Materials Science and Engineering C 47 (2015) 367–375

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

The biocidal effect of a novel synthesized gemini surfactant on environmental sulfidogenic bacteria: Planktonic cells and biofilms

A. Labena [a,*], M.A. Hegazy [b], H. Horn [c], E. Müller [a]

[a] Technical University of Munich, Chair of Urban Water Systems Engineering, Am Coulombwall, 85748 Garching, Germany
[b] Egyptian Petroleum Research Institute (EPRI), Nasr City, Cairo, Egypt
[c] Karlsruhe Institute of Technology, Engler-Bunte-Institut, Chair for Water Chemistry and Water Technology, Engler-Bunte-Ring 1, 76121 Karlsruhe, Germany

ARTICLE INFO

Article history:
Received 5 July 2014
Received in revised form 10 September 2014
Accepted 30 October 2014
Available online 1 November 2014

Keywords:
Mild steel
Weight loss
Microbiological corrosion
Biofilm

ABSTRACT

A cationic gemini surfactant was synthesized and characterized. The surfactant was successfully applied as a biocide against environmental sulfidogenic bacteria in the bulk phase (planktonic) and on the surface (biofilm). The activity of the synthesized surfactant was discussed based on the redox potential and the sulfide productivity in the bulk phase. The cultivated biofilm structure analysis and corrosion rate were estimated on the metal surface. The lowest metal corrosion rate was recognized at a concentration of 1 mM with a metal corrosion inhibition efficiency of 95%. The synthesized gemini surfactant prevented the biofilm formation at a concentration of 0.1 mM. The synthesized gemini surfactant displayed a broad spectrum antibacterial activity against Gram-positive and Gram-negative bacteria.

1. Introduction

Sulfidogenic microbial community is a group of anaerobic microorganisms that reduce sulfate (SO_4^{2-}) to sulfide (S^{2-}) during a process termed a dissimilatory anaerobic respiration process. These microorganisms are highly problematic in the petroleum industries where they induce pitting corrosion of pipelines and tanks [1]. The corrosiveness of sulfidogenic microorganisms is related to metabolites they produced such as hydrogen sulfide. In addition, supposed electrochemical effect, which is termed cathodic depolarization, and microbial colonization (biofilm) on the metal surface are also considered as corrosion induction [2–4]. Sulfidogenic biofilms usually exhibit a localized attack in the form of slimy layers consisted of multispecies microbial community, extracellular polymeric substances (EPS), inorganic material and water. The sulfidogenic biofilms on the metal surface increase the corrosion rate more than their counterpart planktonic cells that are present in the bulk phase. This is attributed to the nature of biofilms which are much more difficult to kill or inhibit by a normal biocide due to their strong adherence to metal surfaces and their high resistance to antimicrobial agents [5]. Corrosion inhibitor is the most practical application for metal corrosion mitigation. Surfactant "surface active agent" is defined as, in a particular system, a chemical substance (at a definite concentration) which partially or completely adsorbs to an interface (metal/liquid) [5]. A monomeric surfactant made up of a hydrophilic polar head linked to a hydrophobic non-polar tail. When surfactant molecules dissolve in the water, they escape from water bulk phase to an available interface as a result of their hydrophobic attitude. A surfactant, as a metal corrosion inhibitor, has been widely used to protect metal surface from the corrosive environment [6]. Furthermore, a surfactant can be applied as a biocide to protect the metal surface from the microbial growth and their activities. Changes in the molecular structure of a monomeric surfactant in order to improve its surface activity have attracted the attention of the scientists [7]. Gemini surfactant displays a new class of surfactant; it consisted of at least two heads (hydrophilic groups) attached to two chain tails (hydrophobic groups) and linked by a spacer [8]. Previous researches revealed that gemini surfactants possess a lot of physicochemical properties that are superior to those of corresponding monomeric surfactants such as a remarkable low critical micelle concentration (C_{CMC}), unusual aggregation morphologies, high dependence on spacer structure and strong hydrophobic micro-domain therefore those characteristics provide more metal surface protection [9]. Metal corrosion inhibitors and biocides are widely applied in the Egyptian Petroleum industries, however, the corrosion problems still occur. This is due to the fact that sulfidogenic biofilms that are present in water tanks induce sever pitting corrosion. Therefore a water sample was collected from an Egyptian petroleum company's water tank (Western desert, Qarun area). The water sample was enriched and used to cultivate sulfidogenic bacterial community. In order to protect the metal surface from the environmental sulfidogenic bacteria in the bulk phase (planktonic) and on the metal surface, a novel cationic gemini surfactant was synthesized and characterized. The activity of the synthesized surfactant, as a biocide against environmental sulfidogenic bacteria, was discussed on the basis of changing the

* Corresponding author.
E-mail address: labena.labena@gmail.com (A. Labena).

http://dx.doi.org/10.1016/j.msec.2014.10.079
0928-4931/

redox potential and sulfide productivity in the reactor's bulk phase. In addition changing on the metal surface was estimated by elucidating the changes in the structure and constituents of the sulfidogenic biofilms. The metal corrosion rate and the metal corrosion inhibition efficiency were estimated and confirmed by scanning electron microscopy. In addition, antibacterial activity (against Gram-positive and Gram-negative strains) of the synthesized surfactant was carried out.

2. Materials and methods

2.1. Synthesis

In this work, a novel cationic gemini surfactant was synthesized through three main reactions. The first reaction was alkylation reaction between 1 mol of 1-bromododecane and 1 mol of 2-(dimethylamino) ethanol in ethanol at 70 °C for 12 h. The mixture was left to cool and precipitate. Then the obtained precipitate was purified by diethyl ether and afterwards recrystallized from ethanol. The second reaction was a reaction between 2 mol of N-(2-hydroxyethyl)-N,N-dimethyl-dodecan-1-aminium bromide and 1 mol of phthalic acid until 2 mol of water was produced. In this reaction, p-toluene sulfonic acid was used as a dehydrating agent and toluene as a solvent. Furthermore, the reaction mixture was distilled under vacuum to completely remove the solvent. The third reaction was a reaction between 1 mol of the resulted product and 2 mol of potassium hydroxide in ethanol at 70 °C for 2 h. The reaction mixture was left to cool for 1 h. At the end, the obtained pale brown precipitate was recrystallized twice from ethanol. The chemical structure of the synthesized gemini surfactant was proven by Fourier transform infrared (FTIR) and nuclear magnetic resonance (NMR) spectroscopy (Bruker, Vortex 70, 400 MHZ NMR spectrometer, Avance DRX 400 for FTIR and NMR, respectively).

2.2. Surface active characteristics

2.2.1. Surface tension (γ)

The surface tension was detected at different concentrations of the synthesized gemini surfactant according to the De Nöuy ring method, Tensiometer (Kruss Type 6). Calibration was carried out with pure distilled water (with surface tension 72 mN m^{-1}) at 25 °C.

2.2.2. Effectiveness (π_{CMC})

The effectiveness value was calculated using the surface tension value (γ) at C_{CMC} point as follows [10]:

$$\pi_{CMC} = \gamma_o - \gamma_{CMC} \quad (1)$$

where γ_o and γ_{CMC} are the surface tension of pure distilled water and the surface tension at C_{CMC} point, respectively.

2.2.3. Surface excess concentration (Γ_{max})

The surface excess concentration of the synthesized gemini surfactant was determined according to Gibbs' adsorption equation [10]:

$$\Gamma_{max} = \left(\frac{-1}{nRT}\right)\left(\frac{d\gamma}{d\ln C}\right) \quad (2)$$

where Γ_{max} is the surface excess concentration of surfactant ions, n is the number of species ions in solution, R is the gas constant, T is the absolute temperature, γ is the surface tension at a specific concentration and C is the concentration of a surfactant. A surfactant that reduces the surface energy thus exists in excess concentration near the interface.

2.2.4. Minimum surface area (A_{min})

An area occupied by one molecule in nm^2 at the interface is defined as a minimum surface area (A_{min}). A_{min} was calculated as follows [10]:

$$A_{min} = \frac{10^{14}}{N_A \Gamma_{max}} \quad (3)$$

where N_A is the Avogadro's number and Γ_{max} (mol m^{-2}) is the maximal surface excess of the adsorbed surfactant molecules to the surface.

2.2.5. Conductivity (K)

The conductivity (K) evaluation of the synthesized gemini surfactant was detected using a conduct meter (LF 191 WTW) at 25 °C in order to estimate the C_{CMC} value and the degree of counter ion dissociation (β).

2.2.6. Standard free energy of micellization (ΔG^o_{mic})

The standard free energy of micellization (ΔG^o_{mic}) per mole of the synthesized gemini surfactant was estimated as follows [11]:

$$\Delta G^o_{mic} = (2-\beta)RT \ln C_{CMC} \quad (4)$$

where β is the degree of counter ion dissociation, R is the gas constant, T is the temperature, and C_{CMC} is expressed the molarity of the surfactant.

2.3. Biocidal effect of the synthesized gemini surfactant on environmental sulfidogenic bacteria

2.3.1. Sulfidogenic microbial-community and growth conditions

The problem statement of this work was related to an Egyptian petroleum company in the Western desert, Qarun field, Egypt. Although, corrosion inhibitors and biocides are applied, the company water tanks still suffered from the corrosion problems. Therefore, water sample was collected from the water tank outlet. The water sample was named Hamra. The Hamra water sample was characterized by a low salinity (0.23% NaCl) and neutral pH (7.1). The Hamra water tank was characterized by its high metal corrosion rate that is due to the sulfidogenic bacteria present in the water tank. Therefore onsite inoculation of the water sample was done on the modified Postgate's-B medium according to Postgate [12]. Modification of Postgate's-B medium was achieved by applying the salinity (NaCl) and pH of the original water sample during preparation. The medium was prepared, sparged with nitrogen gas as described in Hungate's technique for anaerobes [13]. In addition 0.0002% (w/v) resazurin was applied as a redox potential marker for the anaerobic cultivation. The cultivated media were inoculated with 10% (v/v) water sample and incubated for 14 days at 37 °C. The black pellet (iron sulfide) was proposed as an indicator for the sulfidogenic bacterial activity during cultivation. The inoculated water sample was enriched many times using the modified Postgate's-B medium and used as inocula for further experiments.

2.3.2. Experimental design and the sulfidogenic bacterial activity estimation: planktonic cells (bulk phase) and biofilms (surface)

In order to estimate the effect of the synthesized gemini surfactant on the environmental sulfidogenic bacterial activity, the batch reactor experiments were accomplished by using the modified Postgate's-C medium [12]. Modification of Postgate's-C medium was done using the salinity (NaCl) and pH of the environmental water sample. A mild steel coupon (CS1018 3″ × 1/2″ × 11/6″ strip, Cormon LTD) with a chemical composition previously reported [14] was used as a sole iron source for the environmental sulfidogenic bacterial cultivation. The experiments were evaluated using different reactors. (i) Blank reactor (cultivated medium not inoculated with the sulfidogenic bacteria), and (ii) control reactor (cultivated medium inoculated with enriched sulfidogenic bacteria without synthesized gemini surfactant) and surfactant reactors (cultivated medium inoculated with the enriched sulfidogenic bacteria supplemented by different concentrations of the