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Original Research Article

Efficiency Of Silver Nanoparticle Coated Filtering Device For The Control Of Bacterial Pathogen In Ornamental Fish Culturing System

Enilda Rexy Antonykennedy[1], Sivanadanam Vaithilingam[2], Serinemichael Michaelbabu[1], Citarasu Thavisimuthu[1], Selvaraj Thangaswamy[1], Mary Josephine Punitha Satniuslas[1], Michaelbabu Mariavincent[1*]

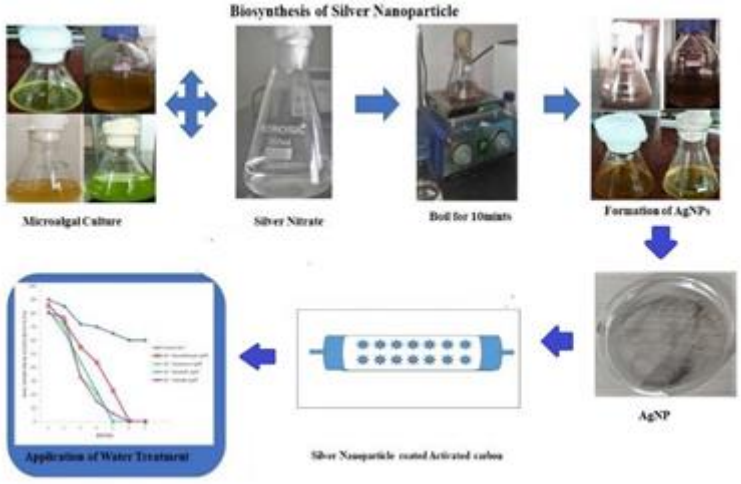
[1]*Centre For Marine Science And Technology, Manonmanium Sundaranar University, Rajakkamangalam, Kanyakumari District, Tamil Nadu, India

[2]Lekshmipuram College Of Arts And Science, Neyyoor, Kanyakumari District, Tamil Nadu.

***Corresponding Author:-** Dr. Michaelbabu Mariavincent

*Assistant Professor, Planktology And Aquaculture Division, Centre For Marine Science And Technology, Manonmaniam Sundaranar University, Rajakkamangalam - 629502, Tamil Nadu, India.
Email. ID; Michaelmsu@Live.Com

GRAPHICAL AB STRACT



ABSTRACT

Nanoparticles are of great interest because they are extremely small in size and large in surface-to-volume ratio, which lead to both chemical and physical enhancements in their properties compared to their bulk composition. Tremendous growth in nanotechnology has opened up novel, fundamental, and applied frontiers in material science and engineering, nanobiotechnology, surface-enhanced Raman spectroscopy, and applied microbiology. In this study, the cell free extract of four micro algae were used for the biosynthesis of Ag-NPs. The size, shape and antimicrobial activity were also determined. The effectiveness of the four silver nanoparticles (Ag-NPs) produced by microalgae and biosynthesized and coated on activated carbon (AC) granules was evaluated in fish culture tanks. The cell-free extract derived from the various species of microalgae influences the size, shape, and antibacterial activity of the silver nanoparticle. Scanning electron microscopy was used to characterise the Ag-NPs/AC that was produced (SEM). The synthesised Ag-NPs/AC had effective antibacterial action against aquatic diseases, and its efficacy may be increased by adding a cell-free extract from the chosen algal species. A silver nanoparticle-coated activated carbon filter

fabricated in this study was found to be cost-effective and simple-to-use as an antibacterial water filter in ornamental fish culture tanks.

Keywords: Microalgal Biosynthesized silver nanoparticle, Cell free extract, Activated carbon, Nano biotechnology, Antibacterial activity

INTRODUCTION

Nanotechnology is a highly energized discipline of science and technology, which is gaining importance in the new millennium. Because of their unusual optical [1], chemical [2], petrochemical [3], and electronic properties [4] nanomaterials are of great interest. These nanoscale materials have gained importance due to their potential applications in optics, biomedical sciences, mechanics, magnetics and energy science. Hence, one of the various challenges of nanotechnology is the synthesis of nanomaterials with a wide range of chemical compositions and sizes. There is a growing need for and awareness of green technologies in today's world. When compared to the bigger particles of the bulk materials, nanoparticles have completely new or better qualities due to its unique characteristics such as grain size, dispersion, shape, and higher surface to volume ratio [5]. Nanoparticles exhibits size and shape-dependent properties which have potential applications in chemical sensors, biosensors, antimicrobial activity, DNA binding, catalysis, optics, computer transistors, electrometers, wireless electronic logic, memory schemes, medical imaging, nanocomposites, water filtration, drug delivery, and hyperthermia of tumors.

Phyconanotechnology is the upcoming exciting research area, providing widespread opportunity in the synthesis of algae-based nanoparticles [6]. Microalgae, in particular, are attracting a lot of attention because of their diversity, availability, and physiological characteristics. The aim is to biosynthesize nanoparticles using microalgal extracts by highlighting the role of silver nanoparticles in the conversion of silver salts into nanosilver and the lack of use of microalgal extracts in this research. The biological activity of AgNPs depends on the factors including surface chemistry, size, size distribution, shape, particle morphology, particle composition, coating/capping, agglomeration, and dissolution rate, particle reactivity in solution, efficiency of ion release, and cell type, and the type of reducing agents used for the synthesis of AgNPs [7]. The physicochemical properties of nanoparticles enhances the bioavailability of the therapeutic agents after both systemic and local administration [8] and on the other hand it can affect the cellular uptake, biological distribution, penetration into biological barriers, and resultant therapeutic effects [9] and [10]. Therefore, the development of AgNPs with controlled structures that are uniform in size, morphology, and functionality are essential for various biomedical applications [11, 12, 13, 14]. Algal biomass can be easily harvested and the intracellular nanoparticles can be resealed by disrupting the cell by a commercially available processing equipment. Because of its enormous surface area and excellent adsorption capacity, activated carbon is one of the most extensively utilized nanoparticles for water filtration [15].

It has been demonstrated that activated carbon works well as an adsorbent to remove a variety of organic and inorganic pollutants from gaseous and aqueous environments [16] and [17]. The green chemistry method for the synthesis of microalga nanoparticles might be regarded as technically feasible due to its economic viability and low-cost scale-up. Thus, using entire microalga cells as well as boiling algal extract, the current study presents a simple, innovative, and environmentally friendly bio reduction approach for the biosynthesis of SNPs. Besides this possibility, the synthesis of very stable SNPs utilizing a boiled extract of microalgae biomass has a significant advantage over the other biological approaches which are now in use. Biosynthesized SNPs could be used as an effective antibacterial agent in a variety of biological applications due to their high antibacterial activity. The present work aims to synthesize different morphological and functional types of AgNPs mediated by the cell free extract obtained from the different algal species and to fabricate and install an in situ bacterial filter to control pathogens in the ornamental fish culture tanks.

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MATERIALS AND METHODS

Collection of microalgae

To isolate microalgae from the saltpan water, water samples were collected from Puthalam saltpan (Long: 77° 28' 0.7968" E; Lat: 8° 6' 21.9816" N) in Kanyakumari district. The water samples were taken from both the surface and the middle of the water column. The collected samples were packed in transparent plastic bottles and marked for identification then brought to the laboratory for further study. The cultures were maintained through usual sub-culturing techniques under the laboratory conditions at 25° C, under cool white fluorescent light (30 µmol photons m^{2} s^{1}), in Walne's medium. The microalgae identification was done based on the standard taxonomic monograph [18] and [19].

Preparation of cell free extract of Micro algae for nanoparticle biosynthesis

In an Erlenmeyer flask, 1.5 g (wet weight) algal biomass was suspended in 30 ml of double distilled sterile water for 30 minutes at 100°C. The mixture was boiled, then cooled and centrifuged for 15 minutes at 5,000 rpm. The supernatant was collected and kept at 4 ° C for later analysis.

Biosynthesis of Silver Nanoparticles from the isolated microalgae

2ml of pure microalgal extract was added dropwise into 100ml of 1mM of nitrate solution in a 250ml conical flask in the standard synthesis method of silver nanoparticles. For 10 minutes, the reaction mixture was kept at 600°C with constant mechanical stirring. The reduction of Ag ions into AgNPs was completed in less than 10 minutes, showing fast AgNP synthesis, while the pH remained between 4.7 and 5.0 during the reaction. A UV-Visible spectrophotometer was used to monitor the colour change and the production of nanoparticles. The prepared silver nanoparticles were centrifuged at 15,000 rpm for 20 minutes at 40°C. The supernatant was discarded and the pellet was collected. To remove the contaminants, the pellet was rinsed several times with distilled water and then treated with 90% ethanol to produce pure AgNPs powder.

Activated Carbon (AC) Treatment

The AC granules utilized in this investigation (commercial kind) were ground and sieved to a 1 mm size before being purified using hot conc. HNO_3, for at least three times. Prior to being coated with silver nanoparticles, AC was dried in an oven at 110°C. In order to know the efficiency of AgNPs coated activated carbon, the activated carbon packed cartridge was used as a positive control. The experiment was repeated four times with triplicates in order to obtain a consistent and a uniform result

Preparation of Activated and Silver Nanoparticle Coated Cartridge

One feat PVC tube of 1.5 inch diameter was taken and its one end was closed with PVC end cape. One hole was made at the centre of the cape to fix the 'I' joint to connect the tube. After fixing one cape, a round-shaped sponge of 1.5 inch diameter was placed at one end of the tube. This will prevent the adsorbent (activated carbon coated with silver nanoparticle) from exiting. After placing the round sponge at one end of the tube, the adsorbent was packed tightly into the full length of the tube. After ensuring the water flow through the coarsely broken pieces of the activated carbon, another end was also closed with a round-shaped sponge of one inch thickness and then with an end cape. The tube was then connected with the immiscible fish tank water pump. The water circulation rate was monitored by regulating the pump speed. The particulate waste in the water of the culture tank was sieved by a pad of cloth and cotton before the water was sent through the absorbent packed tube. The deposited waste was periodically removed. This will prevent the clocking of the waste particles into the adsorbent and thereby increase the life of the filter (Fig. 1).

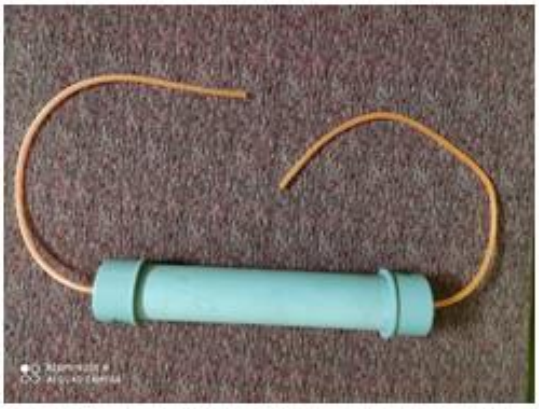

Fig. 1 Fabrication of Filtering Cartridge

Coating of Silver Nanoparticles onto the Activated Carbon Granules

To ensure a thorough coating, 250 ml of a silver nanoparticle solution with varying concentrations (500, 1000, 2000, and 4000 ppm) was impregnated with 50 g of the treated AC granules while being vigorously stirred at room temperature for a whole night. The silver-coated activated carbon was cured in a vacuum oven at 110°C for at least two hours to make sure the silver nanoparticles were thoroughly coated on the carbon. The preparation was verified using the weight difference between the activated carbon granules before and after the coating process as well as the leftover silver in solution.

Evaluation of Biosynthesized Silver nanoparticle coated Activated Carbon - Filtration test

The water containing microorganisms collected from the fish tank inoculated with Vibriyo harveyi was purified using a lab size cylindrical column disinfection system of 50 gm Ag-NPs/AC (20mg Ag-NPs coated onto every one-gram activated carbon granules). At a flow rate of 70–75 ml/h^{-1}, a bacterial (Vibriyo harveyi) suspension with a concentration of approximately 105 CFU/ml was filtered down the column. The system was continuously running, and the filtrate was gathered every hour. Collected samples were plated on TCBS media plates, and following a 24-hour incubation period, the total CFU per plate was measured. This examination was offered three times. The effectiveness of colony forming units (CFU) reduction was assessed based on the CFU counts of the bacteria on the control plate. The cartridge containing various Ag-NP forms made from several species of microalgae were tested independently in a similar manner. The percentage reduction in colony forming units (CFU) efficiency was calculated using the bacterial CFU counts on the control plate:

Efficiency (%): (CFU of Control - CFU of Treatment)/CFU of Control * 100 …..[20]

RESULT AND DISCUSSION

Biosynthesis of Silver Nanoparticles from Maine microalgae

In this study, biological synthesis of AgNPs has been shown from cell-free aqueous microalgal extracts (Nanochloropsis sp., Chaetoceros sp., Dunaliella sp., and Chlorella sp.) When these extracts interact with the silver nitrate salt solution, they produce a dark brown solution due to the reduction of the silver ion to AgNPs, which is followed by a colour shift, suggesting biotransformation of ionic silver to reduced silver and hence the creation of AgNPs in an aqueous medium. This reaction results in the biosynthesis of AgNPs, as seen by a colour change from bright yellow to dark brown at the beginning of the reaction (Fig. 2). The brown colour confirms that it was due to the reduction of Ag+ which indicates the formation of AgNPs. The colour shift was observed visually, and the peak at 420 - 450 nm in the UV-visible spectra revealed the presence of AgNPs, which can flow from surface plasmon vibration excitation in AgNPs. . It is corroborated with the results reported by

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Saravanan et al., [21], Shivaji et al., [22] and Gopinath and Velusamy [23]. The first indicator of bioreduction of Ag ions to silver nanoparticle is the characteristic change in color of the algal suspension.

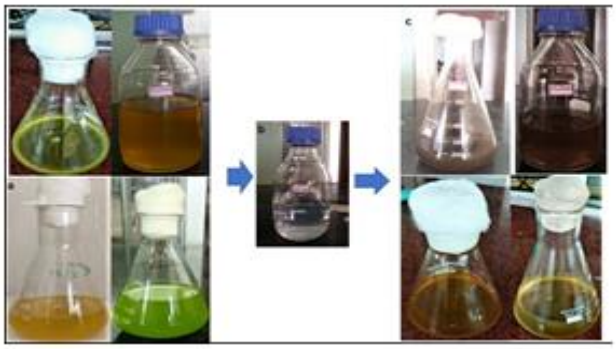

Fig. 2 Color change after the addition of algae extract with 1mm $AgNO_3$ indicates the formation of silver nanoparticles. (a) Microalgal extract b) silver nitrate solution c) Initial color change, (d) after 2hr

Characterization of silver nanoparticles

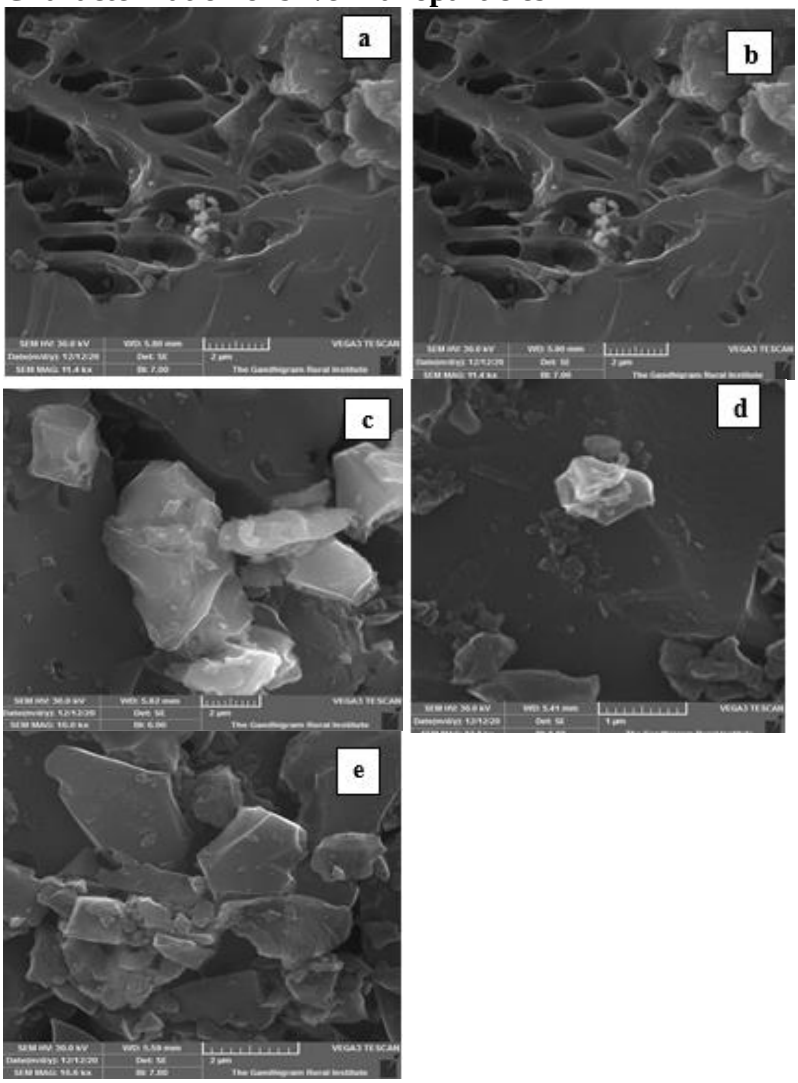

Fig. 3 SEM patterns show the distribution of Ag-NPs on the porous surface of the activated carbon a) AC-Nannochloropsis AgNP b) AC-Chaetoceros AgNP c) AC-Dunaliella AgNP d) AC-Chlorella AgNP e) Untreated activated carbon

On the surface and inside the pores of the activated carbon, AgNPs were distributed fairly uniformly according to SEM images (Fig. 3: a–d), but no AgNPs were seen on the untreated AC (Fig.3: e). AgNPs of different sizes can be found inside the porous area or can aggregate to form bigger particles. Ali et al. [24] used Oscillatoria willi NTDM01 extract in the reduction of the silver ions forming spherical AgNPs ranging from 100–200 nm in size, indicating the role of protein molecules as a capping agent in the synthesis process. The synthesised SNPs in this work were spherical in shape and ranged in size from 157.3 to 183.8 nm, as demonstrated in the SEM image of synthetic SNPs [25]. This report corroborated our findings. As previously demonstrated by Shipway et al. [26], Nie and Emory [26], the form of metallic nanoparticles significantly impacted their optical and electrical properties.

Testing in a dynamic scale-up setting (Filtration test)

Since it is a low-cost, high-efficiency filter, activated carbon (AC) has been effectively utilised for decades to purify water. The special characteristics of AC led to its selection as the filter material to receive a silver nanoparticle coating.

With pre-treated active carbon that had been covered in AgNP, a cylindrical column (100ml) was filled. The model employed in the dynamic experiment was Vibrio harveyi, while the control was untreated AC. According to the findings, the irregular clusters (17.66 nm), spherical (22 nm), truncated triangular (75.06 nm), and cubic (34.21 nm) shapes of the AgNPs-coated AC substrate were capable of killing up to 97-99% of the initial bacterial population within the first hour and continuing for the following five hours (Table 1). Recent results suggests that the silver nanoparticles undergo a shape-dependent interaction with the bacterial cells. Pal et al., [27] demonstrated that the truncated triangular silver nanoplates displayed the strongest biocidal action against E. coli, when compared with the spherical and rod-shaped nanoparticles and also with the silver ions. Similar conclusions were reached by Sharma et al. [28]. The influence of size on antimicrobial activity was also investigated by Baker et al. [29]. In this study, the antibacterial properties were related to the total surface area of the nanoparticles. Smaller particles with larger surface to volume ratios have greater antibacterial activity. Similar results were published by Choi and Hu [30] and these reports support our findings.

Table 1 General properties and Bactericidal Efficiency (%) of Microalgae mediated silver nanoparticle.

SI.NO	Microalgae mediated silver nanoparticle	Size of the AgNP	Shape of the AgNp	Bactericidal Efficiency (%)
1.	Nannochloropsis AgNP	17.66 nm	Irregular clusters	99%
2.	Chaetoceros AgNP	34.21 nm	Cubic	97.03%
3.	Dunaliella AgNP	75.06 nm	Truncated triangular	98.09%
4.	Chlorella AgNP	22 nm	Spherical	99.01%

After 24 hours of filtration, the efficiency stayed at 95%. (Figure 4a - j). In the control (filter with AC substrate but no AgNP coating), it was noted that the bacteria was also decreased by more than 90% at first, but the efficiency quickly fell to about 30% after 10 hours of filtration because the bacteria gradually clogged the porous carbon. As a result, the AC only filter has a relatively short lifespan. The results showed that by eliminating the bacteria that had attached to the filter surfaces, adding AgNPs to the filter substrate might greatly extend the filter's lifespan and improve its antimicrobial effectiveness. Once more, no Ag+ was found in the water that had been filtered. Overall, our research has shown that the pathogens were effectively eliminated from the air during filtering using AgNP-coated AC material. The impact of silver nanoparticles on microorganisms is seen in Fig.4. The growth was observed in the control (activated carbon), as shown in Fig. 5, while overall microbial counts were decreased to zero due to the antibacterial effect of the nano silver

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particles in 5 hours after filtration. Overall, our research has found that pathogens were successfully destroyed by AgNP-coated AC material during filtering. Even after 5 hours of filtration, up to 97 to 99 percent of bacterial cells were destroyed. The mechanism by which the nanoparticles are able to penetrate the bacteria is not understood completely, but the previous studies suggested that when Vibrio harveyi was exposed to silver, changes in its membrane shape occurred, leading to a huge increase in the permeability, which hampered the correct transport across the plasma membrane, rendering the bacterial cells which was incapable of effectively regulating transport across the plasma membrane, resulting in cell death. As a result, larger silver ion concentrations have a stronger bactericidal impact, and the antibacterial activity is time and concentration dependent. The current study shows that the activated carbon composites have the potential to be used in water purification and in controlling bacterial pathogens in small fish culture tanks.

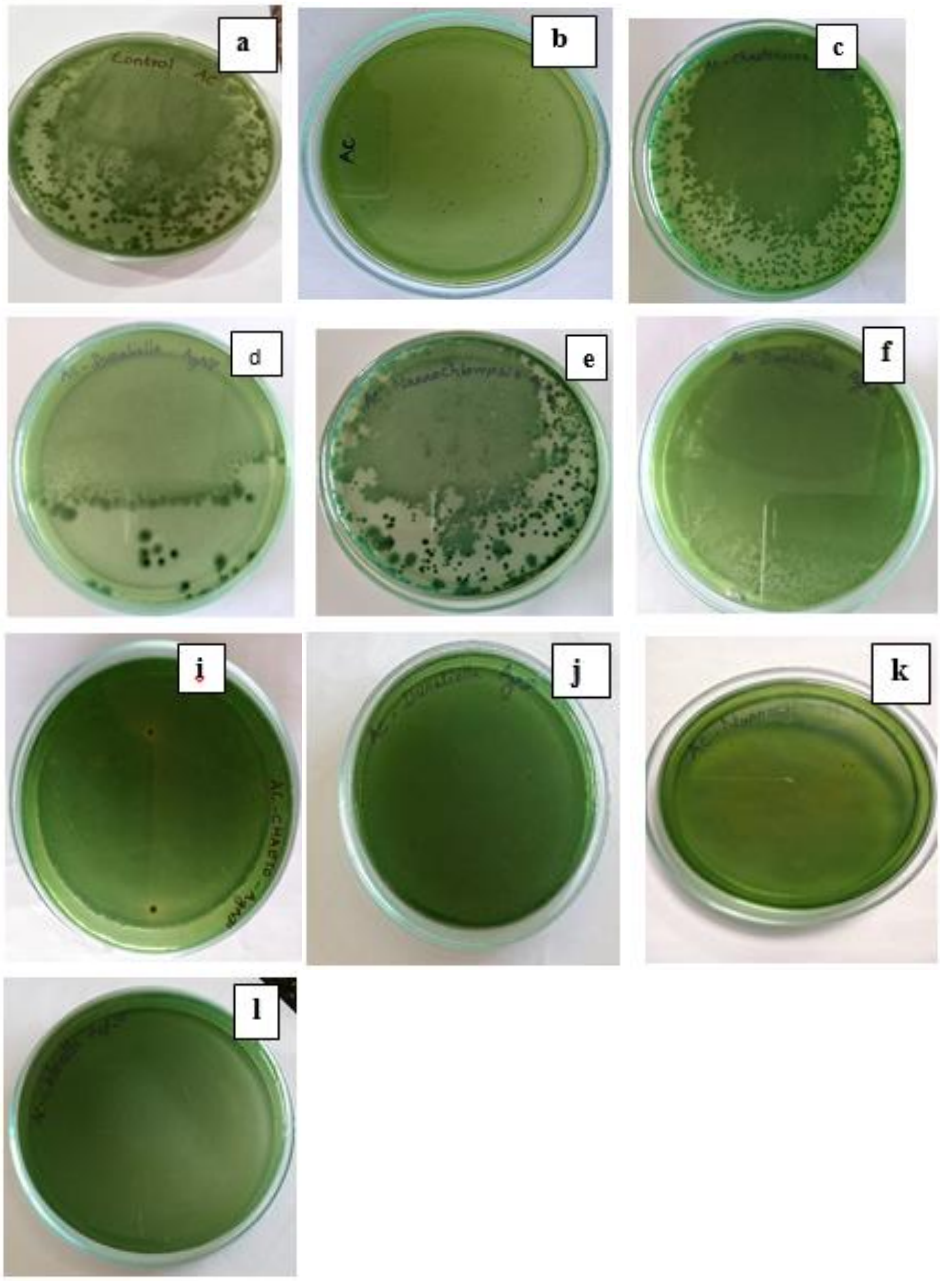

Fig. 4 Flow test result for the wastewater samples. (a-j) After passing through the blank activated carbon. (a&b) After passing through the nanoparticle-silver coating activated carbon. The bacterium count is clearly visible in (a-f), while the count was zero in (i-l).

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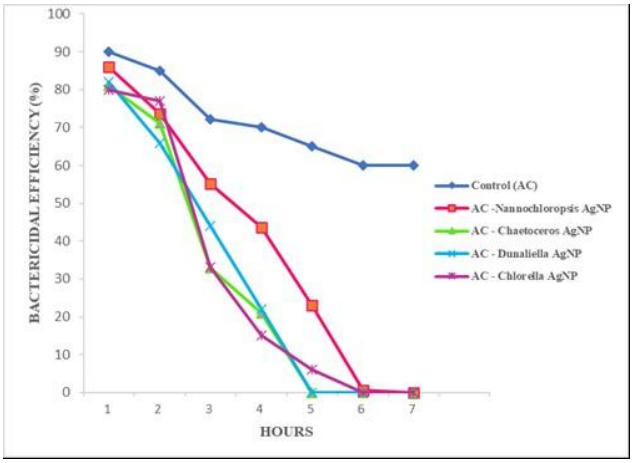

Fig. 5 Scale-up (100 ml) dynamic flow system efficiency test (AgNP -coated AC material against a bacterium Vibrio harveyi and Control - Filter column filled with uncoated AC)

Soaking time of the cartridge

To know the efficiency of the Silver nanoparticle coated activated carbon packed cartridge, outlet water from the ornamental fish culture tank was subjected to the filter. The total number of bacteria before and after filtering through the cartridge were tested and tabulated in Table 2. In the first day at 24 hours of filtration, the control had the bacterial count of 243 CFU/ml. In the experiment, activated carbon coated with Nannochloropsis AgNP, Chaetoceros AgNP, Dunaliella AgNP and Chlorella AgNP packed cartridge had the output bacterial count of 0.3, 0.5, 0.4 and 0.4 CFU/ml respectively.

On the 30th day the control had the bacterial count of 195 CFU/ml. In the experiment, activated carbon coated with Nannochloropsis AgNP, Chaetoceros AgNP, Dunaliella AgNP and Chlorella AgNP packed cartridge had the output bacterial count of 0.3, 0.5, 0.4 and 0.5 CFU/ml respectively. On 60th day, the control had the bacterial count of 157 CFU/ml. In the experiment, the activated carbon coated with Nannochloropsis AgNP, Chaetoceros AgNP, Dunaliella AgNP and Chlorella AgNP packed cartridge had the output bacterial count of 1.5, 2.3, 2.5 and 2.1 CFU/ml respectively. On 90th day the control had the bacterial count of 162 CFU/ml. In the experiment, activated carbon coated with Nannochloropsis AgNP, Chaetoceros AgNP, Dunaliella AgNP and Chlorella AgNP packed cartridge had the output bacterial count of 2.02, 2.5, 3.2 and 2.4 CFU/ml respectively. On 120th day the control had the bacterial count of TNTC. In the experiment, activated carbon coated with Nannochloropsis AgNP, Chaetoceros AgNP, Dunaliella AgNP and Chlorella AgNP packed cartridge had the output bacterial count of 2, 3, 4 and 2.5 CFU/ml respectively. On 150th day the control had the bacterial count of TNTC. In the experiment, activated carbon coated with Nannochloropsis AgNP, Chaetoceros AgNP, Dunaliella AgNP and Chlorella AgNP packed cartridge had the output bacterial count of 5, 6, 6 and 6 CFU/ml respectively. On 180th day the control had the bacterial count of TNTC. In the experiment, activated carbon coated with Nannochloropsis AgNP, Chaetoceros AgNP, Dunaliella AgNP and Chlorella AgNP packed cartridge had the output bacterial count of 7, 8, 7.5 and 8 CFU/ml respectively.

Among the different size and shape of the silver nanoparticle coated activated carbon, the silver nanoparticle obtained from microalgae (Nannochloropasis sp., Chaetoceros sp., Dunaliella sp. and Chlorella sp.) absorbed the maximum bacteria from the outlet water which was sent through the cartridge. The filter loses it absorbing effect from 150 days onwards. On 150th day, the control had the bacterial count of TNTC. But the silver nanoparticle obtained from Nanocholoropsis AgNP, Chaetoceros AgNP, Nannochloropsis AgNP and Chlorella AgNP released the output bacterial number of 5, 6, 6 and 6 CFU/ml respectively. This shows the soaking effect of the filter. From the 180 th day onwards, the holding capacity of silver nanoparticle coated activated carbon reduces and

therefore this type of cartridge filter can be used only for 150 days. When referring to the previous study conducted by the different laboratories there is no evidence given regarding the soaking effect and the life of the filter. Furthermore, these studies demonstrated that the silver nanoparticles penetrates the bacteria and causes harm by interacting with the phosphorus and sulfur-containing components such as DNA, triggering bacterial suppression [31] and [32]. Dosoky et al. [33] evaluated the bactericidal efficiency of AgNPs by its application in different concentrations to the water sample, and allowed to interact with the bacteria for different durations. Then, the bactericidal efficiency of AgNPs was determined by comparing the counted bacteria before and after the treatments, resulting that the highest concentration of AgNP exhibited highest bactericidal efficiency in total bacteria count (TBC), where, after two hours, 0.1, 0.05 and 0.01 mg/L AgNP was found to be sufficient to inhibit 91.85, 89.14 and 74.92% TBC from the surface water [34].

Table 2 Effect of Silver nanoparticle coated activated carbon packed cartridge on the removal of bacteria

Time (days)	No. of Bacterial colony after filtration (CFU/ml)				
	Control (AC)	Nannochloropsis AgNP	Chaetoceros AgNP	Dunaliella AgNP	Chlorella AgNP
1st day	243±52	0.3 ±0.12	0.5± 0.14	0.4± 0.15	0.4±0.13
30th day	195±47	0.3±0.02	0.5±0.03	0.4±0.01	0.5±0.03
60th day	157±38	1.5±0.07	2.3±0.07	2.5±0.03	2.1±0.05
90th day	162±31	2.02±0.04	2.5±0.02	3.2±0.01	2.4±0.01
120th day	TNTC	2±0.01	3±0.02	4±0.06	2.5±0.03
150th day	TNTC	5±0.04	6±0.05	6±0.04	6±0.12
180th day	TNTC	7±0.21	8±0.03	7.5±0.05	8±0.07

Values are expressed as mean ± SEM, n=3

The green chemistry process used to make the microalgal nanoparticles may be regarded as technically feasible due to its commercial feasibility and potential for scale-up at a reasonable cost. The possibility of removing microorganisms from groundwater using materials coated with AgNP was examined [35].

CONCLUSION

In our study, it is investigated that the Size and Shape of the silver nanoparticle determine the antimicrobial activity as well as controlling the bacteria in the aquaculture system when immobilized on activated carbon. The size and shape of the algal biosynthesized silver nanoparticle is in relationship with the species of algae from which the extract was taken. The microalgae extracts can be employed as an effective capping and reducing agent for the synthesis, and the synthetic approach using algal sources is an environmental friendly procedure. Silver nanoparticle coated activated charcoal packed filtering system developed by our study will be a boon for the fish culturist to control the bacterial pathogen without using the antibiotics. The effect of the bacterial removal of the cartridge falls down after 150 days of its application and hence it is advised to remove the cartridge from the ornamental fish culture system after 150 days. Large-scale synthesis of these environmental friendly nanoparticles with the antibacterial and other medical applications will be possible in the future.

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CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

Enilda Rexy AnthonyKennedy: Conceptualization, Methodology, and Writing - original draft, Serinemichael MichaelBabu: Formal analysis and Visualization. Vaithilingam Sivanadanam and Mary Josephine Punitha Satniuslas**:** Validation. Mariavincent Michael Babu: Conceptualization, Supervision. Thavisimuthu Citarasu and Thangaswamy Selvaraj: Writing - review & editing. All authors read and approved the final manuscript.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY STATEMENT

The data obtained from the study are saved as hard and soft storage. This data can be shared on request.

PUBLISHER'S NOTE

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