Green biocide for mitigating sulfate-reducing bacteria influenced microbial corrosion

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Abstract

In the present investigation, *Polyalthia longifolia* plant extract (PLAE) was used as biocide to control corrosion in the presence of sulfate-reducing bacteria (SRB). Transmission electron microscopy showed the damage of SRB outer cell membrane which lead to cell destruction and disturbed membrane permeability. The scanning electron microscopy also confirmed the cell shrinkage due to green biocide, and energy-dispersive Fourier transform infra-red spectroscopy indicated the decrease in sulfide concentration in the presence of biocide. Potentiodynamic polarization of mild steel showed the lower in corrosion rate due to the decrease in cathodic reduction kinetics of SRB in the presence of biocide PLAE. The gravimetric mass loss also showed corrosion rate dropped from 0.064 millimeter per year (mm/year) to 0.013 mm/year with and without biocide. The present study showed that *P. longifolia* extract could be a novel biocide against the growth of the SRB to control corrosion in oil and gas industries.

Keywords *Polyalthia longifolia* · Sulfate-reducing bacteria · Biocide · Microbial corrosion · Transmission electron microscopy

Introduction

The petroleum products, water- and gas-transporting pipelines are suitable environments for microorganisms to survive and also affect the product transport and storage (Gayosso et al. 2004). Corrosion in oil- and gas-transporting pipelines due to microbial corrosion was about 40% (Parthipan et al. 2017). The microorganisms need nutrition comprising primarily of four main components to thrive: a carbon source, water, an electron donor, and an electron acceptor. The hydrocarbons act as an excellent nutrient source and an electron donor for a wide variety of bacteria. Sulfate-reducing bacteria (Mohd Ali et al. 2016) iron-reducing bacteria (Herrera and Videla 2009) are the main bacteria associated with corrosion of metals in pipeline systems.

The inner environments of the pipeline systems typically have anaerobic and/or low oxygen concentrations and it favors the SRB to be the major candidate for accelerating the corrosion process (Dinh et al. 2004). The SRB under anaerobic conditions oxidize organic compounds utilizing sulfate (SO_4^{2-}) as an electron acceptor and generate sulfide (S^{2-}). The sulfide reacts with Fe^{2+} and form iron sulfide as the corrosion product along with hydroxide of iron.

The SRB can utilize the molecular hydrogen for the breakdown of organic compounds or oxidize the organic compounds which act as an electron donor to SRB for their growth in the anaerobic environment. Venzlaff et al. (2013) reported that cathodic reaction in microbial corrosion of iron due to the direct uptake of electron by sulfate-reducing bacteria. Here, iron sulfides acts as a semiconductor and plays an important role in anaerobic corrosion by mediating electron flow from metal surface to SRB.

In addition to the corrosion of pipeline, the sulfide production by the SRB can also alter the chemical properties of oil or petroleum products. Therefore, it is essential to prevent the attack on the internal structure of pipeline from the biological attack by suppressing the growth of bacteria. This can be achieved by the addition of suitable biocides into the systems to prevent the unwanted bacterial growth

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(Telegdi et al. 2000; Konstantinou and Albanis 2004; Little et al. 2007). The biocides are often preferred to control the harmful corrosive bacteria and to protect the metal structures from the biofouling for certain degree. Attractive biocides such as D-methionine, a naturally occurring biodegradable D-amino acid, has been used for biofilm mitigation in oil and gas industries and water utilities (Xu et al. 2013). The combination of green biocide tetrakis hydroxymethyl phosphonium ($C_8H_{24}O_{12}P_2S$) and a naturally occurring chemical D-tyrosine has also been used as large-scale green biocide which is low cost and has no impact on environment (Xu et al. 2012). The non-oxidizing biocides such as glutaraldehyde ($C_5H_8O_2$) and tetrakis hydroxymethyl phosphonium ($C_8H_{24}O_{12}P_2S$) are also used to avoid the chemical corrosion (AQUCAR™ THPS 75 2009).

The key question is to find a biocide which should be environmentally safe and effectively controls the microbial corrosion of pipelines. Most of the high anticorrosion synthetic compounds are environmental toxic (Verma et al. 2015; Haque et al. 2016; Singh et al. 2016). Thus, it is mandatory to move towards the natural, non-toxic, eco-friendly, easily available, cost effective, and biodegradable biocides compounds for cleaner environment to protect the metals from deterioration by the microorganisms (Chambers et al. 2006). The specific chemical structure found in the natural plant extract such as the furan and/or lactone ring is claimed to have good biocidal nature (Malladi et al. 2017; Winska et al. 2018). Shaily et al. (2014) investigate the inhibition behavior of neem extract against sulfate-reducing bacteria. Recently, Parthiban et al. (2017) studied the green inhibitor behavior for microbial corrosion of carbon steel API 5L X in hypersaline environment of neem extract. Extracts from various plant derivatives are reported to control chemical corrosion, for instance, *Lemon verbena*, *Dryopteris cochleata* leaf extracts, bamboo leaves, orange peel extract, *Phyllanthus amarus*, *Chromolaena odorata* extracts, Marigold flower, *Musa paradisiaca*, aloe vera, and garlic extract (Fattah-alhosseini and Noori 2016; Nathiya and Raj 2016; Li et al. 2014; Mhiri et al. 2016; Anupama et al. 2016; Aribio et al. 2016; Mourya et al. 2014; Ji et al. 2015; Abiola and James 2010; Parthipan et al. 2018).

Hence, our group tried to investigate to sort out that the problem arises due to the use of toxic chemical biocide against microorganisms and suggests an alternate environmental friendly biocide. *Polyalthia longifolia* has many advantages, including availability and low cost. Pharmacologic studies on the bark and leaves of *P. longifolia* plant show effective antimicrobial activity, cytotoxic function, antiulcer activity, hypoglycemic activity, and hypotensive effect (Katkar et al. 2010). To the best of our knowledge, no literatures are available related to *P. longifolia* extract for mitigating sulfate-reducing bacteria influenced microbial corrosion. In the present study, the application of green plant

extract of *P. longifolia* (PLAE) to control the SRB growth and reduce the corrosion was discussed. The effective testing for the biocide against the consortium of microorganism is required for the success in preventing the corrosion of structures. However, the current preliminary work focuses only on the sulfate-reducing bacteria.

Materials and methods

Green biocide extraction

The source for the green biocide was the *P. longifolia* plant, which was grown in the premise of CSIR-Central Electrochemical Institute laboratory, Karaikudi, Tamil Nadu, India. The healthy, disease free, and mature leaves were collected and washed in clean water thoroughly 2–3 times and followed by sterile deionized water and dried under shade. After few days, dried leaves were collected and powdered finely using sterile mortar and pestle and kept in air-tight containers and stored in refrigerator for further studies. The plant material (*P. longifolia*) (50 g) was added with methanol (400 mL) for 24–48 h at room temperature. Under reduced pressure, methanol was evaporated to yield dark-brown syrup, and between $CHCl_3$ and water, the syrup was partitioned. Then, using 3% HCl, the $CHCl_3$ partition was extracted and the $CHCl_3$ solution was evaporated to obtain the green viscous residue (Chen et al. 2000; Lee et al. 2009; Sashidhara et al. 2009).

Bacterial growth conditions

Sulfate-reducing bacteria (SRB) isolated from the marine environment which was used in the present study. The bacteria were grown in Postgate medium B for 3 days at 30 °C under anaerobic condition in 30 mL sterile screw cap tubes (Postgate 1984). The Postgate medium B consists of the following compounds in g/L: 0.5 g KH_2PO_4 , 1 g NH_4Cl , 1 g $CaSO_4$, 2 g $MgSO_4 \cdot 7H_2O$, 3.5 g sodium lactate, 1 g yeast extract, 0.1 g ascorbic acid, 0.1 g thioglycollic acid, 35 g sodium chloride, and 0.5 g $FeSO_4 \cdot 7H_2O$. The pH of the media was adjusted between 7 and 7.5.

Corrosion study

Mild steel specimens were used in the present study to evaluate the biocidal property of green biocide in sulfate-reducing bacterial environment. The chemical composition of mild steel was C: 0.24%, Pb: 0.04%, S: 0.045%, N: 0.009%, and rest Fe. The surface preparation of mild steel substrates involved polishing using various grades of SiC paper and finally with diamond paste up to mirror finish and degreased with acetone to remove residual oil-based

Scheme 1 Chemical structure of the extract of *P. longifolia* used as biocide against sulfate-reducing bacteria

the SRB grown electrolyte for 240 h in the presence and the absence of biocide (PLAE). $1 \times 1 \text{ cm}^2$ size specimens were used for electrochemical and morphological observation. The corrosion rate was calculated based on the following equation (Revie 2011):

$$\text{Corrosion rate (mm/year)} = (87.6 \times W) / (D \times A \times T), \quad (1)$$

where W —weight loss (mg), D —density of mild steel (7.86 gm/cm^3), A —area (cm^2), and T —time (h).

Instrumentation

The green plant extract was characterized using NMR spectroscopy (^1H -NMR (400 MHz) and ^{13}C NMR (100 MHz)

Fig. 1 **a** ^1H NMR (400 MHz) spectral data of biocidal extract. The top figure is the whole spectrum. **b** ^{13}C NMR (100 MHz) spectral data of biocidal extract. The inset figure shows the isolated compound which is responsible for biocidal activity

contamination using ultrasonicator for 10 min. The mirror-polished samples of dimension ($2.5 \times 2.5 \times 0.5 \text{ cm}^2$) were used for gravimetric study. The initial weight of the samples was measured using Shimadzu AUM220D-weighting balance (0.01 mg accuracy). Mild steel specimens were immersed in

Bruker instrument), and deuterated chloroform was used as a solvent. Determining the nature of the pure plant extract and biocide activity against SRB was employed by FTIR spectroscopy (model Nexus 670). The sulfate-reducing bacterial

culture in the presence and the absence of plant biocide (5%) was used for FTIR analysis. After 3 days of incubation period, the bacterial cell was separated by centrifugation at 6000 rpm for 20 min. The process was repeated thrice using deionized water. The sample was placed directly in the KBr crystal, and in the transmittance mode, the spectrum was recorded.

The surface morphology of mild steel after exposing to the SRB environment with the presence and the absence of biocide (5%) was observed under Hitachi, S-3000H Scanning electron microscope (SEM) (operating voltage: 0.3–30 kV) attached with energy-dispersive X-ray analyzer (EDS). The standard procedure adapted by Sridharan et al. (2013) was followed for SEM sample preparation. The corrosion product present over the mild steel surface after exposing to the SRB environment was also characterized using EDAX analysis.

The biocidal action of biocide PLAE was also investigated by transmission electron microscopy (model TecnaiTM G2 F20, FEI). After the incubation period, in the presence and the absence of biocide, the culture was filtered aseptically using 0.2 μm membrane filter to remove the unwanted particles present in the culture medium. The cells were placed on the TEM copper grid for observation.

Electrochemical measurement

The electrochemical study was carried out using computer-controlled Autolab PGSTAT 302 potentiostat in a corrosion cell. A three-electrode system was used, which contains mild steel as a working electrode, platinum foil as an auxiliary electrode, and saturated calomel electrode (SCE) as the reference electrode. Polarization experiment was carried out by sweeping voltage from cathodic to anodic (−0.200 V to +0.200 V) at a sweep rate of 0.5 mV/s. Each experiment was done with a replica at ambient room temperature (28–30 °C).

Results and discussion

NMR spectroscopy

The plant extract contains various chemical compounds such as furan and lactone ring (Scheme 1) has the capability to show biocidal activities (Chen et al. 2000; Lee et al. 2009; Sashidhara et al. 2009). In the present study, the ^1H NMR spectrums' four methyl groups are found to be at δ 0.584, 0.916, 1.133, and 0.607 (Fig. 1a, b). A triplet signal at δ 5.099 can be attributed one oxymethine group. In addition, two singlet signals δ 5.894 and 7.193 were confirmed the presence of one γ -hydroxy- α,β -unsaturated- γ -lactone moiety (Table 1). The OH group was present at δ 3.382. ^{13}C NMR

spectra showed that 20 distinct signals are present in the spectra. In that, 4 methyl carbons are present at δ 18.73, 20.96, 21.01, and 22.64. Six methylene carbons are present at δ 20.74, 24.08, 26.20, 30.11, 32.46, and 37.11. Five methane carbons are present at δ 39.07, 41.39, 79.82, 102.08, and 119.47, and five quaternary carbons are present at δ 40.91, 53.41, 80.14, 173.63, and 174.44. For the above spectral

Table 1 ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of biocide

Position	δH (multiplicity)	δC (multiplicity)
1	0.681(m), 1.594(m)	20.74(s)
2	1.265(m), 2.067(tt)	30.11(t)
3		79.82(d)
4		53.41(s)
5		80.14(s)
6	0.719(m), 1.498(m)	32.46(t)
7	0.748(m), 1.436(m)	26.20(t)
8	0.791(m)	39.07(d)
9		40.91(s)
10	1.652(dd)	41.39(d)
11	1.352(m), 1.401(m)	37.51(t)
12	1.955(br s)	24.08(d)
13		173.63(s)
14	5.894(s)	119.47(d)
15		174.44(s)
16	7.193(s)	102.08(d)
17	0.607(d)	18.73(q)
18	1.133(s)	22.64(q)
19	0.916(s)	20.96(q)
20	0.584(s)	21.01(q)

Fig. 2 Postgate SRB medium without and with biocide addition

Fig. 3 FTIR spectroscopy of pure plant extract, SRB with and without PLAEC biocide

data, signals at δ 5.894, 7.193 with respect to ^1H NMR and signals at δ 102.08, 119.47, 173.63, and 174.44 with respect to ^{13}C . NMR demonstrated the presence of γ -hydroxy- α,β -unsaturated- γ -lactone moiety. The furan and lactone moiety of the active component of plant extract were identified by the NMR spectroscopy which was similar to the report in the literature (Xu et al. 2012).

Figure 2 shows the isolated bacteria inoculated Postgate medium B with and without biocide treatment. Without biocide, black color was observed in the culture tube due to the presence of SRB. Sulfate-reducing bacteria convert sulfate to sulfide, and the sulfide reacts with iron which are present in the medium and form FeS. Besides, in the presence of green biocide the black color formation will be very less. The biocidal property of the green biocide suppresses the SRB growth and reduces the sulfate conversion. Figure 3 shows the FTIR spectrum of pure plant extract, SRB cells and SRB cells treated with plant (PLAE) biocide. The pure

Fig. 4 Surface analysis by scanning electron microscopy of SRB cells with and without biocide. SRB cells on mild steel in the absence of PLAEC biocide shows **a** dense SRB biofilm, **b** high porous bio-sulfide,

and in the presence of PLAEC biocide shows **c** loosely bound biofilm with less cells and sulfide **d** cell shrinkage and membrane damage

plant extract FTIR spectra represent the peaks at 1644 cm^{-1} , 1418 cm^{-1} , and 1063 cm^{-1} and were amide I arise due to carbonyl stretch in proteins, $\text{C}-\text{C}$ -stretching (in ring) aromatic and $\text{C}-\text{N}$ -stretching vibration of amine, respectively (Kaviya et al. 2011). The spectra of the SRB system clearly show peak at the 640 cm^{-1} , 1150 cm^{-1} , 1637 cm^{-1} , 2934 cm^{-1} , and 3436 cm^{-1} for $\text{C}-\text{O}-\text{S}$ vibration of sulfates, $\text{S}=\text{O}$ -stretching vibration of sulfate esters, protein amide I, $\text{C}-\text{H}$ stretching, and OH groups, respectively (Alexander et al. 2013). FTIR spectra for the SRB with biocide system show the reduction in the peak intensity for protein amide I ($\sim 1631\text{ cm}^{-1}$), $\text{C}-\text{O}-\text{S}$ vibration of sulfates ($\sim 576\text{ cm}^{-1}$), $\text{S}=\text{O}$ -stretching vibration of sulfate esters ($\sim 1050\text{ cm}^{-1}$), and OH groups ($\sim 3457\text{ cm}^{-1}$). This analysis clearly shows the interaction between the SRB and the plant biocide. SEM image 4a shows the extent of colonization with a higher number of SRB cells found in the mild steel surface, and it is due to the absence of biocide in the system, and in Fig. 4b, SRB cells with salt like structures were observed and it was due to the presents of sulfide salts present in the medium. In Fig. 4c, SRB cell shrinkage/deformation was noticed in the SEM images of the biocide treated SRB cells; due to

the cell shrinkage, the permeability of the cell membrane might be increased. Chen and Cooper (2002) reported that biocide has high positive charge density, and it displaces the membrane surface divalent ions and bind with the phospholipid membranes. It causes the slight change in membrane permeability. High concentration of cationic biocide can be disintegrating the bacterial membrane. The similar results were reported earlier (Tyagi and Malik 2010) on *E. coli* cells treated with the lemon grass oil (LGO) and its vapour confirmed by SEM and TEM. Figure 4c, d clearly shows that the disruption of the biofilm, reduced sulfide formation, and also SRB cell envelope damage were observed. The similar observation was made earlier (Jayaraman et al. 1999) by antimicrobial peptides inhibiting the growth of SRB on 304 L stainless steel and reduction in H_2S production. The results indicated that the increased cell membrane permeability of the SRB cells confirmed that the pits formed on it due to the green biocide action on the cell walls.

Figure 5a, b shows the results obtained from EDAX spectroscopy for corrosion product formed over the mild steel specimens exposed to both the absence and the presence of biocide systems, respectively. Figure 5a clearly shows

Fig. 5 EDAX of SRB cells on mild steel with and without PLAE biocide. **a** Increased sulfide concentration of SRB cells in the absence of PLAE biocide and **b** considerable decreased concentration of sulfide on SRB cells in the presence of PLAE biocide

the higher concentration of sulfide and other elements of untreated SRB on mild steel. Figure 5b indicates remarkable decrease in the corrosive sulfide after biocide-added/treated system. In the absence of PLAE biocide, sulfate conversion was higher and formation of FeS also higher. Besides, bacterial metabolism was suppressed due to the addition of PLAE biocide and its affects the sulfate conversion.

Transmission electron microscopy

The biocidal action and bacterial morphological changes of the PLAE biocide subjected SRB cell structure were assessed by transmission electron microscopy (Figs. 6, 7). Figure 6a–d shows that the undamaged whole healthy

cell of the SRB was observed. The healthy usual cell shape with the unaltered cell structure of the inner and the curved intact outer membrane, also the space between the outer membrane and the periplasm was clear and the consistent appearance was observed. Figure 6c, d shows the SRB flagellated and a helical-shaped cell structures, respectively. The PLAE-treated SRB cells (Fig. 7a–d) were disrupted and it was mainly noticed near to the cell membrane which has undergone severe damage. Figure 7a shows a certain extent of deformed cell membrane of the SRB. However, a remarkable morphological and cytological change was observed (Fig. 7b) due to the severe cell envelope breakage. Figure 7c infers that the membrane damage causes the protoplasmic aggregation at one end of the cell, and in Fig. 7d,

Fig. 6 Transmission electron microscopy of the SRB cells without biocide **a** well-defined SRB untreated cells, **b** SRB whole cell with distinct cell membrane and clear cytoplasm, **c** SRB cell with flagellum and distinct cell boundary, and **d** SRB cell-helical shaped

the complete whole cell disintegration of the sulfate-reducing bacteria was noticed. The large amount of cytoplasmic inclusions of the SRB cells was oozes out due to cell wall damage. The membrane damage leads to the collapse in the enzyme activity, cell content leakage due to permeability and also affects the reproductive activity of the living cell.

The TEM results clearly show the alterations in the morphology of the SRB cells after the treatment with PLAE biocide, and distinctly shows the cell lysis which indicates that the PLAE was effective biocide against the SRB. The similar cell damage in the SRB was found by Korenblum et al. (2012) using the AMS H₂O (Antimicrobial substance) which is a mixture of four surfactin-like homologues produced by *Bacillus* sp. alternate to the chemical biocide which has the

strong biocidal and surfactant property on the cell membrane alteration in SRB.

Biocidal effect on corrosion

The TEM study clearly depicts the role of decimating nature of PLAE on the SRB. Relationship between the biocidal property of the PLAE biocide and its influence on corrosion behavior of mild steel was studied by non-electrochemical (gravimetric) and electrochemical method. The weight loss measurement (Fig. 8) shows corrosion rate 0.037 and 0.038 mm/year in the presence and the absence of PLAE biocide in Postgate medium, respectively. The insignificant variation in the corrosion rate, corrosivity of biocide on mild

Fig. 7 Transmission electron microscopy shows the biocidal effect of PLAE on SRB **a** wall damage, **b** protoplasm aggregation, **c** oozing out of cell inclusion, and **d** whole cell disintegration

steel was nil. Due to SRB growth, the corrosion rate was (0.064 mm/year) higher than the medium alone and medium with biocide without bacteria. It reveals that SRB involves on steel corrosion in the presence of organic and inorganic nutrients. It is well known that while adding biocide, SRB activity was lower with low corrosion rate (0.013 mm/year) when compared to the absence of biocide. We should consider that SRB reduces S^{2-} and it may form as FeS on the metal surface, which may reduce the corrosion rate. Since the bacterial activity was severe in medium with SRB, the corrosion rate was higher. EDAX analysis also reveals that sulfide concentration was lower in the presence of bacteria with biocide-added system. In the presence of bacteria without biocide, the surface film contains high concentration of sulfur. Hence, it can be concluded that 80% of corrosion rate was decreased in the presence of green biocide. Bhola et al. (2014) reported that neem extract at 4% concentration reduced corrosion rate up to 50% and effectively reduced the sulfate-reducing bacterial growth.

Figure 9 shows the potentiodynamic polarization data for the mild steel corrosion in the presence of Postgate medium containing SRB (red line) and in the presence of green biocide PLAE (green line) in SRB inoculated Postgate media. The corrosion potentials (E_{corr}) of Postgate medium (black line) increased from -727 mV vs SCE to -820 mV SCE, in the presence of SRB in Postgate medium (red line) and decreased to -800 mV due to the effect of green biocide PLAE (green line) in SRB media. It can be seen that potential decreases, i.e., moves positive direction when biocide was added into the test solutions. This was due to decrease in cathodic kinetics of SRB in the presence of biocide PLAE, where a formation of biogenic film may shift the

Fig. 8 Weight loss measurement for mild steel in the presence and the absence of biocide PLAE in SRB medium

Fig. 9 Potentiodynamic polarization measurement for mild steel in the presence and the absence of biocide PLAE in SRB medium

potential to positive side. TEM and SEM data confirmed the cell death of SRB which in turn reduces the corrosion activity on metal surface. The mild steel showed corrosion current density (j_{corr}) around 9.37×10^{-6} A cm $^{-2}$ which increased to 23.4×10^{-6} A cm $^{-2}$ in the presence of SRB. However, the current density for biocide-added mild steel in SRB medium was 11.8×10^{-6} A cm $^{-2}$. The biocide added onto the solution resulted in the detachment and limiting the growth of bacteria which resulted in lower corrosion activity. This may be due to the formation of biogenic film which reduces corrosion current. The cathodic curve for mild steel exposed in SRB culture with biocide was around one order of magnitude lower than that of mild steel exposed in SRB medium indicating the reduction in cathodic reaction. The polarization curves show the evidence of initial dissolution of mild steel in anodic direction and later inhibition (sight passivation) due to the action of biocide on SRB. The sulfides generated by the reduction of sulfate by SRB react with mild steel to produce iron sulfide as corrosion product. The presence of organics and HS^- ions also determines the anodic reaction. Besides, the formation of FeS with organics also forms as film which reduces the cathodic current. The porous nature of iron sulfide allows the diffusion of cathodic reactants and also nutrients to the bacteria which accelerate the corrosion. However, the addition of plant extract to the SRB culture during the polarization experiment definitely reduced the corrosion activity of SRB. Based on the results, a detailed mechanism has been proposed on the mitigating SRB-induced microbial corrosion by green *P. longifolia* in product transporting pipelines (Fig. 10).

Fig. 10 Schematic illustration of microbial corrosion mechanism of product pipeline (mild steel) by sulfate-reducing bacteria and its control by novel green biocide

Conclusions

The present study confirmed that the extract from the plant origin possesses the effective biocidal property. The selected green biocide (PLAE) was investigated for their biocidal efficacy. Our findings reported that the PLAE severely damaged the cell membrane of the selected sulfate-reducing bacteria, which resulted in cell death was proven by transmission electron microscopy. The cellular membrane damage which results in disruption of biofilm and cell death and also the inhibitory property against mild steel revealed by scanning electron microscopy. The corrosion study clearly showed the decrease in corrosion rates due to the reduction of SRB population which hindered the formation of sulfides. These preliminary results suggest that the extract of *P. longifolia* constitutes a suitable biocide to inhibit the growth of SRB, so the incorporation of this biocide study in antifouling paint and toxicity study to aquatic organisms or environmental damage continue in the group.

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Compliance with ethical standards

Conflict of interest All the authors have checked the manuscript and there are no conflicts of interests.

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