Engineering Failure Analysis 36 (2014) 92–103

Contents lists available at ScienceDirect

Engineering Failure Analysis

journal homepage: www.elsevier.com/locate/engfailanal

Neem extract as an inhibitor for biocorrosion influenced by sulfate reducing bacteria: A preliminary investigation

Shaily M. Bhola [a,*], Faisal M. Alabbas [a], Rahul Bhola [a], John R. Spear [b], Brajendra Mishra [a], David L. Olson [a], Anthony E. Kakpovbia [c]

[a] Department of Metallurgical and Materials Engineering, Colorado School of Mines, Golden, CO 80401, USA
[b] Department of Civil and Environmental Engineering, Colorado School of Mines, Golden, CO 80401, USA
[c] Inspection Department, Saudi Aramco, Dhahran 31311, Saudi Arabia

ARTICLE INFO

Article history:
Received 19 June 2013
Received in revised form 17 September 2013
Accepted 20 September 2013
Available online 2 October 2013

Keywords:
Neem extract
Biocorrosion
SRB
Linepipe steel

ABSTRACT

This work investigates the inhibition effect of Neem (*Azadirachta indica*) extract on microbiologically influenced corrosion (MIC) of API 5L X80 linepipe steel by a sulfate-reducing bacterial (SRB) consortium. The SRB consortium used in this study included three phylotypes; *Desulfovibrio africanus*, *Desulfovibrio alaskensis* and *Desulfomicrobium* sp. Steel coupons were incubated in the presence of the SRB consortium without and with 4 wt.% Neem extracts for different periods of time. The morphology, compositions of the interfaces and subsequent corrosive pitting were characterized with field emission scanning electron microscopy (FE-SEM) coupled with energy dispersive spectroscopy (EDS). In addition, electrochemical impedance spectroscopy (EIS), linear polarization resistance (LPR) and open circuit potential (OCP) were used to investigate the in situ corrosion behavior under the two different conditions. The results revealed that Neem extract has the capability to reduce the biocorrosion rate by approximately 50%. Neem has significantly reduced the propensity of linepipe steel to SRB caused MIC by minimizing the cell growth and has subsequently suppressed the sulfide productions, sessile cell density and biofilm development.

1. Introduction

Microbiologically influenced corrosion (MIC) or biocorrosion is a considerable problem for the oil and gas industry. MIC is considered one of the most damaging mechanisms to pipeline steel materials. Microorganisms are thought to be responsible for greater than 20% of pipeline systems failures [1]. The main types of bacteria associated with metals in pipeline systems are sulfate-reducing bacteria (SRB), iron and CO_2 reducing bacteria and iron and manganese oxidizing bacteria [1,2]. Among these, SRB have received much attention in the oil and gas industry and MIC investigations have revealed that these microorganisms have several detrimental metabolic activities including the ability to: (1) oxidize hydrogen as an electron donor for metabolic life [1,2], (2) use O_2 and Fe^{3+} as a terminal electron acceptor [3], (3) utilize aliphatic and aromatic hydrocarbons as a carbon source [4], (4) use very low levels of water for cellular maintenance and growth [4], (5) couple sulfate reduction to the intracellular production of magnetite [4] (6) compete with nitrate-reducing/sulfur-oxidizing bacteria (NRB–SOB) (since they may have a nitrite reducing activity) [5,6] (7) and cause elemental oxidation of iron [7].

Basically, prevention and treatment of MIC is aimed mainly on destroying the microbial cell and/or preventing the development of biofilms [8]. Various commercial mitigation techniques have been used in the oil and gas industry to control

* Corresponding author. Tel.: +1 (303) 875 1642; fax: +1 (303) 273 3795.
E-mail address: malhotra.shaily@gmail.com (S.M. Bhola).

1350-6307/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.engfailanal.2013.09.015

MIC. These techniques include mechanical (i.e. pigging), chemical (i.e. biocides), electrochemical (i.e. cathodic protection) and biological (i.e. microbial injection of more beneficial microbiota) approaches [1,2,8]. Among these techniques, the biocide is considered the most effective method. Biocides, however, are not only expensive but also pose considerable hazard to the environment and field personnel owing to their toxicity [1,2,8].

The conventional criteria governing the selection of an effective biocide include: (i) proven efficacy against a broad spectrum of microorganisms; (ii) ability to penetrate and disperse microbial slime; (iii) chemical and physical compatibility with other products (e.g. corrosion inhibitors) and the environment (e.g. pH effects); (iv) safe easy use and storage; (v) appropriate biodegradability; (vi) cost effectiveness [1,9,10]. Biocides, however, are inherently toxic and most of the times difficult to degrade. They may thus have a negative impact on the environment if used without a proper environmental risk assessment [10]. Moreover, in the past few years, the ineffectiveness of biocides against sessile organisms have been documented [11]. This is probably due to the inability of the chemical to penetrate thick biofilms, in addition to physiological differences between sessile and planktonic cells [12]. It has also been reported that biocides' sensitivity can be altered up to 1000-fold by changes in nutrients and growth rates [11].

Use of naturally occurring compounds such as plant extracts might be considered as an environmentally benign way of treating MIC. A number of plant oils and aqueous plant extracts have been shown to have inhibitory activity against yeast, filamentous fungi and bacteria [13–15]. Indian species such as clove, cinnamon, horse raddish, cumin, tamarind, garlic, onion, are use as preservatives, disinfectants and antiseptics [16]. Vitamins [17] and other biomolecules [18] have also been used as potential corrosion inhibitors for steel and nickel in acidic media.

Neem tree (*Azadirachta indica*) is well known for its unusual biological properties. Its bark and leaves are known to possess diverse and multifarious therapeutic uses for the treatment of many diseases [19]. The most important bioactive principal constituent in Neem is Azadirachtin [16,19]. Neem leaf extract has been widely explored as a potential corrosion inhibitor for alloys such as mild steel, carbon steel and zinc in acidic medium [19–21].

Despite this knowledge, there is lack of information on the effects of Neem extract on biocorrosion. The present investigation focuses on the use of Neem extract as a MIC inhibitor. The effect of 6% w/w azadirachtin towards SRB caused microbiologically influenced corrosion of API 5L grade X80 carbon steel has been explored.

2. Materials and methods

2.1. Organisms and culture

The SRB mixed cultures of *Desulfovibrio africanus* sp. (ATCC 19997), *Desulfovibrio alaskensis* (ATCC 14653) and *Desulfomicrobium* sp. (Accession # KC756851) [7], were used in this study. Both *D. africanus* sp., *D alaskensis* were obtained in freeze dried samples obtained from American Type Culture Collection (ATCC) while *Desulfomicrobium* sp. were isolated from water samples obtained from an oil well located in Louisiana, USA. The SRB cultures were cultivated in supplemented enriched artificial sea water. The growth medium was composed of magnesium sulfate (2.0 g), sodium citrate (5.0 g), calcium sulfate di-hydrate (1.0 g), ammonium chloride (1.0 g), sodium chloride (25.0 g), di-potassium hydrogen orthophosphate (0.5 g), sodium lactate 60% syrup (3.5 g), and yeast extract (1.0 g). All components were per liter of distilled artificial seawater. The pH of the medium was adjusted to 7.5 using 5 M sodium hydroxide and sterilized in an autoclave at 121 °C for 20 min. The SRB species were cultured in the growth medium with filter-sterilized 5% w/w ferrous ammonium sulfate added to the medium at a ratio of 0.1–5.0 ml respectively. The bacteria were incubated for 72 h at 37 °C under an oxygen-free nitrogen headspace.

2.2. Sample preparation

Pipeline steel (API 5L X80) coupons, provided by SAUDI ARAMCO, Saudi Arabia, were used for this study and composed of the following elements with a weight ratio of 0.073% C, 1.36% Mn, 0.004% P, 0.008% Ti, 0.003% S and balance as Fe. The steel has already been characterized in our previous research [7].

For corrosion evaluation, the coupons were machined to a size of $10 \times 10 \times 5$ mm and embedded in a mold of non-conducting epoxy resin, leaving an exposed surface area with a polished mirror finish of 100 mm^2. For electrical connection, a copper wire was soldered at the rear of the coupons prior to epoxy embedding. The coupons were polished with a progressively finer polishing paper until a final grit size of 600 micro was obtained. After polishing, the coupons were rinsed with distilled water, ultrasonically degreased in acetone and sterilized by exposing to pure ethanol for 24 h.

2.3. Electrochemical tests

The electrochemical measurements were made in a conventional three electrode ASTM glass cell coupled with a potentiostat and a high frequency impedance analyzer (Gamry-600). The electrochemical cell composed of a test coupon as a working electrode (WE), a graphite electrode as an auxiliary electrode and a saturated calomel electrode (SCE) as a reference electrode. All glasswares were autoclaved at 121 °C for 20 min at 20 psi pressure and then air dried for an initial aseptic condition. Graphite electrodes, purging tubes, rubber stoppers and needles were sterilized by immersing in 70 vol.% ethanol for 24 h followed by exposure to a UV lamp for 20 min. Two solutions were used in this experiment, without Neem (M1) and