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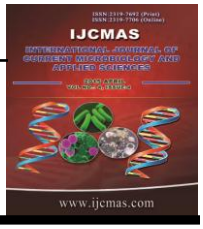

Original Research Article

Antifouling effects of silver nano particles synthesized from tropical seaweeds

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Keywords

Seaweeds, Silver nano particles, Marine biofilm, Anti-microfouling, Field trail

A B S T R A C T

Biogenic silver-nano particles (AgNPs) are reported to have exceptional antibacterial and antifungal activities. The present work focuses on the biological synthesis of silver nano particles (AgNPs) and their application to prevent marine biofouling. Among the eleven seaweeds studied, the yield of AgNPs synthesized by *U.lactuca* was high and exhibited excellent micro-fouling activities.The UV-Visible spectra showed a peak at 430 nm corresponding to silver, which was confirmed by SEM and EDS studies. The surface potentials of AgNPs were measured to be -15 mV, and the marine biofilm consortia varied between -39 mV to -45 mV. The AgNPs were spherical in shape witha sizeof 20 to 50 nm. The biogenic AgNPs coated on PVC coupons exposed for 45 days in natural seawater, inhibited micro-fouling. The application of biogenic AgNPs as an effective anti-foulant against consortia of marine biofilms has not been reported elsewhere in the literature.

Introduction

The undesirable accumulation of micro- and macro-foulers on the submerged structure in natural seawater is a perennial problem, causing a million-dollar expenditure to tackle the same. Since, the formation of biofilm is the prerequisite for the subsequent colonization by other organisms, any ideal antifouling product should target the prevention of biofilm formation without being toxic to other non-target organisms. The ban on tin-based antifouling paints by International Maritime Organization in the year 2008 led to the use of fewer toxic herbicidal antifouling compounds like Irganol 1051, Diuron, etc. Though these compounds are reported to be effective anti-foulants, their half-life and degradation process is not encouraging (Yebra et.al 2006). Hence, the multipronged search for novel antifoulants to combat biofouling is in progress worldwide.

The present work focuses on the biological synthesis of silver nano particles (AgNPs) and their application to prevent marine biofouling. AgNPs have already been reported to inhibit biofilm formation in

medical devices (Hee-Jin Park et.al 2013). AgNPs have high affinity towards microbial cell membrane than many other metal nanoparticles (Ivan Sondi et.al 2004). Though the silver ion possesses antimicrobial activity, it easily gets deactivated by precipitating as silver chloride in the marine environment. Nano silver in zerovalent form, acts as a betteralternative for ionic silver (Silver et.al 2006), (Russell et.al 1994)as well as nano silver is less toxic to higher animals (Melaiye et.al 2005). Though AgNPs can be synthesized by means of chemical methods, it requires the addition of reducing and stabilizing agents (Zaheeret.al 2011). However, whensynthesized biologicalit does not require any external stabilizing agents and is less time consuming too.

Marine natural products, like seaweeds (Rajesh et.al 2012), sponges (Inbakandan et.al 2013),and mangrove plants (Gnanadesigan et.al 2012), have the ability to reduce metallic silver to AgNPs. The biogenic AgNPs have a wide range of applications like antibacterial (Hajipour et.al 2012) and antifungal agents (Kumar et.al 2013).Poly-phenols, proteins, and other active phytochemical in the seaweeds have the ability to reduce and stabilize noble metals like silver, gold, platinum, and lead (Devi et.al 2012). The application of metal nanoparticles to prevent marine biofilm formation is scarce. Though biofouling occurs in any material immersed in natural seawater, there is a substrate-specific variation in fouling load (Palanichamy et.al 2012). The most commonly used material in the seawater includes, concrete for the building of marine structures, mild steel in power plant cooling systems, PVC and stainless steel, in oceanographic research equipment, aquaculture cages, and ship hulls, wood in boats. These materials are highly subjected to fouling communities and cause severe technical problems and environmental issues.

An effective antifoulant should inhibit biofouling in all the materials used in the marine atmosphere. Hence, it is essential to study the effect of antifouling product against microbial consortia from different materials. The biofilm consortia from materials, like concrete, wood, SS-316, mild steel and PVC, are isolated and the effect of AgNPs on the same has been reported in this study. The application of biogenic AgNPs as an effective anti-micro-fouling agent against marine biofilm consortia has not been reported elsewhere in the literature barring the work of (Inbakandan et.al 2013) and (Ramkumar et.al 2014). Further, all the work done so far has concentrated on the antibacterial activity of biogenic AgNPs against pure cultures of biofilm forming bacteria. In nature, biofilm is composed of varieties of bacteria, belonging to both gram-positive and gram-negative bacteria. The growth of gram-negative bacteria was more profoundly inhibited by the AgNPs than that of the gram-positive bacteria (Amanulla et.al 2010).Inbakandan et.al 2013 demonstrated the species-to-species variation in the antibacterial activity of AgNPs synthesized from sponges.Therefore, for effective control of both micro- and macro-fouling, the antifoulant should have a broad spectrum of anti-microfouling property. This report is a novel approach which carries the application of biogenic AgNPs to field testing in natural seawater.

Materials and methods

Collection of seaweeds

The brown seaweeds, *Acanthophora najardiformis, Sargassum wightii, Padina boergesenii,* the red seaweeds, *Gracilaria corticata, Kappaphycus alvarazii* and the

green seaweeds *Caulerpa peltata, Gracilaria edulis, Ulva reticulata, Caulerpa scalpelliforms, Ulva lactuca,* and*Enteromorpha intestinalis* were collected from hare island (8°46'25.9"N 78°11'16.1"E), Tuticorin, India. The seaweeds were washed with tap water to remove the debris, epi-fauna, and flora, followed by a final rinse with double-distilled water, and wereshade dried at room temperature for a fortnight.

Preparation of aqueous seaweed extract

The dried seaweeds were coarsely ground and cleaned three times in an ultra-sonicator bath to remove the free salts and other debris. These seaweeds were then blotted dry using filter paper and were air dried. One gram of powdered seaweed was added to 100 ml of de-ionized water,was heated, and was maintained at 60^0C for 20 minutes. The aqueous extract thus prepared was filtered using Whatmann no 1 filter paper, and the filtrate was used for the synthesis of biogenic AgNPs.Meanwhile; 100 ml of aqueous extracts was prepared separately and concentrated using a rotary evaporator, and was freeze dried to make it as a fine powder.The dried aqueous extract powder was used for antimicrobial and anti-microfouling studies.

Biosynthesis of silver-nano particles (AgNPs)

Biogenic AgNPs were prepared by following the method adopted by(Kumar et.al 2013) with minor modifications. The prepared aqueous seaweed extract was mixed with 250 µl of 1 M silver nitrate (Merck) solution and maintained at, 60 ^{0}C for 20 minutes. The reaction mixture was then left at room temperature for 24 h for the reduction and stabilization of silver ions to occur. After the development of dark-brown color, the solution was centrifuged at 10000 rpm for 10 minutes. The pellet, thus formed was rinsed and centrifuged thrice by replacing the supernatant with de-ionized water. The pellet was then rinsed with extra pure acetone, and was air dried to make it as a fine powder. The pellet was later mixed with double-distilled water to attain the required concentration.The control solution was prepared by adding 250 µl of 1 M silver nitrate in 100 ml of de-ionized water.

Isolation And Identification Of Marine Biofilm Bacteria

Coupons of different materials like SS-316, mild steel, PVC, and natural materials,like wood and concrete, were immersed into the seawater below the CECRI's offshore platform at Tuticorin ($8^0 8^{'}$N; $78^0 13^{'}$E) for a period of seven days. Using sterilized cotton, identical area of 1 cm^2 from each material was swabbed, collected in sterilized seawater, and cultured in nutrient broth (Hi-Media) prepared using sterilized seawater. The marine biofilm consortia from different materials were maintained with uniform agitation (150 rpm) at 37^0C for 24 h. The marine bio-film culture broth was then serially diluted and plated on Zobell marine agar (Hi-Media) and was incubated at 37^0C for 24-48 h. The bacterial population was enumerated, and the morphologically distinct colonies were isolated. The strains were physiologically characterized by the methods described by (Simbert et.al 1997) and (Sneath et al. 1986).

Antimicrobial effects of biogenic AgNPs and Aqueous seaweed extracts

To assess the antimicrobial activity of biogenic AgNPs and aqueous seaweed extract, the disc diffusion method was followed. The sterile discs were loaded with 50 µgof aqueous extracts, biogenic AgNPs

and50 µlcontrol solution. The discs were thenplaced on Mueller Hinton agar (Hi-Media) plates cultured with marine biofilm consortia from different materials, having the population of 10^6 CFU/ml and incubated for 24 h for 37^0C.The clear zones around the discs were measured from the edge of the disc to the edge of the zone.

The microbicidal effects of biogenic AgNPs were studied by measuring optical density (OD) using a UV-Visible spectrophotometer (Shimadzu UV 1800). To the various aliquots of 10 ml, sterile nutrient broth (Hi-Media), 100 µgof aqueous extracts, biogenic AgNPs,and 100µlcontrol solution were mixed with 100 µl of culture broth containing marine bio-film consortia. The control broth was cultured only with the marine bio-film consortia. Optical densities of both control and test systems were measured after 24 h at 600 nm.

Anti-microfouling activity of biogenic AgNPs

Anti-microfouling effects of biogenic silver nano particles were studied using an Epi-fluorescence microscope (80i Nikon). The sterilized SS-316, PVC, and mild steel coupons were placed in a beaker containing 100 ml of nutrient broth (Hi-Media) prepared using sterile seawater. To the beakers, 100 µg of synthesized AgNPs and 1 ml broth cultures of marine bio-film consortia from SS-316, PVC, and mild steel were added and kept in an orbital shaker at 150 rpm for 24 h.

Simultaneously,the control systems were maintained without AgNPs. The coupons for Epi-fluorescence microscopy studies were first gently rinsed in autoclaved sterilized seawater to remove the loosely adherent cells and debris if any. The coupons were then fixed in 3% glutaraldehyde for an hour and stained with acridine orange dye and were examined under UV excitation, using the filter type C-FL Epi-FL and filter block type B-2A in conjunction with a calibrated eye piece reticule. Acridine orange stained live cells, were green and dead cells, were orange. Images were captured using a Nikon eclipse 80i fluorescence microscope. Randomly chosen fields were photographed using 20 X objectives (400 X magnifications) on an Evolution MP camera.

Characterization of biogenic AgNPs

The bio-reduction of silver nitrate to AgNPs after 24 h was monitored using a UV-Visible spectrophotometer (Shimadzu UV 1800). The powdered pellet was coated thoroughly in a carbon coated copper grid, and the presence of the AgNPs was examined under a scanning electron microscope (SEM) (Vega 3 Tescan) at different magnifications.The elemental composition was monitored using EDS (Bruker quantae). The exact size of the AgNPs was obtained using a transmission electron microscope (TEM) (FEI,- Tecnai 20 G2). The surface potentialof AgNPs and biofilms were measured using the zeta potentiometer(Malvern zeta sizer nano-ZS). FT-IR analysis (Bruker Tensor 27 FTIR spectrophotometer) was carried out to study the predominant functional groups present in the aqueous extract.

Field trail

The biogenic AgNPs, 5 mg,were incorporated with 5 ml of acrylic resin using an ultra sonicator and coated on a PVC coupon of 2”x3” Size and a PVC coupon, coated only with acrylic resin served as the control. Both the coupons were mounted on a wooden raft and exposed to natural seawater for 45 days below the CSIR-CECRI’s Offshore Platform in Tuticorin.

Toxicity studies

Toxicity studies were performed in a static bioassay system using freshly hatched brine shrimp, *Artemia salina*. In the test system 15 nos of brine shrimp larvae were cultured in 50 ml of filtered seawater containing50 µg of AgNPs. In the control system was served only withseawater. The number of survivors, dead and immobile, in both the systems was observed on an hourly basis. The assay was performed in triplicate, and the mean value was noted.

Result and Discussion

Synthesis of Biogenic AgNPs particles

In the present study, all the seaweeds were able to synthesize AgNPs with variations in the color intensity with respect to time. It is well known that AgNPs exhibit brown color in water (Sastry et.al 1998) which is attributed to the excitation of surface plasmon vibrations in metal nanoparticles. The formation of dark-brown color after the addition of silver nitrate to the aqueous seaweed solution is believed to be due to the synthesis of AgNPs. This is further confirmed by UV-Visible spectral peak (Fig 1) at 430 nm corresponding to the surface plasmon resonance of the silver nano particles (Sastry et.al 1998).

Bacteria in the marine biofilm consortia

The predominant bacterial colonies from all the materials were identified to be *Pseudomonas sp., Flavobacterium sp., Aeromonas sp., Micrococcus sp., Cornybacterium sp.,* and *Achromobacter sp*.Total bacterial population from wood, PVC, mild steel, concrete, and stainless steel coupons was found to be $20x10^5$, $14x10^6$, $17x10^6$, $20\ x10^6$, and $15x\ 10^5$ per cm^2, respectively.

Antimicrobial effects of biogenic AgNPs

The degrees of the antimicrobial activities of AgNPs synthesized by various seaweeds are illustrated in Fig. 2.Among the seaweeds studied, the AgNPs synthesized by *U.lactuca* showed the highest zone of inhibition against marine biofilm consortia isolated from different materials. Furthermore, the yield of AgNPs synthesized by *U.lactuca* was high among all seaweeds. Hence, *U.lactuca* was chosen as the best candidate species and was used for characterization and antifouling studies.

Further, AgNPs from green seaweeds showed good anti-microbial activity as compared to the brown and red seaweeds against marine bio-film consortia**.** This report is the first of its kind that no literature is available to compare the antimicrobial activity of AgNPs synthesized from seaweeds against the consortia of marine bio-film bacteria.

Microbicidal effect of biogenic AgNPs is presented in Fig.3. The synthesized AgNPs from *U.lactuca* showed an excellent microbicidal effect against biofilm consortia from all materials. The microbial population from SS-316, PVC, mild steel, wood, and concrete was reduced to 95-99%. The aqueous extracts showed the least inhibitory effects against the marine biofilm consortia of PVC, mild steel, and wood. Similarly, (Inbakandan et.al 2013) reported the bactericidal effect of silver nano particles synthesized from the marine sponge against different species of marine biofilm forming bacteria. The reduction of bacterial population is attributed to the effect of AgNPs.

The application of AgNPs in a wide range of products as a powerful antibacterial agent has been well recognized (Susan et.al 2009).

The mode of action is still in debate (Kittleret.al 2010). Different views have been proposed, surface area could be one of the main factors in nanoparticles toxicity (Johnston et.al 2010) andthe adhesion of nanoparticles to the bacterial surface, altering the membrane properties (Wong et.al 2010).

The small size and extremely large surface area of nanoparticles enable them to a make strong contact with the surface of microorganisms. However, the direct particle-specific antibacterial activity of nanosilver has also been ruled out specifying that silver ions are definitive molecular toxicants (Xiu et.al 2012).

It was also postulated that the antibacterial activity of silver ions is caused by the synergistic effect between the binding of silver ions to the cell wall, their uptake and subsequent accumulation in the cell, and their interference with critical biomolecules within the cell (Samberg et.al 2011).

Anti-microfouling effects of biogenic AgNPs

The anti-microfouling effect of AgNPs is shown in the (Fig. 4). Micro-fouling was found to be appreciable in the control coupons of PVC, mild steel, and SS-316, indicating the uninterrupted biofilm formation; while in the AgNPs treated systems,a very negligible amount of bio-film formation could be observed indicating the anti-microfouling effects of AgNPs. Similarly, (Inbakandan et.al 2013) has demonstrated the anti-microfouling effects of AgNPs by crystal violet staining method against different biofilm bacteria.

Characterization of biogenic AgNPs

The images of SEM studies showed the presence of nano-sized particles, which were confirmed as AgNPs by the EDS data (Fig. 5). According to TEM, the morphology of biogenic AgNPs was observed to be spherical with an average size of 25 nm (Fig. 6). It is evident from the Fig. 6 that all the silver nano particles were not agglomerated; thereby, they render more reactive surfaces. According to (Inbakandan et.al 2013), silver nano particles sizing between 14 to 34 nm will enhance the contact area by 10^9. The surface potential measurements disclosed that the biogenic AgNPs are highly stable with a value of –15 mV , whereas the surface potential of marine biofilm was ranging between -39 mV to -45 mV. The difference in the surface potential will also enhance the attachment of biogenic AgNPs with marine biofilm. The qualitative phytochemical analysis of *U.lactuca* revealed the presence of saponins, alkaloids, flavonoids, sterols, tannins, and terpenoids. From the FT-IR studies of *U.lactuca*, the IR bands at 3418 cm^{-1}, 2922.59 cm^{-1},2849 cm^{-1},1634 cm^{-1}, 1425 cm^{-1},1118 cm^{-1} and 621 cm^{-1} correspond to the functional groups like alcohols, alkanes, primary amines, aromatics, aliphatic amines, and alkyl halides. The alcohols and amine groups act as stabilizing and capping agents of metal nano particles (Inbakandan et.al 2013) and (Kumar et.al 2013).

Field trail

The biogenic AgNPs coated PVC coupon exhibited antifouling property, whereas the control coupon showed micro-fouling by bacteria and macro-fouling by algal biomass and barnacles.Fig. 7 shows the photographs of both control and test PVC coupons after 45 day exposure to natural seawater. The algal biomass coverage was found to be high in the control coupon, while the biogenic AgNPs coated PVC coupon showed no algal biomass coverage (Fig. 8), however, very few numbers of barnacles could be seen. The bacterial density in the control coupon

was 10^6 times higher than the AgNPs coated coupon. As biogenic AgNPs exerted excellent antimicrobial activity, the process of macro fouling development on the study material could not be observed. This study again confirms the general perception that microbial biofilm is a prerequisite for macro-fouling to take place.

Toxicity studies

Ecological effects regarding the toxicity of AgNPs have become prevalent (Nowack et.al 2010). Hence, it is necessary to study the response of biogenic AgNPs against non-targeted species. When *A.salina* is cultured in laboratory conditions, natural death could be possible. Biogenic AgNPs also had a minimal lethal effect on *A.salina*. During the first hour of observation, 85 % of the populations were alive; gradually, the survival rates in both control and test systems were almost equal. This study indicates that the brine shrimp *A.salina* is susceptible to AgNPs due to sudden initial shock when a foreign material is added to the system.

From the overall results, it could be concluded that *U.lactuca* is the best candidate species for the biosynthesis of AgNPs. Further, the biogenic AgNPs exhibited appreciable antimicrobial, anti-microfouling activities. Hence, biogenic AgNPs based antifouling coating could be used in minor equipment. With regard to the antifouling studies made in the present investigation, a period of 45 days is too short by which no claim could be made for its antifouling activity. For evaluating the antifouling effects of AgNPs in the field conditions, a detailed study is required, which should address some of the properties of the coatings like the quality of the binder,the thickness of coating, surface contact angle, release rate, maximum endurance period of the coating as well as biocide, toxicity to non-target organisms, etc.

Fig.1 UV-Visible spectra of biogenic AgNPs

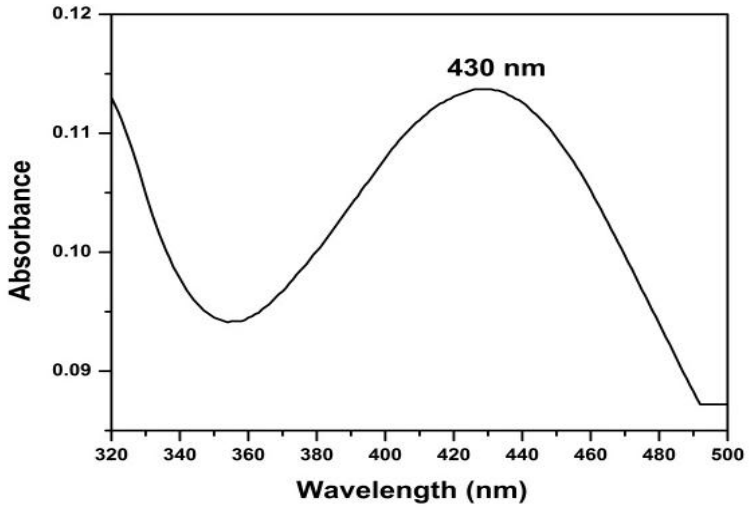


Fig.2 Zone of inhibition (ZOI) exhibited by the biogenic AgNPs from brown, red and green seaweeds against marine biofilm consortia

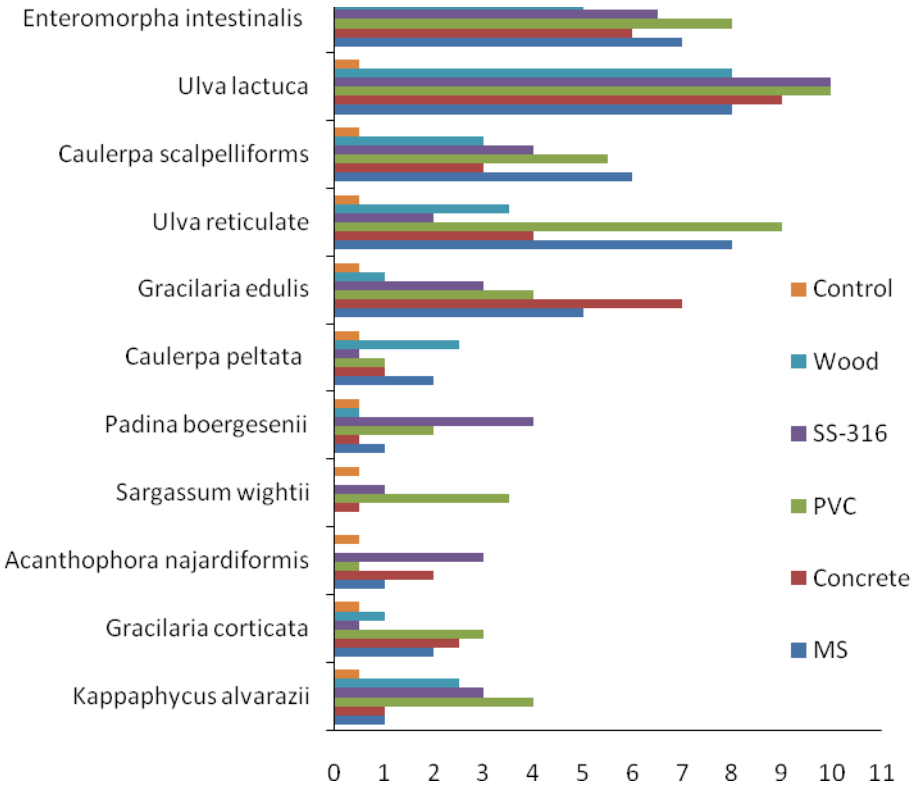


Fig.3 Microbicidal effect of AgNPs synthesized by U.lactuca

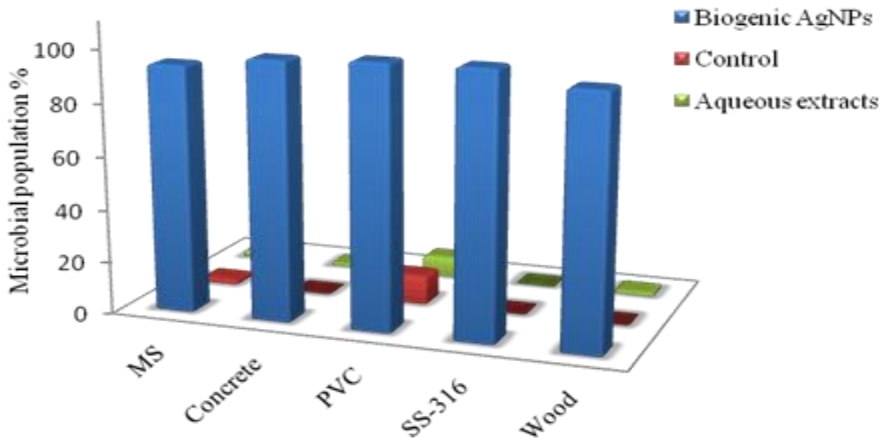


Fig.4 Epi-fluorescence images showing anti-microfouling effect of biogenic AgNPs - A, B and C indicate, the formation of marine bacterial bio-film on mild steel, PVC and SS-316 without AgNPs treatment; D, E, F indicate the Anti-microfouling effects of silver nano particles synthesized fromU.lactucaon mild steel, PVC and SS-316 respectively

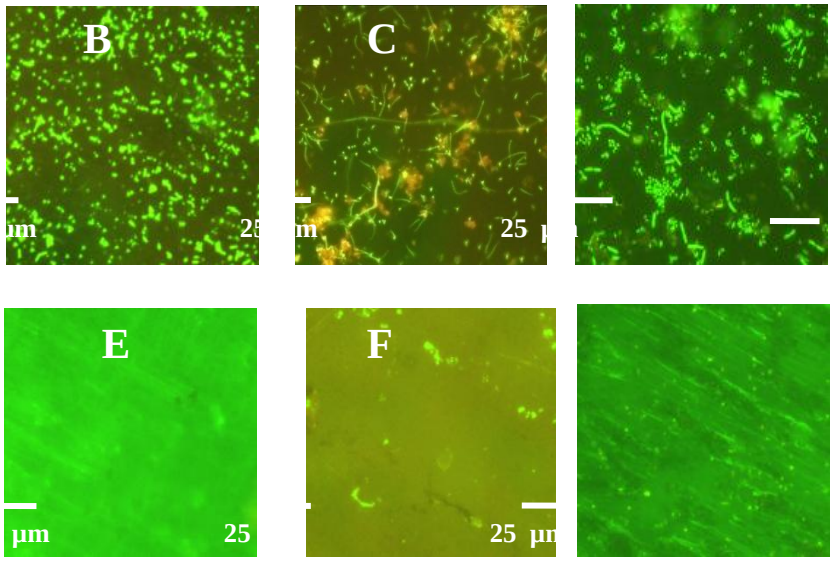


Fig.5 Scanning electron microscope (SEM) and EDS images of AgNPs synthesized by U.lactuca.

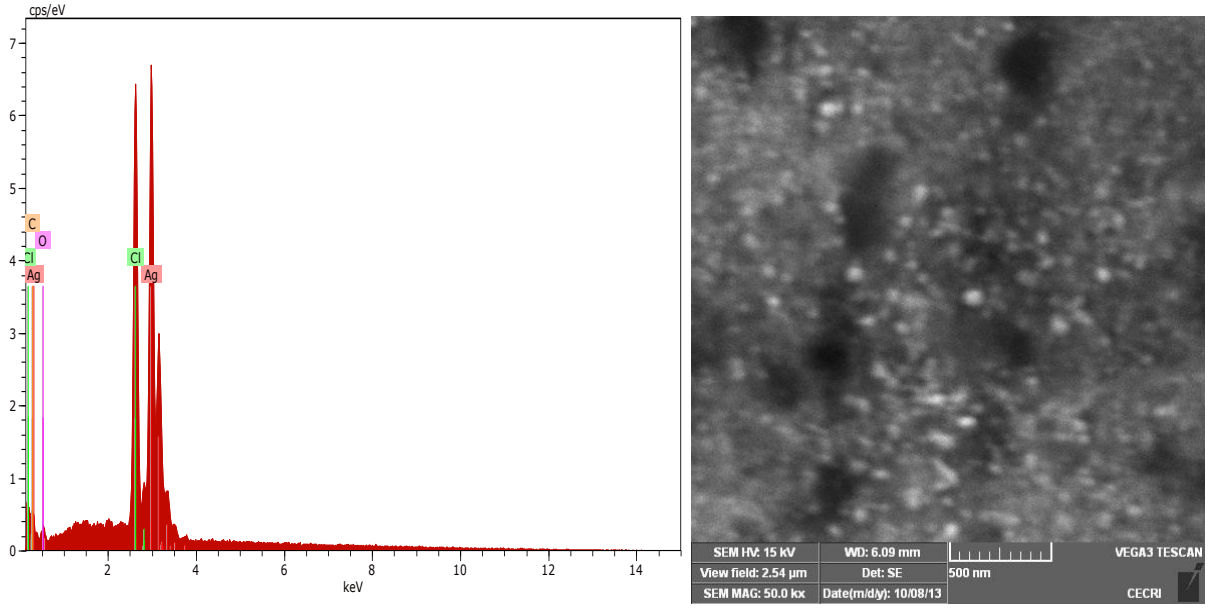


Fig.6 Transmission electron microscope (TEM) images of silver-nano particles synthesized by *U.lactuca*

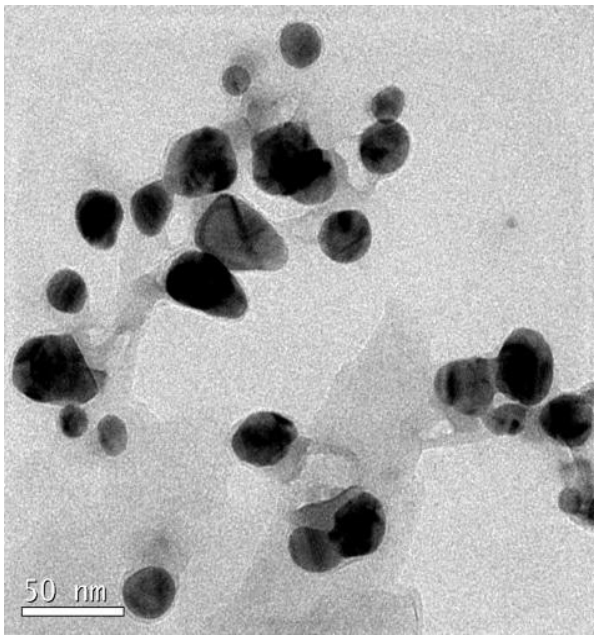


Fig.7 Antifouling effects of biogenicAgNPs coated in PVC coupons in natural seawater after 45 days exposure; A- Coating without AgNPs; B- Coating with AgNPs

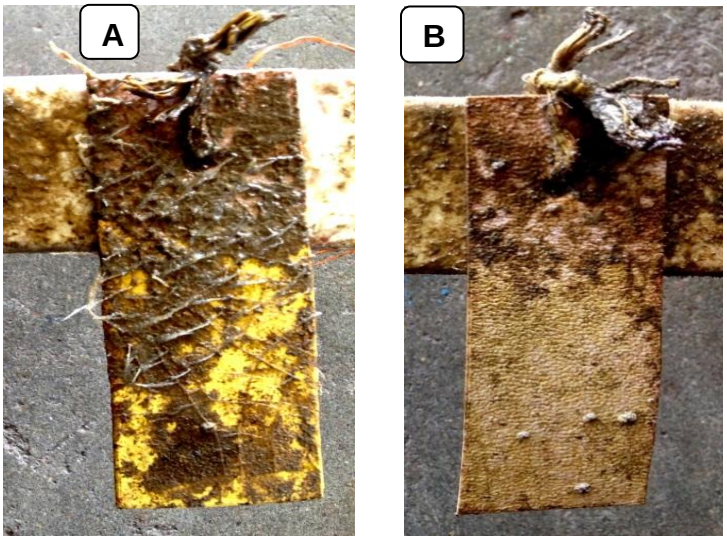
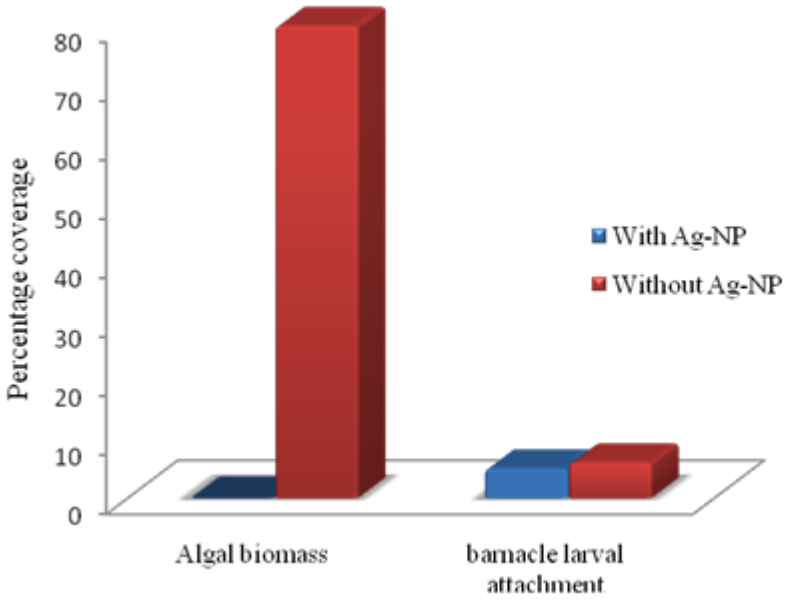
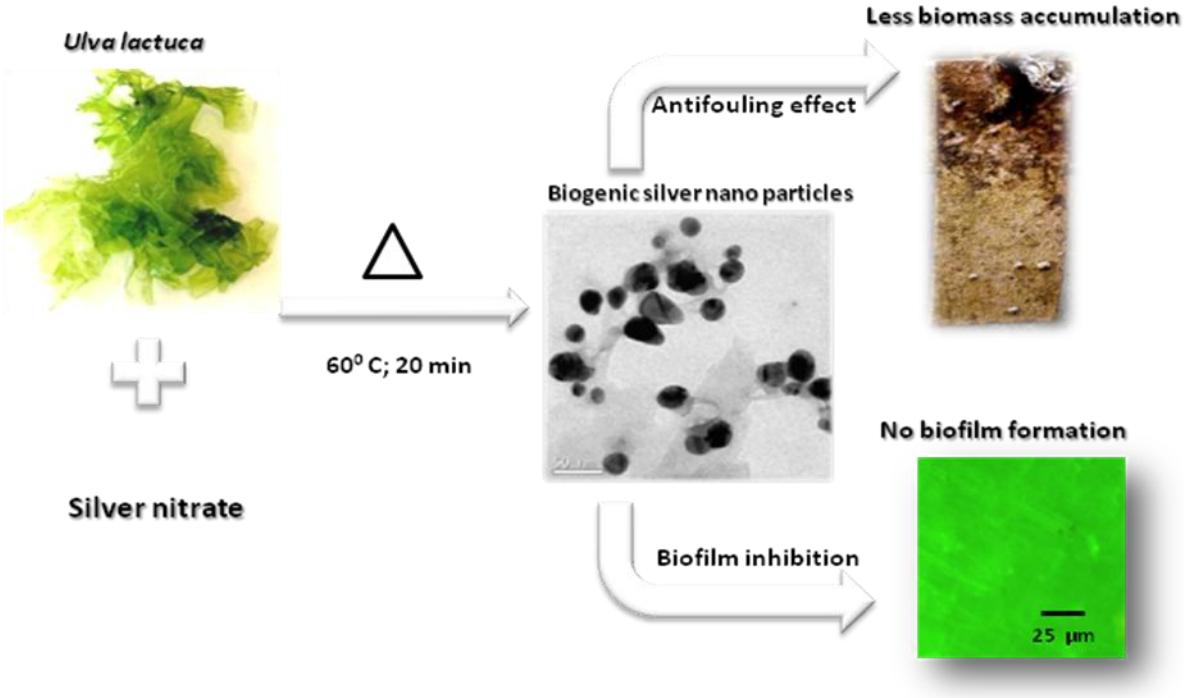


Fig.8 Percentage coverage of macro-foulants on coupons coated with and without biogenic AgNPs





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References

Amanulla Mohammed Fayaz, Kulandaivelu Balaji, Morukattu Girilal, Ruchi Yadav, Pudupalayam Thangavel Kalaichelvan, Ramasamy Venketesan. 2010. Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics. Nanomedicine: Nanotechnology, Biology and Medicine. 6:103-109.

Yebra, D. M., S. Kiil, K. Dam-Johansen,2004. Antifouling technology—past, present and future steps towards efficient and environmentally friendly antifouling coatings, Progress in Organic Coatings. 50: 75–104.

Devi, J. Saraniya Bhimba, B. Valentin Peter, D. Mages.2012 Production of biogenic Silver nanoparticles using *Sargassumlongifolium* and its applications.Indian Journal of Geo-Marine Sciences.42:125-130.

Hee-Jin Park , Soomin Park , JinkyuRoh, Sujin Kim, Kyunghee Choi, Jongheop Yi, Younghun Kim , Jeyong Yoon,2013. Biofilm-inactivating activity of silvernanoparticles: A comparison with silver ions, Journal of Industrial and Engineering Chemistry. 19:614-619.

Inbakandan, Kumar C, Abraham LS, Kirubagaran R, Venkatesan R, Khan SA.2013. Silver nanoparticles with anti microfouling effect: A study againstmarine biofilm forming bacteria. Colloids Surfaces B Biointerfaces.111:636-643.

Ivan Sondi,Branka Salopek-Sondi,2004. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria, Journal of Colloid and Interface Science. 275:177-182.

Johnston.H.J, Hutchison, G.Christensen, F.M.Peters, S.Hankin, S.Stone, 2010.A review of the in vivo and in vitro toxicity of silver and gold particulates: Particle attributes and biological mechanisms responsible for the observed toxicity. Critical reviews in toxicology.4:328–346.

Kittler.S,Greulich. C,Diendorf .J,Köller.M, Epple.M, 2010.Toxicity of silver nanoparticles increases during storage because of slow dissolution under release of silver ions. Chemistry of Materials.22: 4548–4554.

Gnanadesigan, M., M. Anand ,S. Ravikumar, M. Maruthupandy ,M. Syed Ali ,V. Vijayakumar A. K. Kumaraguru. 2012. Antibacterial potential of biosynthesised silver anoparticles using *Avicennia marina* mangrove plant. Applied Nanoscience.2:143-147.

Melaiye,W. J. Youngs, . 2005. Silver and its application as an antimicrobial agent. Expert Opinion on Therapeutic Patents. 15:125-130.

Mohammad J. Hajipour, Katharina M. Fromm, Ali Akbar Ashkarran, Dorleta Jimenez de Aberasturi, Idoia Ruiz de Larramendi, TeofiloRojo, VahidSerpooshan, Wolfgang J.Parak , MortezaMahmoudi. 2012. Antibacterial properties of nanoparticles. Trends in Biotechnology.30: 499-511.

Nowack.B, Krug.H.F, Height.M, 2010.120 years of nanosilver history: Implications for policy makers. Environmental Science and Technology, 45:1177–1183.

Kumar, P., S. SenthamilSelvi, M. Govindaraju.2013. Seaweed-mediated biosynthesis of silver nanoparticles using *Gracilaria corticata* for its antifungal activity against *Candida sp*. Applied Nanoscience.3:495-500.

Russell, A.D, Hugo, W.B.1994. Antimicrobial activity and action of silver. Progress in Medicinal Chemistry.31:351-370.

Palanichamy, S., G. Subramanian,M. Eashwar. 2012. Corrosion behaviour and biofouling characteristics of structural steel in the coastal waters of the Gulf of Mannar(Bay of Bengal), India. Biofouling. 28:441-451.

Rajesh, S., D. Patric Raja , J.M. Rathi and K. Sahayaraj. 2012. Biosynthesis of silver nanoparticles using *Ulvafasciata* (Delile) ethylacetate extract and its activity against *Xanthomonas campestris V. malvacearum.* Journal of Biopesticides .5 :119-128.

Samberg, M.E.; Orndorff, P.E.; Monteiro-Riviere, N.A.2011. Antibacterial efficacy of silver nanoparticles of different sizes, surface conditions and synthesis methods. Nanotoxicology .5:244–253.

Sastry.M, Patil.V,Sainkar.S.R, 1998. Electrostatically Controlled diffusion of carboxylic acid derivatives silver colloidal particles in thermally evaporated fatty amine films. Journal of Physical Chemistry .B. 102; 1404-1410.

Silver, S., Phung, L. T., Silver, G. 2006. Silver as biocides in burn and wound dressings and bacterial resistance to silver compounds .Journal of Industrial Microbiology and Biotechnology.33:627-634.

Simbert, R.M. N.R. Krieg, 1997. Phenotypic characterization. In: Gerhardt, P., Murray, R.G.E., Wood, W.A., Krieg, N.R. (Eds.), Methods in General and Molecular Bacteriology. American Society for Microbiology, 607–653.

Sneath, P.H.A., N.S. Mair, M.E. Sharpe and J.G. Holt, 1986. Bergey's Manual of Systematic Bacteriology,Williams and Wilkins, Baltimore, US, 2:965–1599.

Sri Ramkumar Vijayan, Prakash Santhiyagu, Muthukkumarasamy Singamuthu, Natarajan KumariAhila, Ravindran Jayaraman, and Kannapiran Ethiraj.2014.Synthesis and Characterization of Silver and Gold Nanoparticles Using Aqueous Extract of Seaweed, *Turbinariaconoides*, and Their AntimicrofoulingActivity,The Scientific World Journal.2014: 1-10

Susan W.P. Wijnhoven , Willie J.G.M. Peijnenburg , Carla,Herberts ,

Werner. Hagens ,Agnes G. Oomen , Evelyn H.W. Heugens , Boris Roszek, Julia Bisschops , IlseGosens, Dik van de meen. 2009. Nano-silver—A review of available data and knowledge gaps in human and environmental risk assessment. Nanotoxicology.3:109-138.

Wong, K.K.Y.; Liu, X.2010 Silver nanoparticles- The real silver bullet‘‘in clinical medicine MedChem Comm.1:125–131.

Xiu.Z, Zhang.Q, Puppala, H.L.Colvin, V.L, Alvarez, P.J.J. 2012, Negligible particle-specific antibacterial activity of silver nanoparticles. Nano Letters. 12:4271–4275.

Zaheer Khan, Shaeel Ahmed Al-Thabaiti, Abdullah Yousif Obaid, A.O. Al-Youbi.2011. Preparation and characterization of silver nanoparticles by chemical reduction method. Colloids and Surfaces B: Biointerfaces.82:513-517.