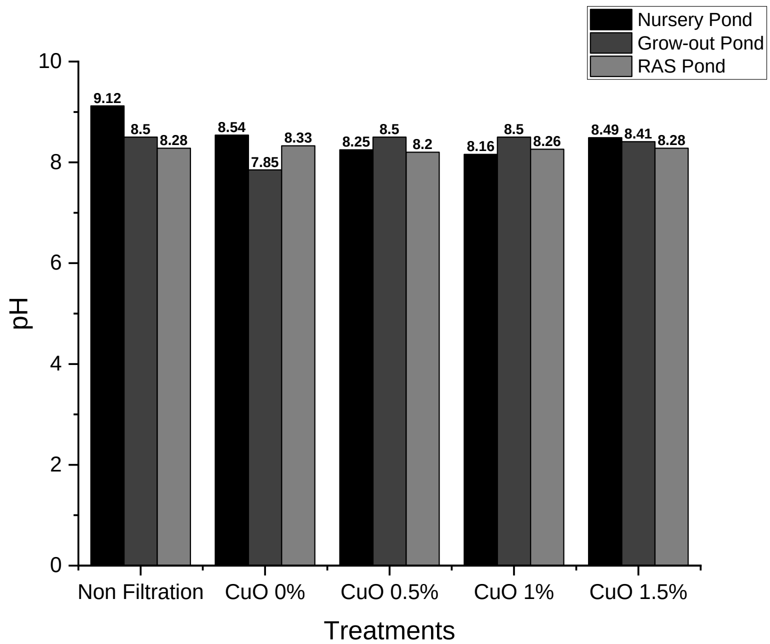

Fig. 4. pH measurement result (non-filtration divided to nursery pond, grow-out pond, RAS pond)

B. Total Organic Matter (TOM)

Total Organic Matter (TOM) is an indicator of the amount of organic matter in water primarily through food and excretions (feces and urine) which can affect the aquatic ecosystem [33]. TOM also indicates the dissolved oxygen consumption required to oxidize dissolved organic matter in water. High levels of organic matter in aquatic environments continue to pose significant challenges in fish farming, as this condition is a primary cause of pathogenic infections. In the nursery, grow-out and RAS pond used for goldfish farming, TOM values before filtration were 6.32, 3.792, and 2.528, respectively. The measurements of TOM values show a significant difference ($p<0.05$) before and after filtration using nanofilters BC and nanofilters with the addition of 0.5%, 1%, and 1.5% CuO-NPs can be seen in Figure 5. TOM values in water are considered good if they are less than 20 mg/L [34]. The TOM values in the nursery and grow-out pond were obtained through filtration with a BC/CuO-NPs 1.5%wt nanofilter, while the best TOM values for the RAS pond were achieved using a BC/CuO-NPs 1%wt nanofilter. Nevertheless, the TOM values obtained remained within the optimal range. This indicates that the BC/CuO-NPs nanofilter has pores capable of filtering dissolved organic matter, keeping the values within the optimal range for each treatment.

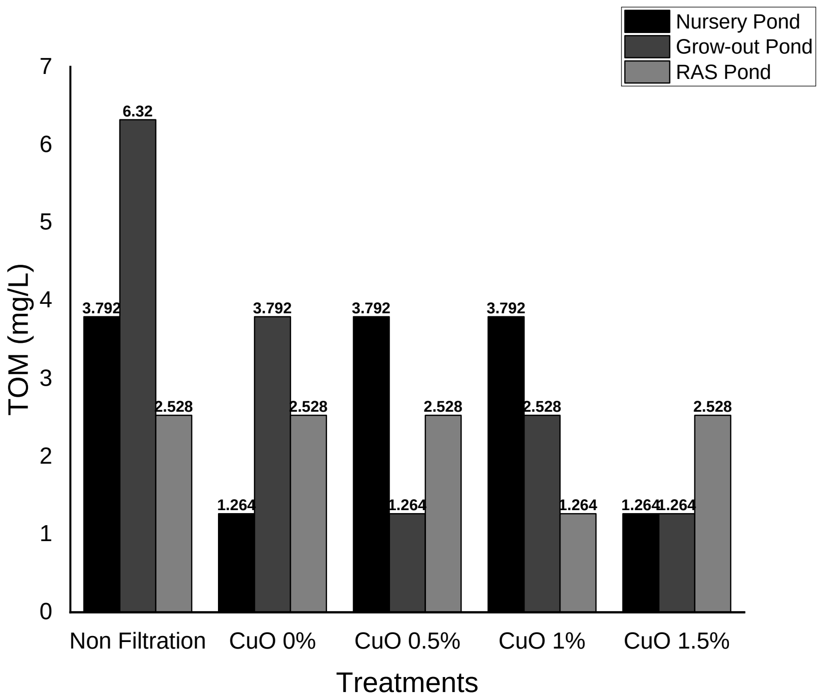

Fig. 5. Total organic matter measurement results (Non-filtration divide to nursery pond, grow-out pond, RAS pond)

C. Total Dissolved Solid

TDS naturally occur in water, comprising organic molecules and minerals that offer nutrients, and introduce contaminants like organic pollutants and toxic metals to indicate the concentration of dissolved substances in water[35]. Before filtration, the TDS values in ponds A, B, and C for goldfish farming were 334 mg/L, 412 mg/L, and 331 mg/L, respectively. Measurements of TDS show a significant difference ($p<0.05$) before and after filtration using BC nanofilters and nanofilters with 0.5%, 1%, and 1.5% CuO-NPs additions can be seen in Figure 6. The optimal TDS value for fish farming is <1000 mg/L [32]. After filtration with nanofilters, the TDS values in goldfish farming water decreased to approximately 150-244 mg/L, with the most optimal TDS value achieved when filtering with the BC/CuO-NPs 1%wt nanofilter. The presence of this means that the BC/CuO-NPs nanofilter has small pores that can filter not only insoluble particles but also dissolved particles to maintain optimal levels of dissolved substances [36]. Furthermore, lower concentrations of dissolved substances are more beneficial for fish maintenance. High TDS levels can cause stress in fish, disrupt osmoregulation, and increase the risk of disease, while low TDS levels can lead to deficiencies in essential minerals needed for fish growth.

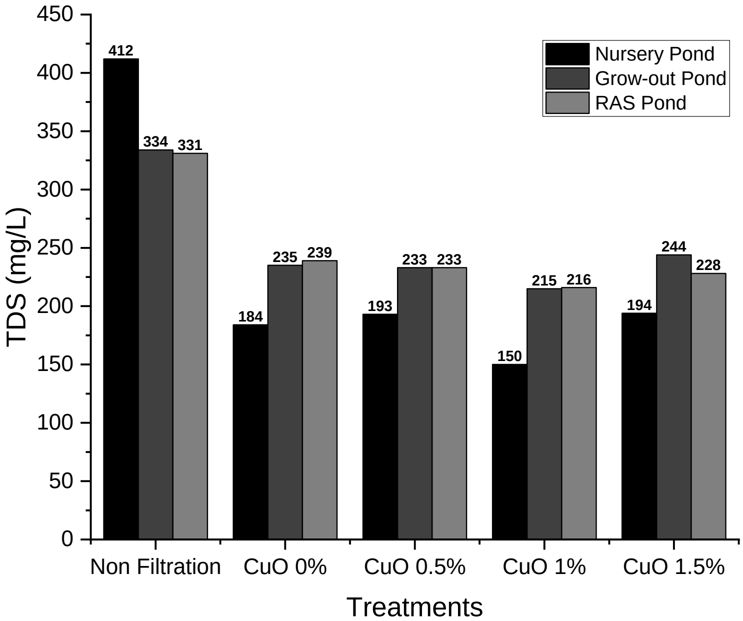

Fig. 6. Total dissolved solid measurement results (Non-filtration divided to nursery pond, Grow-out pond, RAS pond)

D. Total Suspended Solid

TSS refers to particles that are large enough to be retained by a filter, preventing them from passing through with the water[37]. Before filtration, the TSS values in ponds A, B, and C for goldfish farming were 0.014, 0.088, and 0.032, respectively. The measurements of TSS values show a significant difference ($p<0.05$) before and after using nanofilter BC and nanofilter with additions of CuO-NPs 0.5%, 1%, and 1.5% can be seen in Figure 7, the ideal conditions for water in fish farming based on TSS values are <25 mg/L, with a maximum of 80 mg/L [32] [36], while the results after filtration range between 0.008-0.046 mg/L, and the most effective for filtering out insoluble solids is BC/CuO-NPs 1%wt nanofilter. Suspended solids typically refer to substances like plankton, fish waste, uneaten fish feed, or clay particles in water that gradually settle out over time from accumulated water[34]. An increase in TSS values can be caused by water turbidity, which can hinder light penetration into aquaculture water. This study indicates a decrease in TSS, which means the BC/CuO-NPs nanofilter can reduce suspended particles in aquaculture water values after filtration with nanofilters. TSS (Total Suspended Solids) concentrations can rise in turbid water due to the buildup of organic and inorganic particles, causing an imbalance in bacterial populations, increasing the concentration of hazardous chemicals, and affecting fish health. Inadequately handled suspended particles can cause direct injury to fish by blocking their gill function, creating stress, and weakening their immune systems [3]

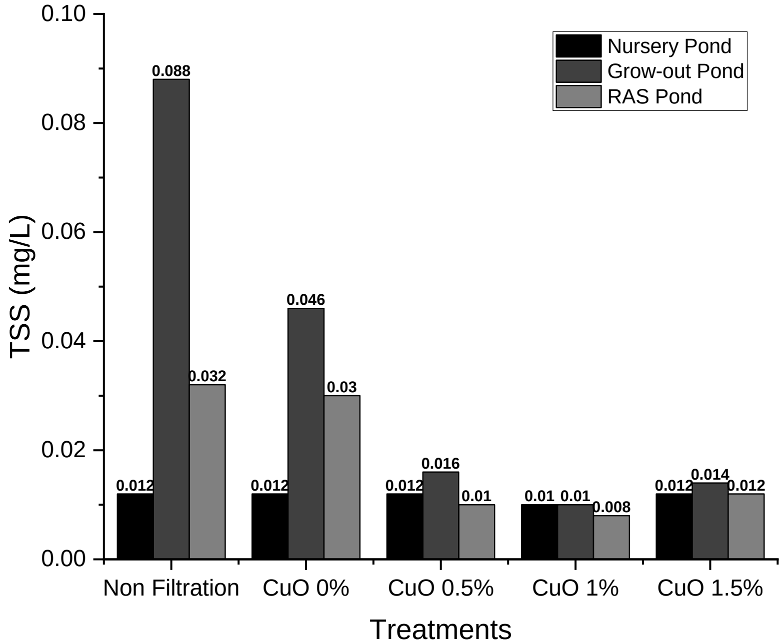

Fig. 7. Total suspended solid measurement result (Non-Filtation divide to Nursery pond, Grow-out pond, RAS pond)

IV. Conclusions

This study shows that bacterial cellulose grown on Sargassum sp. extract becomes an alternative material with good characteristics for use as a membrane nanofilter, and the addition of CuO-NPs also has potential for filtering pond water in goldfish (*Carassius auratus*) farming to improve water quality for more optimal conditions, thereby increasing the productivity of goldfish. Post-filtration results show that the nanofilter, particularly BC/CuO-NPs 1%, effectively filters organic and inorganic pollutants that could degrade water quality in terms of physical, chemical, and biological aspects. In this regard, further research is needed on the use of membrane nanofilters in relation to the physiology, biology, and production of goldfish, as well as other fish species.

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