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Antifouling assessments on biogenic nanoparticles: A field study from polluted offshore platform

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ABSTRACT

Turbinaria ornata mediated silver nanoparticles (TOAg-NPs) were evaluated for antibacterial activity against 15 biofilm forming bacterial isolates. A field study in natural seawater for 60 days showed antifouling activity of TOAg-NPs on stainless steel coupons (SS-304) coated with Apcomin zinc chrome (AZC) primer. Though TOAg-NPs showed broad spectrum of antibacterial activity, the maximum zone of inhibition was with *Escherichia coli* (71.9%) and a minimum with *Micrococcus* sp. (40%) due to the EPS secretion from Gram-positive bacteria. Compared to control coupons (18.9 [$\times 10^3$], 67.0 [$\times 10^3$], 13.5 [$\times 10^4$] and 24.7 [$\times 10^4$] CFU/cm^2), experimental biocide coupons (71.0 [$\times 10^2$], 32.0 [$\times 10^3$], 82.0 [$\times 10^3$] and 11.3 [$\times 10^4$] CFU/cm^2) displayed lesser bacterial population density. Toxicity studies revealed 100% mortality for *Balanus amphitrite* larvae at 250 μg ml^{-1} concentration within 24 h, while 56.6% recorded for *Artemia marina* at the same concentration indicating less toxicity to non target species. It proved that AZC + TOAg-NPs prevent biofouling by its Ag-NS affinity and antimicrobial effectivity.

1. Introduction

In the marine environment, competition for living space is intense and all surfaces living or innate are susceptible to fouling. micro and macroorganisms commonly adhere to natural or man-made aquatic surfaces and form sessile multicellular communities known as biofilms (Dalton and March, 1998; Dahms et al., 2004a,2004b; Camps et al., 2011). The formation of biofilms commonly begins with the adsorption of organic macromolecules to form a conditioning film (Wahl, 1989; Dahms et al., 2012; Halder et al., 2014). This is followed by the attachment of bacteria and other unicellular organisms (Dobretsov et al., 2006, 2013). Biofilms may mediate the settlement of macroorganisms and both together can lead to biocorrosion (Satuito et al., 1997; Wieczorek and Todd, 1997). Biofouling can cause serious operational problems with economic losses, among others for the shipping industries, industrial equipment, offshore platforms, underwater pipelines and desalination plants (Evans and Clarkson, 1993; Dahms et al., 2004a,2004b; Inbakandan et al., 2013).

The chemical agents which are generally used to prevent/protect metallic structures from biofouling/biocorrosion are generally highly toxic and can have negative impacts, and hence pollute the aquatic environment after use (Daniel et al., 2010). So far, the most effective methods of biofouling control are based on the application of toxic substances like tributyltin (TBT) and triphenyltin (TPT), or copper and organotin compounds (OTC) (Turner, 2010). The Marine Environmental Protection Committee (MEPC) and International Maritime Organization (IMO) were strongly opposing the continued use of TBT, TPT or other substances containing tin as biocides in antifouling paints (Dafforn et al., 2011). Hence, there is an urgent need for the development of environmentally less harmful non-toxic antifoulants.

The number of natural products isolated from marine organisms can be used as replacements for chemicals commonly used in antifouling coatings (Clare, 1996a) and several hundreds of new compounds are discovered every year. Many potential antifouling compounds have been isolated from sponges (Kubanek et al., 2002), corals (Kelman et al., 1998), algae (Rothman et al., 2013), ascidians (Wahl et al., 1994), sea grasses (Jensen et al., 1998), sea stars (Iken et al., 2002), bacteria (Bernbom et al., 2011), fungi (Gao et al., 2013), and Macroalgae (seaweed) (Girling et al., 2015). Qian et al. (2015) compiled a mini review about marine natural compounds with structure, merit and demerit properties of the antifouling compounds. Similarly, in South Korea alternative antifouling biocides from polluted coastal sediments were reported (Kim et al., 2015).

Although physical/chemical methods are more popular in the synthesis of silver nanostructures (Ag-NS), the use of toxic chemicals

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greatly limits their environmental applications. The development of reliable, nontoxic and eco-friendly methods in the synthesis of silver nanoparticles Ag-NPs would be important (Abbott Chalew et al., 2012). A standard method as outline by Vivek et al. (2011) was followed in the present study for the biosynthesis of Ag-NPs. This method is reliable and nontoxic because no chemical is used as capping and reducing agent. Furthermore, the spectral absorbance of biosynthesized nano particles resembles exactly the spectrum of Ag-NPs, *e.g.* showing its peak value at 420 nm. Hence, this is a reliable and eco-friendly method. Recently, Ag-NPs or Ag^+ ions were intensively screened as biocide agents for several applications such as applying them to water filter membranes, fabrics, thin layers, anti-fouling thick coatings, dental materials and surface modified implants (Lara et al., 2010).

Ag-NPs are also widely investigated owing to their broad range of applications against several microorganisms of clinical interest such as antibacterial, antifungal, and antiviral agents (Melaiye and Youngs, 2005; Vignesh et al., 2014). Ag-NPs are one of the less-toxic and safe antimicrobial agents to higher animals (Ananda Priya et al., 2013). An innovative attempt to accomplish this goal is the use of naturally-produced compounds, such as synthesized nano-scale TOAg-NPs that are environmentally acceptable, durable and resistant to damage. These compounds are also repairable, low maintenance, easy to apply, hydraulically smooth, compatible with existing anticorrosion coatings and non-toxic to non-target species. Hence the present study made an attempt to find anticorrosive and antimicrobial effect of TOAg-NPs against biofilm microbes and *Artemia*/*Barnacles* at both *in-situ* and *in-vitro* conditions.

2. Materials and methods

2.1. Study area

The present study was conducted at the Offshore Platform and Marine Electrochemistry Center (OPMEC), of the Central Electrochemical Research Institute (CECRI), Tuticorin, (longitude 8° 45′ north and latitude 78° 13′ east) located at the ocean front in the new harbor area of the Gulf of Mannar region, India. Tuticorin Port is one of the 12 major ports in India and the second-largest port in Tamil Nadu, and fourth-largest container terminal in India. Tuticorin coastal regions drains untreated domestic/industrial wastes like discharges of coolant waters, harbor activities such as dredging, cargo handling, dumping of ship wastes, spilling of cargo's chemicals, metal ores, fishing activities and ballast waters.

2.2. Preparation of metal coupons

Stainless steel (SS 304) (Cr-19.11%, Fe-72.74%, Ni-8.15%) was used as a substratum for field study. The SS304 coupons (1″ × 3″ dimension) were pickled in 10% nitric acid at 60 °C for 30 min, polished on motor wheels and degreased in acetone before immersion (Palanichamy et al., 2002). The treated SS 304 coupons were mounted on an acrylic holder raft using PVC washers and insulated brass bolts and nuts, and exposed to natural seawater for 7 days (168 h) (Fig. 1a) below the offshore platform in the new harbor at Tuticorin, Tamil Nadu, India.

2.3. Isolation and characterization of marine biofilms

After 7 days of immersion, the biofilm developed on SS 304 coupons were optically characterized by fluorescence microscopy (Fig. 1b). The biofilms from coupons were scrapped using sterile cotton swabs and immediately transferred to culture tubes containing sterilized aged seawater (ASW). From the mixture (biofilm isolates + sterile aged seawater), bacterial strains were isolated and enumerated by pure culture techniques (spread plating method — 0.1 ml) on selective medium plates with suitable (10^{-1}, 10^{-2}, 10^{-3} and *etc.*) dilutions (Vignesh et al., 2012, 2014, 2015). Nutrient and selective media plates were prepared with the addition of sterile aged sea water (aged seawater was sterilized by autoclaving at 15 lbs pressure at 121 °C for 15 min). Bacterial parameters were characterized and listed in Table 1. The average bacterial counts of the replicates were noted and the mean values were recorded. Morphologically dissimilar, distinct colonies were randomly selected and inoculated into rapid microbial limit test kits (Hi-media Laboratories Limited, India) for identification of bacterial strains (Allegrucci and Sauer, 2007).

2.4. Collection and extraction of algae

Fresh specimens of the brown alga *Turbinaria ornata* were collected from the Mandapam coastal region (latitude 78° 8′ east and longitude 9° 17′ north) in the Gulf of Mannar at the Bay of Bengal by using sterile polyethylene bags. Samples were cleaned thoroughly with sterile seawater followed by distilled water to remove adhering debris, associated epifauna/epiphytes. After cleaning, they were dried in the shade at room temperature for a week. The shade dried algal material was macerated as to make coarse powder using mortar and pestle. The 20 g of algal powder were mixed with 200 ml of distilled water and kept in a boiling water bath at 60 °C for 10 min. After cooling, the crude algal aqueous extract (AAE) was filtered through a Whatman No.1 filter and was stored in a refrigerator at 4 °C until further analysis (Vignesh et al., 2013).

2.5. Biosynthesis and characterization of silver nanostructures (Ag-NPs)

Aqueous solution of 1 mM silver nitrate ($AgNO_3$) (analytical grade — Merck, India) was used for the synthesis of silver nanoparticles (Vivek et al., 2011). The reaction mixture was prepared by adding 5 ml of AAE to 95 ml of 1 mM $AgNO_3$ solution in a 250 ml Erlenmeyer flask and kept in a boiling water bath at 70 °C until the color changed to dark brown. The formation of brown color indicates the biosynthesis of *T. ornata* silver nanoparticles (TOAg-NPs), which were confirmed by UV–Vis spectroscopy (Shimatzu 160). The size/morphology, composition and functional groups of the nanoparticles were elucidated by

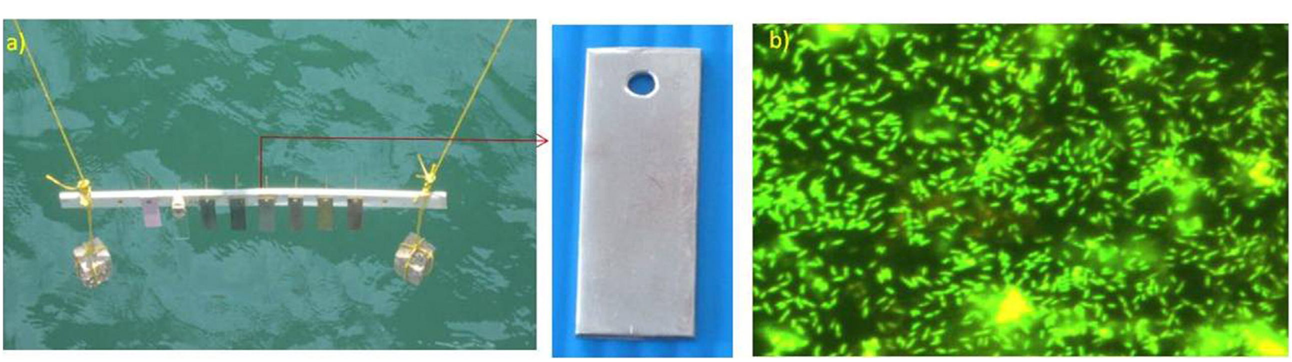

Fig. 1. (a) SS 304 coupons exposed to natural seawater, (b) biofilm formed coupons were optically characterized by fluorescence microscopy.

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