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Neem extract as a green inhibitor for microbiologically influenced corrosion of carbon steel API 5LX in a hypersaline environments

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ABSTRACT

In this study, the efficiency of neem extract to inhibit the corrosion of carbon steel API 5LX in the presence of different bacterial strains (*Bacillus subtilis* A1, *Streptomyces parvus* B7, *Pseudomonas stutzeri* NA3 and *Acinetobacter baumannii* MN3) were evaluated. 150 ppm of *Azadirachta indica* leave extract (AILE) was identified as minimal inhibitory concentration for the bacterial strains *B. subtilis* A1, *S. parvus* B7, *P. stutzeri* NA3 and *A. baumannii* MN3. Weight loss, electrochemical studies including potential measurements, potentiodynamic polarization and impedance and surface analysis includes Fourier transform infrared spectroscopy (FT-IR) were used to examine the corrosion inhibition efficiency (IE) of AILE on carbon steel API 5LX. The individual bacterial strains and mixed consortium had showed severe corrosion of carbon steel API 5LX in hypersaline environment. In contrast with AILE, the IE of abiotic control and mixed consortia were about 81% and 72% respectively. The corrosion rate was observed as increased trends in presence of all bacterial strains (0.179 mm/y) when compared to abiotic system with AILE system (0.012 mm/y). X-ray diffraction spectroscopy (XRD) confirms that all bacterial strains has accelerates the corrosion by the production of higher intensity peaks of corrosion products when compared to AILE systems. FT-IR data reveals that AILE extracts consists of azadirachtin and other phytochemical compound which was play a key role in control of bacterial biofilm on metal surface and thus leads to inhibit corrosion process. This is the first report on the application of AILE as a natural, eco-friendly corrosion inhibitor with antibacterial activities to control microbiologically influenced corrosion in hypersaline environment of oil reservoir.

1. Introduction

Microbiologically influenced corrosion (MIC) is an electrochemical process where microbes accelerate/cause metal deterioration by their action of metabolism on the metal surface [1–4]. As a result of microbiological action in reservoir fuel contamination, fuel turbidity, metal deterioration in storage tanks and pipelines, and souring of oil products occurs [5,6]. The presence of microorganisms is one of the key factors responsible for the corrosion issues associated with the oil industries [6–9]. MIC is one of the most important features for pipeline failure, and one of the key factor involved in operation and maintenance cost issues of crude oil industry [10,11]. Overall, about 40% of internal corrosion in gas and oil industries is originated from microorganisms [12–14]. Carbon steel is a widely used engineering material for the transportation and storage of the oil products and many researchers have reported the biocorrosion of carbon steel [1,6,15].

Control and anticipation of microbial corrosion have potential importance in at the moment in sight of the harshness of the problems caused. The electrostatic charge accumulation on metal surface, which are irregular and positive discrimination for biofilm development. Biofilm is a formed by the mixture of microbial cells, spores and their metabolites along with grime materials which are harden to a greasy film by the action of cellular secretions [4]. The microbial activity leads to formation of products which are very corrosive to the metal surfaces. Different microbial communities (aerobic/anaerobic) in a system acts on surface of metals in different behavior [3,16].

Using inhibitors is an important method of protecting materials against deterioration due to corrosion [7,17]. The influence of heterocyclic compounds containing oxygen, sulfur and nitrogen on the corrosion of different steel in acidic environments has been investigated by some researchers [17–20]. Many of the synthetic compounds give high anticorrosive performance, but most of them are extremely toxic to environment [21–23]. The protection and ecological concerns of synthetic

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corrosion inhibitors in industries have constantly exist as global apprehension [24]. The known hazardous effect of most synthetic corrosion inhibitors has motivated scientists to use natural products as corrosion inhibitors as they are inexpensive, readily available and renewable sources of materials, environmentally friendly, and ecologically acceptable [7,25–27]. Up to date, many corrosion inhibitors have been synthesised, and they sort from rare earth elements [28] to organic material [29,30].

Recently many of the plant derivatives were used as green corrosion inhibitor for the control of chemical corrosion in steels, such as *Areca catechu*, *Syzygium cumini*, *Tragia plukenetii*, *Azadirachta indica*, *Calotropis gigantea*, *Gossypium hirsutum*, *Artemisia pallens,* garlic peel extract, Papaya seeds and etc., [3,24,26,31–39]. Among these natural products neem (*Azadirachta indica)* only used as inhibitor with biocidel activities for control of the microbial corrosion in API 5L X80 linepipe steel and copper [3,15].

Neem tree is distinguished for its extraordinary biological properties around the world. Neem oil, leaves and bark are acknowledged to acquire diverse medical uses for the healing of many diseases [40]. Extracts of neem tree contains several compounds, such as not limited to: azadirachtin, gedunin, nimbin, nimbandiol, azadirone, nimbolide, nimbinene, nimbolin, nimonol, margolone, vilasanin, flavonoids and etc. [15,31,40–42]. Among identified neem compounds azadirachtin has highest antimicrobial activity against both Gram positive and Gram negative bacteria [15,40]. Previously neem was used as potential inhibitor for acid corrosion of the different metals such as mild steel, copper, zinc, X80 carbon steel [31,32,40,43,].

The present research focused on the application of *Azadirachta indica* leaves extract (AILE) as green inhibitor to control MIC of carbon steel API 5LX (CS) in the hypersaline environment. Weight loss test, electrochemical studies (polarization and impedance spectroscopy), X-ray diffraction spectroscopy (XRD) and Fourier transform infrared spectrum (FT-IR) were utilized to characterize the role of AILE on control of MIC of carbon steel API 5LX.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Four bacterial strains were exploited in this study namely *Bacillus subtilis* A1, *Streptomyces parvus* B7, *Pseudomonas stutzeri* NA3 and *Acinetobacter baumannii* MN3 which were isolated and identified from an Indian crude oil reservoir. These strains were identified by 16S rDNA sequencing and deposited under NCBI Genbank accession numbers KP895564, KP895570, KU708859 and KU708860 respectively. All the strains were retrieved from glycerol stock and sub-cultured in Luria–Bertani (LB) agar plates (g/L: 10.0 tryptone, 5.0 yeast extract, 10.0 sodium chloride with 15.0 agar (Himedia, Mumbai, India)) and incubated at 37 °C for 24 h. Further sub cultured by single colony inoculation method using LB broth (pH: 7.0) and incubated in an orbital shaker (150 rpm) for 24 h at 37 °C.

2.2. Corrosion inhibitor preparation

Fresh *Azadirachta indica* leaves were collected from Thiruvalluvar University campus and surface cleaned with deionised water and grained with ethanol (99.9%). The liquid thus acquired was sieved via Whatman filter paper to eliminate the dregs. The filtered solution was rotary evaporated and sterilized using autoclave prior to the further uses and stored at 4 °C [3]. The functional groups present in *Azadirachta indica* were qualitatively characterized by Fourier transform infrared spectrum (FT-IR). The samples were pulverized with the addition of potassium bromide in a 1:100 ratio and the pellet was fixed in the sample container, and analyzed in the mid IR region 400–4000 cm^{-1}.

2.3. Antibacterial properties of AILE

2.3.1. Agar-well diffusion assay

To assess the antibacterial activity of the AILE, Mueller Hinton Agar (MH) (Himedia-India) plates was prepared as per manufacturer's instructions. Six different concentrations at 20 ppm, 50 ppm, 100 ppm, 150 ppm, 200 ppm and 250 ppm of the AILE were selected to evaluate their bactericidal activity of the four bacterial strains (*B. subtilis* A1, *S. parvus* B7, *P. stutzeri* NA3 and *A. baumannii* MN3). All strains were inoculated separately on the MH agar plates and evenly spread over the entire surface of MH agar. Wells of 6 mm diameter with 5 mm deepness were made on the solid MH agar using a sterile glass borer. 50 μL of AILE at the different concentrations were loaded to each respective well. 10 μg gentamycin was used as a positive control because of its broad range bactericidal activity. Sterile ethanol was used as negative control and each experiment was done in triplicates and incubated at 37 °C for 24 h. Zone of the inhibition was measured and expressed in millimetres (mm).

2.3.2. Biofilm inhibition assay

Overnight bacterial cultures of *B. subtilis* A1, *S. parvus* B7, *P. stutzeri* NA3 and *A. baumannii* MN3 in Luria Bertani (LB) medium were diluted in 1:20 ratio with fresh LB medium with different concentration of AILE such as (100 ppm, 150 ppm, 200 ppm and 250 ppm) and further these diluted cultures (100 μL) were transferred into 96-well microtiter plate and incubated at 37 °C for 24 h. For comparison same culture broth was preferred without AILE and served as control. The suspended culture medium were removed and the 96-well plate was washed with phosphate buffered saline (137 mM NaCl, 2.7 mM KCl, 10 mM $NaHPO_4$, 2 mM $KHPO_4$, pH:7.2), 120 μL of crystal violet were added to each well and left for 20 min. Finally, 125 μL of acetic acid was added over the crystal violet and incubated for additional 15 min at 37 °C. Visible changes in appearance were observed to confirm the biofilm inhibition capability of the AILE at the lowest concentration [44].

2.4. Corrosion studies

2.4.1. Carbon steel coupon preparation

MIC behavior of carbon steel API 5LX was investigated as described by Rajasekar et al. [6]. with the modification in the systems design as follows: produced water collected from an oil reservoir was served as corrosive medium. Collected produced water contained higher amount of the inorganic corrosive substances such as chloride (36 g/L), sulphate (354 mg/L), iron (32 mg/L) as major components and other chemicals as trace amounts like magnesium (529 mg/L), calcium (1800 mg/L) among others. Carbon steel API 5LX coupons (metals components in (wt%): C 0.070, Si 0.195, Cr 0.03, Ni 0.02, Mn 1.05, Cu 0.05 and Al 0.029, balanced with Fe) was cut into 25 × 25 × 0.4 mm and 10 × 10 × 1 mm size coupons for weight loss studies and electrochemical measurements (impedance and potentiodynamic polarization studies) respectively. All the coupons were sequentially smoothened with different grade silicon carbide papers (180, 500, 800, 1200, and 1500) and finally polished with 0.3 μM alumina powder [6]. The polished carbon steel API 5LX which are acted as working electrodes (WE) with an exposed area of 1.0 cm^2 were used for EIS studies. The electrodes were sterilized by UV light prior to the experiment.

2.4.2. Weight loss experiments

For weight loss measurements, initial weights of the each coupon (in triplicate) were recorded before immersion into the corrosion systems. The coupons were first dipped into the deionised water and degreased with trichloroethylene. System I consisted of coupons (in triplicates) immersed in a 500 mL Erlenmeyer flask with 300 mL sterile corrosive medium (produced water) which acted as abiotic control systems. System II –XII are similar to that of system of I. The system II was added with 150 ppm of ethanolic extract of AILE, system III, IV, VII, IX were