Synthesis of silver nanoparticles from Indian red yeast rice and its inhibition of biofilm in copper metal in cooling water environment

Muthukumar Suganya · Parameswaran Sujatha Preethi · Jayaraman Narenkumar · Arumugam Arul Prakash · Sandhanasamy Devanesan · Mohamad S. AlSalhi · Aruliah Rajasekar · Ayyakkannu Usha Raja Nanthini

Abstract
The development of environmentally acceptable benign techniques using purely natural methods is a cost-effective procedure with long-term benefits in all areas. With this consideration, myco synthesized silver nano particles (AgNPs) were studied and it acted as an impending corrosion inhibitor in the environment. Initially, AgNPs were evaluated by physical and surface characterizations and this evidence demonstrated that RYRE’s water-soluble molecules played an essential role in the synthesis of AgNPs in nano spherical size. The myco synthesized of AgNPs has showed an antibacterial activity against corrosive bacteria in cooling water system (CWS). Hence, the AgNPs were used in biocorrosion studies as an anticorrosive agent along with AgNO₃ and RYRE was also checked. For this experiment, the copper (Cu) metal (CW024) which is commonly used was selected, the result of corrosion rate was decreased, and inhibition efficiency (82%) was higher in the presence of AgNPs in system IV. Even though, AgNO₃ and RYRE had contributed significant inhibition efficiency on Cu at 47% and 61%, respectively. According to XRD, the reaction of AgNPs on Cu metal resulted in the formation of a protective coating of Fe₂O₃ against corrosion. EIS data also indicated that it could reduce the corrosion on the Cu metal surface. All of these findings point out the possibility that the myco-synthesized AgNPs were an effective copper metal corrosion inhibitor. As a result, we encourage the development of myco-synthesized AgNPs, which could be useful in the industrial settings.

Keywords Red yeast rice extract · Silver nanoparticle · Copper metal · Biocorrosion · Cooling water system

Introduction
Bio-corrosion is one of the leading industrial problems that can cause extreme damage to expensive equipment/instruments and consequently bring about great economic loss in the maintenance at industrial sector (Guo et al. 2018; Narenkumar et al. 2017a). Due to the microbial activity, metal deterioration appears in the cooling tower which is known as microbial influenced corrosion (MIC). The microorganisms can change the chemical compounds on the surfaces of metals by metabolic activity, which can affect the metal’s interface chemical bond (Narenkumar et al. 2018a; Beech and Sunner 2004). There are many negative impacts influenced by microorganisms and their by-products on heat transfer in the cooling tower(Rajasekar and Yen-Peng 2014), whereas increasing the bacterial community’s resistance to heavy metals is a significant risk worldwide (Ianieva 2009; Wood and Wang 1983).

Cu is the most commonly used metal for cooling towers in many industries as they have good conductivity,
higher corrosion resistance behavior, and excellent insulating properties (Narenkumar et al. 2019). Although on the contrary, several research work have found that Cu alloys are subjected to MIC-induced pitting and crevice corrosion. (Javed et al. 2016; Lytle and Nadagouda 2010; Warraky et al. 2004). The metal content of copper with the minimum amount of nickel alloys is susceptible to several types of localized corrosion (Little et al. 1991). Meanwhile, microbes accelerate the material deterioration which causes the high release of Cu byproducts in the water bodies (Critchley et al. 2004; Reyes et al. 2008). Therefore several techniques (mechanical, chemical, electrochemical and biological etc.) have been performed to reduce the biocorrosion in CWS (Li et al. 2018; Narenkumar et al. 2017a). Aside from the natural methods, most of these approaches are expensive and deemed detrimental to the environment (Dalmoro et al. 2012). Consequently, researchers are involved in exploration of the natural by-products as potent corrosion inhibitors due to the tremendous biological availability in the environment.

Myco-nanoparticle synthesis is one of the biosynthetic methods, which involves fungus for the synthesis of nanoparticle due to their large biomass which allows for easy handling during biosynthesis. A large number of fungal strains are capable of synthesizing metal nanoparticles (Dinarvand et al. 2017). A large number of physical and chemical methods which is used to synthesize nanoparticles are expensive and possess serious threat to the biological system. Relatively successful, biosynthetic methods can be suitable alternative, as they are safe, more effective, and eco-friendly (Ahmed et al. 2016) and they have risk-free synthesis procedures (Khalilzadeh and Borzoo 2016). In that way, myco-synthesized nanoparticles have more potential activities due to their bioavailability, cost-effective and less toxicity (Azmath et al. 2016; Madakka et al. 2018). The beneficial fungi, Monascus ruber, produces more secondary metabolites which have many medicinal properties (Lachenmeier et al. 2012), and it has been used for producing fermented food product known asangakak or red yeast rice in East Asian countries. It is also used as traditional medicine for over 1000 years (Wang and Lin 2007). The present study has been to synthesis silver nanoparticles using red yeast rice (RYR) and to evaluate their anti-biocorrosion activity on Cu metal in cooling water system.

Materials and methods

Fungal culture and chemical

The Monascus ruber (460) was obtained from MTCC, Chandigarh, India. The culture kept at 4°C on Potato Dextrose Agar (PDA) and was sub cultured periodically. Silver nitrate chemical (AgNO₃) was obtained from Sigma Aldrich.

Preparation of red yeast rice powder and extract

The culture of M. ruber was inoculated in steamed rice and was kept for solid-state fermentation in 250-mL Erlenmeyer flask for 14 days at 30°C. After that, the fermenting rice was dried and ground (Ferdes et al. 2009). The red yeast rice extract (RYRE) was obtained by extracting the final product of RYR with water (ratio 1:10 w/v) at 70°C for 15 min.

Silver nanoparticles synthesis

A reaction mixture of RYRE and 1.5-mM AgNO₃ solution in a ratio of 1:9 was used. The primary confirmation of colour change observed by visual observation and was confirmed at 420 nm in UV Vis spectrophotometer. In centrifugation, sedimentation was separated at 12,000 rpm for 15 min. To ensure purity, the collected sedimentation was re dispersed in deionized water and centrifuged again (Phongtongpasuk et al. 2016) and, then, dried the final particles and analyzed the characterization by FTIR, XRD, and TEM.

Biocorrosion studies

Cultures

In this experiment, Bacillus thuringiensis (EN2), Terribacillus aidingensis (EN3), and Bacillus oleronius (EN9) strains were used; these strains are found to be dominant in the biofilm sample of CWS and was isolated and identified by Narenkumar et al. (2018b). EN2, EN3, and EN9 sequences were submitted to the National Center for Biotechnology Information (NCBI) with the accession numbers KR183873, KR183874, and KR183880. All of the strains were kept in glycerol stock and maintained the sub cultured in sterile nutrient agar (NA) plates.

Agar well diffusion assay

The antibacterial activity of AgNPs and RYRE on Muller Hinton Agar was determined using the well diffusion method. MHC agar plates were prepared and 0.1 mL of bio-corrosive strains (EN2, EN3, and EN9) was uniformly spread under aseptic condition. The wells were cut by cork borer and filled with 50ppm of RYRE and 10, 50, and 100ppm of AgNPs, respectively. In the remaining wells, as a positive control, Tetracycline (10 μg) was used, because it is having wide range of bacterial resistance and as a negative control, sterile distilled water was used on the plate. These plates were performed in triplicates and incubated for 24 h at 37°C. After that, the diameter of the inhibition zones was measured.
Biofilm formation assay

This biofilm assay technique on AgNPs was done by following Narenkumar et al. (2018a). Twenty-four-hour culture of EN2, EN3, and EN9 was inoculated in nutrient broth (NB) medium diluted to 1:20 ratio with fresh NB medium. A 100-μl mixed consortium (10⁴ CFU/mL) was transfer into a 96-well microliter plate followed by 50ppm of AgNPs which were added and incubated at 37°C for 24 hrs. After the incubation, the culture was removed and rinsed with phosphate buffer saline (137mMNaCl, 2.7 mMKCl, 2 mM KHPO, pH 7.2) and then with distilled water. After that, 120 μl of crystal violet was added and incubated at 37°C for 20 min then rinsed with distilled water. In the end, 125 μl of acetic acid added and the mixture incubated at 37°C for 15 min. The final product was examined using UV–visible spectrophotometer set to 600 nm.

Bio‑corrosion studies

For this study, Cu CW024 was chosen and tested for corrosion rate (CR); Electrochemical Impedance Studies (EIS); and surface analysis. The coupon size was around 2.5cm² and 1.5cm², and the metal composition wt (%) was zinc 0.03, tin 0.01, manganese 0.65, nickel 6.09, silicon 0.004, iron 1.41, lead 0.01, and the remaining 91.79 Cu. The experimental system strategy is given in Table 1. Briefly, the copper metal coupons were polished, and the coupons were immersed in 400-mL CTW containing 1% of sterile NB medium in 500-mL Erlenmeyer flask and were denoted as control. The system I consisted of control inoculated with 1mL of each bio corrosive strains (consortia) at 1.3 × 10⁷ CFU/mL in a 500-mL Erlenmeyer flask. System II comprised of system I and added 50ppm of AgNO₃ alone, whereas, system III comprised of system I with 50 ppm of RYRE, and system IV comprised of system I with 50 ppm of AgNPs. To analyze corrosion rates, all experiments were performed in triplicate coupons and kept in room temperature for 14 days in a static condition (Narenkumar et al. 2016).

Corrosion rate (CR) and weight loss (WL) measurements

After the incubation, the surface biofilm was scraped off from the Cu coupons in respective bio-corrosive systems. Collected samples were dried and crushed into fine powder then the samples were characterized by FTIR and XRD (Bruker-8030). To analysis the WL, the Cu coupons were soaked with pickling solution (1 L of H₂Oand 500 mL of HCL) for 25 min at room temperature, and then, the average WL and standard deviation (SD) were calculated after that CRs of the metals were calculated by using a standard method (Preethi et al. 2019).

Electrochemical measurement

EIS contains three types of electrode systems (CH Instrument Inc., USA model CHI-608E): Cu coupons were chosen as the working electrode, while Ag/Agcl was used as the reference electrode and for counter electrode platinum wire was used. These electrodes were used to detect impedance and Tafel polarization. (Bellon-Fontaine et al. 1996).

Result and discussion

Biosynthesis of AgNPs

The preparation of RYR (Fig. 1a) and RYRE were carried out by the procedure mentioned in material and methods. A reaction mixture color was then changed from pale red to dark brown as seen visually (Fig. 1b). This brown color indicated the formation of AgNPs as a result of Ag⁺ ion reduction in the reaction solution (Gurunathan et al. 2009), which was confirmed by UV-Vis spectroscopy at 420 nm with specific excitation of surface plasmon resonance in Fig. 1c (Sharma et al. 2018). It was concluded that the cell-free extract of RYRE is suitable for synthesis of silver nanoparticles because it contains high amount of amino acids (Abdul et al. 2017), polysaccharide(Srianta et al. 2014), and total phenols and flavonoids (Hasim et al. 2018). As a reducing and stabilizing agent, these compounds are taking part in the formation of AgNP synthesis (Aritonang et al. 2019; Marslin et al. 2018).

Characterization of AgNPs

FTIR analysis of AgNPs (Fig. 1d) revealed that seven distinct functional groups are present in the sample. The

| S.no | Systems | Experimental plan |
|------|---------|------------------|
| 1 | Control | 400 mL in sterile cooling tower water containing 1% of nutrient broth (NB) |
| 2 | Systems I | 400 mL in sterile cooling tower water containing 1% of nutrient broth (NB) with 1mL of consortium (B. thuringiensis EN2, T. ailingensis EN3 and B. oleronius EN9) |
| 3 | Systems II | 400 mL in sterile cooling tower water containing 1% of nutrient broth (NB) with 1mL of consortium and 50 ppm AgNO₃ |
| 4 | Systems III | 400 mL in sterile cooling tower water containing 1% of nutrient broth (NB) with 1mL of consortium and 50 ppm RYRE |
| 5 | Systems IV | 400 mL in sterile cooling tower water containing 1% of nutrient broth (NB) with 1mL of consortium and 50ppm AgNPs |
O-H stretch of alcohol and phenols is indicated by the prominent and broad transmittance peak at 3433.33 cm\(^{-1}\). The primary amines and secondary amines of amino acids, peptides, and proteins are indicated by the bonds made at 2925.86 cm\(^{-1}\) and 1626.61 cm\(^{-1}\) for C-H stretching alkanes and N-H stretch, respectively, which enhance the stability of synthesized nanoparticles (Tai et al. 2014). The phosphorus or sulfur function groups, which might also link with silver and act as both capping and stabilizing agent of nanoparticles, can be attributed to the peak at 1432.48 cm\(^{-1}\) and 1079.04 cm\(^{-1}\) correlated with C-C stretch (Hamouda et al. 2019). C-O stretch alcohol, carboxylic acid, and 762.82 cm\(^{-1}\) and 669.20 cm\(^{-1}\) corresponded to alkenes. Hence, these biomolecules are considered to be responsible for efficiently capping and stabilizing AgNPs.
The distraction intensity was recorded from 200 to 800 at 2θ angle based on the XRD data (Fig. 1e). It indicates 6 strong intensity peaks at 32.40, 33.58, 38.20, 44.42, 64.59, and 77.53, which corresponded to the Centro symmetric structural planes 122, 212, 123, 312, 241, and 137. All of these peaks are quite similar to the JCPDS file number 00-043-0649. Using the Scherrer formula, the average crystallite particle size was calculated to be 6.46 nm (Fig. 1f). According to the TEM, the particles nano-size ranged from 6.81 to 30.93 nm in diameter, and their shape was spherical (Fig. 1f and g).

**Antibacterial activity**

Myco-synthesized AgNPs were tested for antibacterial activity against bacterial consortium with agar well diffusion method. AgNPs had the highest activity at 100ppm and the lowest activity at 10ppm, with moderate inhibition at 50ppm. When compared to other concentrations of AgNPs, the zone of inhibition shown by RYRE was relatively moderate. According to several reports, AgNPs are an effective antibacterial agent that breaks the chemical bonds of the bacterial wall and causes cell death (Le Ouay and Stellacci 2015; J. Li et al. 2013). Based on the findings, 50ppm of AgNPs was chosen as minimal inhibitory concentration (MIC) for further bio-corrosion study.

**Biofilm formation assay**

Crystal violet was used for the biofilm formation assay on a microliter plate (Toole et al. 1999). The consortium has formed biofilm on the outer surface after 24 h of incubation; in contrast, there was no biofilm formation appearing in AgNPs (Fig. 2). The foregoing results were shown AgNPs had the ability to inhibit biofilm growth on the surface.

**Biocorrosion studies**

**Weight loss and corrosion rate**

WL and CR of copper coupons was calculated (Table 2). In abiotic condition, the average weight loss of the control system was around 0.0016± 1, whereas the system I had maximum amount of weight loss about 0.0039 ± 2. System II and system III had about 0.0008 ± 2 and 0.00060 ± 1, and the inhibition efficiency was about 47% and 61%, respectively. These results were shown that the presence of AgN03 and RYRE compounds is acting against the host bacteria in CWS.

The presence of AgNPs in system IV resulted in a considerable weight reduction of 0.00027 ± 2, and the inhibition efficiency was 82% that is higher when compare than other systems. All corrosion rate were calculated from average weight loss about 0.017, 0.042, 0.009, 0.006, and 0.002 mm/ year, respectively. Metal weight loss generally occurred by the formation of biofilm on metal surface, which caused pitting corrosion, mainly in Cu, due to metal oxidation (Preethi et al. 2019). From these experiments, the results indicated that system IV had lower WL and CR and have found high inhibition efficiency. Finally, myco-synthesized AgNPs have an important role in CWS as an effective inhibitor of biofilm formation as well as a reduction in Cu metal corrosion.

**Surface analysis**

XRD was used to characterize the corrosion products obtained from each experimental system (Fig. 3). The control system listed all of the peaks and their compounds, and that peak intensity was compared to other experimental systems. The high-intensity peaks were observed in 20 ranges of 35, 45, and 55, indicating Cu (OH)2, Cu2O, and NiCuO3 compounds, respectively, and the low-intensity peaks were found at 20 ranges of 65 and 75, indicating the compounds Mn2O4 and Fe2O3, respectively.
The peak intensity of Cu (OH)₂ was found in all experimental system. Besides, Cu₂O peak was significantly reduced from system II to IV due to the activity of AgNO₃, RYRE, and AgNPs, respectively. Consortia and AgNO₃ indicate that system I and system II exhibited a partially reduced peak at NiCuO₃, but NiCuO₃, Mn₂O₄, and Fe₂O₃ were absent in the RYRE-contained system III, demonstrating that RYRE can reduce corrosion. When compared to other systems, the intensity of Cu (OH)₂ and Cu₂O peaks was reduced in system IV, whereas NiCuO₃ and Mn₂O₄ disappeared. Those peak intensities have decreased and vanished, indicating that Cu metal is less corroded. In system IV, the peak in Fe₂O₃ was higher than others, indicating that the adhesion of AgNPs on Cu metal can be reacted with Fe₂O₃ via the oxidation process (Touati 2000). Similarly, Narenkumar et al. (2017a) found that the adsorption of AgNPs on mild steel formed a protective film with Fe compound, acted to protect the metal from corroding. Furthermore, Ag/Fe₂O₃ nanoparticles showed a significantly higher antibacterial effect than Fe₂O₃ alone (Kareem et al. 2018). In system IV, the oxidation process of Fe and AgNPs compound was clearly found to decrease corrosion. As a result, we confirmed AgNPs as a positive inhibitor that created a protective coating on Fe₂O₃ compound of the surface of Cu metal, which inhibits biofilm formation and prevents corrosion in the CWS.

| S.No | Systems | Polarization data |
|------|---------|-------------------|
|      |         | $E_{\text{corr}}$ (mV) | $i_{\text{corr}}$ (A/cm²) |
| 1    | Control | -522              | $3.6 \times 10^{-6}$ |
| 2    | System I| -499              | $4.7 \times 10^{-7}$ |
| 3    | System II| -467             | $3.5 \times 10^{-7}$ |
| 4    | System III| -462            | $3.3 \times 10^{-6}$ |
| 5    | System VI| -495             | $4.6 \times 10^{-6}$ |

$E_{\text{corr}}$ Corrosion potential, $i_{\text{corr}}$ Corrosion current

![Fig. 3 XRD pattern of corrosive Cu product](image)

![Fig. 4 Nyquist plot/AC impedance spectra of Cu metal treated with various corrosion systems](image)
Electrochemical impedance studies (EIS)

Electrochemical impedance results are presented in the Table 3 and Figs. 4 and 5. The control system’s corrosion current ($i_{corr}$) was around $3.6 \times 10^{-7} \text{A/cm}^2$ and in system I mixed consortium was higher at $4.7 \times 10^{-7} \text{A/cm}^2$. In system II, the addition of AgNO$_3$ resulted in an $i_{corr}$ of about $3.5 \times 10^{-7} \text{A/cm}^2$, while in system III, the addition of RYRE resulted in an $i_{corr}$ of about $4.6 \times 10^{-6} \text{A/cm}^2$. The corrosion current in system IV with AgNPs was about $3.3 \times 10^{-6} \text{A/cm}^2$. System I had the higher corrosion current because of the growth of microbes on the Cu surface; therefore, anodic cite current density was shifted to cathodic cite that indicates the higher level of corrosion occurred in Cu metal Narenkumar et al. 2017b; Rajasekar et al. 2007). According to Preethi et al. (2019) and Rajasekar et al. (2005), when corrosion occurs, the Cu ions breakdown on the metal surface, which stimulates the formation of pits. According to the results, when compared to other systems, system IV showed lower corrosion current density indicating that it had achieved less metal corrosion. System III outperforms system II by the $i_{corr}$ value. From this EIS data, it is clear that AgNPs have the capability to prevent corrosion on Cu metal used in CWS.

Conclusion

Current investigation highlighted the significance of an industrial complication, which is commonly found in CWS and dealt with the issue by using fungi-based RYRE-mediated AgNps. We successfully developed an environmentally friendly synthesis method for AgNps, with formulation and characterization confirmed by physical examination. In bio-corrosion experiments, AgNps exhibited strong antibacterial properties against the corrosive bacteria of CWS and have been found to be a possible corrosive inhibitor against biofilm formation, resulting in a reduction in Cu metal CR. According to XRD results, AgNPs reacted with Cu metal to form Fe$_2$O$_3$ compound, which serves as a protective coating on the Cu surface; in addition to, EIS data also proved that the significant indication on Cu metal. Furthermore, RYRE and AgNO$_3$ were found to be capable of minimizing Cu corrosion rate and have the evidence of inhibition efficiency in all studies. As a result, we hypothesized that myco-synthesized AgNPs could be an effective inhibitor to prevent corrosion from corrosive bacteria in CWS and that it could be used in the development of anti-biofilm agents, a promising corrosion application.

Acknowledgment The authors are grateful to the Deanship of Scientific Research, King Saud University, for funding through the Vice Deanship of Scientific Research Chairs.

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Environmental Science and Pollution Research (2022) 29:77800–77808

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