Synthesis of silver and gold nanoparticles for the prevention of biofouling in waste water

R.Vivetha*, R.Shanthini[1], M.Vijay Pradhap Singh[2]

Department of Biotechnology

Vivekanandha College of Engineering for Women (Autonomous)

Namakkal, Tamilnadu, India.

ABSTRACT

Biosynthesis of silver and gold nanoparticles is extracted by plant leaves of *M. Piperita* and *C. latifolium* and the synthesis of nanoparticles is analyzed by UV–Vis spectroscopic. The antimicrobial activity of Ag-NPs and Au-NPs are analyzed by gram-positive and gram-negative bacteria. Bacterial biofilms represent a significant problem in wastewater. Biofilm is embedded in its own microbial-originated matrix of protective and adhesive of extracellular polymeric substances (EPSs). The nanoparticles have inhibited the biofilm and the anti-biofilm activity against the biofilm forming in wastewater. The silver nanoparticles are succeeded to form relatively low adherent biofilm. The pretreatment of Ag-NPs was aimed at controlling biofilm formation on the surface in water.

Keywords: silver and gold nanoparticles, wastewater treatment, biofilm, *M.Piperit,* and *C. latifolium.*

1. INTRODUCTION:

Water is a very important substance in living beings. A dependable and property approach to scrub and safe thought-about to be one amongst the foremost required for people in general. These days water scarceness is that the major drawback of society. Pollution could be attire for scare rate primarily caused by man-made activities of polluting water that square measure domestic, industrial and agriculture waste. Nanoparticles outline materials that the structural parts are sized between one and one hundred nm. Nanoparticles have a large risk to be employed in waste treatment. It's have an associate uncommon characteristic of high extent is often used with efficiency for removing hepatotoxic significant metal ions, disease-causing microorganisms from the water

Nanomaterial is active analysis and development and with success applied in several fields like drugs, catalysis, sensing, and biology. Thus waste is treated with nanoparticles and that they are removal of significant metals. Waste material water has unhealthful microorganisms, nitrogen, and phosphorus, heavy metals, detergents, and chemical (Shama Sehar et al., 2016). To introduce innovative technology are used to manage the water waste material. Nanomaterial analysis could be an additional advanced technology that takes advantage of the high extent per volume ratios, uncommon optoelectronic properties, of surfaces and chemical action reactivity to grant assurance of the new water purification approach. Gold metal has curiosity potential to take care of the water waste material problem because the recent analysis for an

additional advanced plan of nanoscale gold is there are less expensive for nanotechnology-based on water treatment. Gold nanoparticles have special properties for inflating the chemical action activity and chemical activity it creates additional helpful than alternative material.

The silver nanoparticles are strong bactericide activity; adversely have an effect on the biological wastewater treatment method. Silver nanoparticles were perceived within the biofilm living thing compound substances. Due to the antibacterial activity of silver nanoparticles is incorporated into many consumer products such as clothing, paints bandages, and food containers. Ag nanoparticles penetrate the microbe cells if some cells are dying once the uptake of Ag-NPs. The silver nanoparticle is toxicity involves membrane disruption, organic phenomenon, energy production, enzyme inhibition, and closely related to the silver dissolution. The widespread use of products containing silver nanoparticles would certainly release the silver nanoparticles into the sewer system and subsequently into municipal wastewater treatment to the removal of biofilms. The antimicrobial activity of silver nanoparticles, the possible effects of Ag-NPs on the bacteria in the environment cannot be overlooked (Linlin Hou et al ., 2011).

2. MATERIALS AND METHODS

The plant extract mediated synthesis and characterization of silver and gold nanoparticles are investigated. Biofouling is the undesired deposition of microorganisms and their Extracellular polymeric substances on various surfaces. Avoiding biofouling in the wastewater and its negative effects on the process performance of water systems is a serious operational challenge in all of the water sectors, including pipes, water distribution systems, filtration processes.

2.1 Synthesis of nanoparticles using plant extracts

Nanoparticles synthesis is distributed by varied chemical and physical ways; however, the utilization of such ways is harmful in one or the opposite way. The photosynthesis of nanoparticles is rising because of the intersection of applied scince and biotechnology. Due to a growing need to develop environmentally benign technologies in material synthesis, it's received increased attention. This has motivated the researchers to synthesis the nanoparticles using this route that enable higher control of shape and size for various applications.

2.2 Preparation of plant extract

The leaves of *M. Piperita* were washed totally thrice with H2O and were shade dried for five days. The fine powder was obtained from the dried leaves by employing a room liquidizer. The leaf powder was sterilized at 121 ◦C for fifteen min. 20 g of powder was taken and mixed with 200ml Q water and kept in boiling water bath at 60 ◦C for 10 minutes. The extracts were filtered with Whatman filter paper No one. The filtered extract was kept within the refrigerator at 4◦C. And another plant *C. latifolium* leaves are dry in the air atmosphere and then grinded by using an electronic blender with a size of 1-2 mm. The resulting (10 g)

of powder was refluxed with distilled water (100 mL) for 1 h. The mixture was filtered and stored in the refrigerator at 4° C

2.3 Biosynthesis of silver and gold nanoparticles

For the synthesis silver nanoparticles, 1.5 milliliters of plant extracts are mixed with 30 milliliters of AgNO3 solution (1 mM/ml) and incubated at 28 ◦C for 24 h. A small aliquot of a solution is used for the UV–Vis spectroscopic analysis and FTIR is performed to the extract that was exposed before and once added to the silver nitrate solution. The mixture is centrifuged at 6000 rpm for 15 min and then the pellet was resuspended a small quantity of sterilized double H2O then a small quantity of suspension was sprayed on a glass slide to create a thin film. The thin film was kept in hot air oven to dry then the thin film was used for the SEM analysis equipped with EDS. The similar procedure is kept for gold (Au) nanoparticles synthesis.

2.4 Biosynthesis and Optimization of Silver and Gold Nanoparticles

The extract was placed in solutions of Ag+ or Au3+ions under stirring in a dark condition at 1200 rpm. The formation of metallic nanoparticles (MNPs) was clearly observed by changes in the color. Optimization of reaction parameters as well as a number of metallic ions (0.5,1.0, 1.5, and 2.0 mM), the reaction temperature in vary from 30-120° C and also the reaction time 180 min was surveying through the measuring of UV-Vis spectra from a range of 200 to 800 nm. Reduction of ions Ag+ and Au3+by the plant extract induced an increase of absorbance at the peaks of around 400 nm and 540 nm, respectively. For more studies, the Ag-NPs and Au-NPs were biosynthesized within the optimized conditions. The solid MNPs were obtained by centrifugation at ten thousand revolutions per minute for ten min and washed thrice with water to remove metallic ions and also the impurities. Finally, the dried powder of MNPs was obtained once heating long at 90° C in an oven

2.5 Physicochemical Characterization of Silver and Gold Nanoparticles

Absorption spectra were decided by a JASCO V-630 spectrophotometer (USA). The functional groups of organic compounds from the aqueous extract were identified by the Fourier transform infrared (FTIR), spectrophotometer (Germany). Transmission electronic microscopy (TEM), Hitachi H8100, and high-resolution transmission electron microscopy (HRTEM), JEOL JEM2100, were used to analyze the morphology and crystal structure of the nanoparticles. Chemical elements were analyzed by energy-dispersive X-ray spectroscopy (EDX) (EMAX ENERGYEX-400, HORIBA). The adsorption spectra of prepared Ag-NPs suspension showed a Plasmon band at 420 nm, which is characteristic of silver nanoparticles. A drop of mixture nanoparticles is coated on a copper plate and dried in an exceedingly hot air oven and examined exploitation field emission Scanning microscopy (FESEM) (Carl Zeiss Germany).

2.6 Antibacterial Activity of Ag and Au Nanoparticles

The antibacterial activity was analyzed with synthesized silver and gold nanoparticles by a well diffusion method against clinically isolated Gram-negative (Escherichia coli) and Gram-positive (Staphylococcus aureus) microorganisms. The pathogenic cultures were taken from broth culture into the antibacterial assay. The 8-mm diameter of the well was built on Muller Hinton Agar plate with the help of gel puncture. If the cultures were swabbed on test media with the sterile cotton swab. 25µl of synthesized particles were inoculated to the well, and then the plates were incubated in the incubator for 38 ◦C for 24h. The antibacterial activity was carried out via the disk diffusion method as previously reported. The two gram-positive being strains (*Bacillus subtilis, staphylococci aureus)* and a couple of gram-negative being strains (*Escherichia coli, Agrobacterium tumefaciens*) acquired arise from the school of Biotechnology, Tan Tao University, Vietnam, were used to take a look at the bactericide activity of the biosynthesized silver nanoparticles and gold nanoparticles. The standard antibiotic ampicillin (0.01mg/mL) and Luria Bertani broth are used by positive and negative control. The antibacterial activity made up our minds by diameters of the inhibition zone around the paper disks in millimeters. And another antibacterial activity of the synthesized Ag and Au NPs screened against marine biofilm bacterial strains using agar well diffusion method. All the biofilm microorganism strains were individually inoculated within the Zobell marine broth and incubated for twelve h. The biofilm bacterial cells (∼1 × 108 cells mL−1) were inoculated using sterile cotton swabs on the Mueller Hinton Agar plates prepared with 50% seawater. The experimental wells of 6 mm diameters on the plates were loaded with a 50µL of colloidal nanoparticles, and then sterilized distilled water was used as a negative control and incubated for 24 h at 37◦ C. The assay was carried out in triplicates. The zone of inhibition was measured from the well to the sting of the clear zone in mm.

2.7 Nanoparticles stability

Particle stability over time was studied by scanning the samples among the, at time zero and once one month. The concentration of nanoparticles was measured for recent nanoparticles and when fortnight distinction by sp-ICP-MS. The thermal stability of fine-grained nanoparticles has been determined by the thermo-hydrometric analysis (TGA), on a Discovery TGA (TA Instrument). For the TGA analysis, nanoparticles were placed in AN aluminum oxide pan and heated from twenty to 700 °C at a ramping time of 10 °C/min.

2.8 Biofilm inhibition assay

Au-NPs and Ag-NPs against biofilm formation, microorganism cultures grownup nightlong were diluted with the lb broth to get 1–2 × 106 CFUs/ml. These samples were placed in 96 well plates and incubated at 37 °C for 5 h. After the incubation period, the old culture medium was replaced with a fresh medium accommodates different concentrations of gold nanoparticles or silver nanoparticles, without physically disrupting the biofilm. Samples were incubated further at 37 °C for 24 h. After a period, the medium was removed; samples were gently washed two times with sterile distilled water to remove the planktonic state/free-floating bacteria and then dried for 30 min at room temperature. The biofilms were stained with a 0.1% solution of crystal violet for 20 min. The excess stain was removed by washing with distilled water for times, and stained biofilms are dried at room temperature for 1 hour. 200 µl of absolute ethanol was added to each stain of biofilm samples, and then the mixture was agitated for 20 minutes to extract the stain.

2.9 Effect on bacterial growth in culture medium

The effect of nanoparticles growth on E. coli and Staphylococcus aureus at sub-MIC levels, bacterial growth was monitored spectrophotometrically at 600 nm against a culture medium blank, at selected time intervals. To taken from100 ml of bacterial suspension from overnight culture was sub-inoculated in the fresh medium and incubated at 37°C to keep in shaking incubator. Silver nanoparticles were tested on bacterial growth.

2.10 Nanoparticle coatings and bacterial adhesion assay

Silver nanoparticles were coated on a glass cover slips by a drop-casting method and then 250 ml of samples with different concentrations of water dispersed nanoparticles were placed on the cover glass and dried at 37°C overnight. 250 ml of bacterial suspension containing 1–2 10^6 CFUs/ml were added to the non-coated and coated cover glass and incubated for 5 h at 37°C. After the incubation period, biofilms were rinsed with distilled water and homogenized by sonication. The homogenized biofilm suspensions were plated on agar plates for CFU enumeration. Moreover, adhesion of the microorganism cells nanoparticles is visualized by fluorescence microscopy. Briefly, after a 5 h period of bacterial adhesion, biofilms were rinsed with sterile water and stained with a mixture of 6.0 lM SYTO 9 and 30 μM potassium iodide from Live/Dead BacLight Viability kit L13152 for 20 min. The imaging of stained biofilms was performed using a fluorescence microscope.

2.11 Biofilm inhibition by nanoparticles

Silver and gold nanoparticles are familiar to possess well designed an antibacterial activity against each gram-positive and gram-negative microorganism. Since the antibacterial impact is simply depending on the strategy of synthesis, size of particles, the release of silver, the kind of microorganism strains, several variations in nanoparticle concentration needed for antibacterial activity effects are reported by completely different studies. We executed an initial antimicrobial assessment of synthesized silver nanoparticles and gold nanoparticles by determination of MIC and MBC against E. coli and Staphylococcus aureus. The obtained results demonstrate a significant antibacterial activity of silver nanoparticles with bacteriostatic and bactericidal effects at the concentration of 50 and 100 μg/ml against Staphylococcus aureus and 100 and 200 μg/ml against E. coli respectively. In contrast, gold nanoparticles used a high concentration by 200 μg/ml, so they did not show any antibacterial activity against either bacterial strain. Smaller nanoparticles to offer a greater surface area to interact with microorganisms or biological components are more effective. Silver nanoparticles release silver ions via oxidation and increase the generation of reactive oxidative species, which damage cell components and cause cell death. The silver nanoparticles are small size 15–30 nm is an additional contributing factor to their antibacterial effect. Then we test both silver nanoparticles and gold nanoparticles in inhibiting the biofilm formation by E. coli and Staphylococcus aureus. Both types of nanoparticles inhibited the biofilm formation by Staphylococcus aureus and E. coli at the sub-MIC concentrations. The inhibition in biofilm formation was observed and then Ag-NPs at a sub-MIC level significantly inhibited the growth rate of both the bacterial strains, suggesting significant levels of sub-lethal damage. Adhesion of bacteria of the biofilm formation at the primary stage, since the adhesion of bacteria

must be affected by several factors such as growth environment, bacterial viability, and material surface characteristics, we justify the inhibitory effect of silver nanoparticles against the bacterial adhesion.

3. CONCLUSION

The silver and gold nanoparticles are synthesized at a high concentration in the range of 3-5mg/ml. We demonstrated that the silver nanoparticles can be effectively inhibition of biofilm formed by E.coli and Staphylococcus aureus. The M. Piperita and C. latifolium extract could be useful for producing nanoparticles in a biocompatible way. Biosynthesis of silver nanoparticles is a smaller size and understanding nanostructure are promising materials for the formation of biofilm.

4. ACKNOWLEDGMENT

The authors are very grateful to the department of biotechnology and waste management and economical industries, faculty of biological science, environmental science were helped in this study.

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