International Biodeterioration & Biodegradation 127 (2018) 178–184

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

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

D-phenylalanine inhibits the corrosion of Q235 carbon steel caused by *Desulfovibrio* sp.

Ee Li[a,b,c], Jiajia Wu[a,c,*], Dun Zhang[a,c,**], Yan Sun[a,c], Juna Chen[a,b,c]

[a] Key Laboratory of Marine Environmental Corrosion and Bio-fouling, Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao, 266071, China
[b] Open Studio for Marine Corrosion and Protection, Qingdao National Laboratory for Marine Science and Technology, 1 Wenhai Road, Qingdao, 266237, China
[c] University of Chinese Academy of Sciences, 19 (Jia) Yuquan Road, Beijing, 100039, China

ARTICLE INFO

Keywords:
Desulfovibrio sp.
D-phenylalanine
Biofilm
Hydrogen sulfide
Carbon steel

ABSTRACT

D-phenylalanine (D-Phe) was tested for its effect on the corrosion of Q235 carbon steel caused by *Desulfovibrio* sp. which belongs to sulfate reducing bacteria (SRB). Weight loss results indicated that the corrosion rate of Q235 coupons exposed to D-Phe was reduced. Much less pits were found on the surface of these coupons. It was found that pH and dissolved oxygen content of bacteria cultures as well as the growth of bacteria were not affected by D-Phe. However, the cell shape of *D.* sp. was changed to a more filamentous or spherical shape by treatment with D-Phe, and the structure of biofilms formed by bacteria with transformed shapes was looser and thinner. Moreover, the hydrogen sulfide production of *D.* sp. was inhibited by D-Phe. These results suggest that D-Phe may inhibit the corrosion process through two ways, i, D-Phe alters the bacteria shape, which inhibits the formation of biofilm and changes the structure of it consequently; ii, D-Phe reduces the production of hydrogen sulfide.

1. Introduction

Microbiologically influenced corrosion (MIC) is a metal deterioration process caused by the presence and/or the metabolic activities of microorganisms (Videla and Herrera, 2005). Due to MIC damages, billions of dollars are lost each year in the US alone (Walsh et al., 1993). Sulfate reducing bacteria (SRB) are generally considered as the major culprits involved in MIC, especially of steel (Xu and Gu, 2014).

Like many other microbes, SRB live in biofilms predominantly. They can adhere to metal surfaces and form biofilms subsequently. The biofilm including bacteria and bacteria-produced extracellular polymeric substances (EPS) can influence the corrosion of metals by changing the physical or chemical properties of the interface (Anandkumar et al., 2016). SRB present in the biofilm are usually associated with pitting corrosion which is more severe than normal uniform corrosion (AlAbbas et al., 2013). Another key feature of SRB is that they can transform sulfate or other partially oxidized inorganic sulfur species to hydrogen sulfide (H_2S). H_2S can lead to electrochemical and anaerobic corrosion, and cause severe corrosion of metals (Lee et al., 1995; Chen et al., 2017). Therefore, the mitigation of corrosion caused by SRB can be achieved via inhibiting the formation of biofilm or production of H_2S.

Proteinogenic amino acids which are ubiquitous in natural environment, except glycine, have two stereoisomeric forms: the levorotatory (L) and the dextrorotatory (D) form. In the evolution process, L-amino acids have been selected as blocks of protein by organisms while D-amino acids have been endowed with diverse biological functions (Cava et al., 2011). For instance, Lam et al. (2009) found that many bacteria could synthesize and release D-amino acids into the bacteria culture when they entered into the stationary phase of growth. In the microenvironment, D-amino acids could accumulate at millimolar concentrations. These D-amino acids, in turn, regulated bacteria metabolism and growth by influencing cellular synthesis of peptidoglycan (PG) (Lam et al., 2009). Kolodkin-Gal et al. (2010) found that these bacterial originated D-amino acids as well as exogenous D-amino acids could inhibit biofilm formation of *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. They also speculated that D-amino acids might be widespread extracellular regulatory signals for biofilm disassembly (Kolodkin-Gal et al., 2010). Recently, many researchers reported that D-amino acids could prevent biofilm formation of several pathogenic microbes and biofouling bacteria effectively (Hochbaum et al., 2011; Xu and Liu, 2011; Yu et al., 2012; Ramón-Peréza et al.,

* Corresponding author. Key Laboratory of Marine Environmental Corrosion and Bio-fouling, Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao, 266071, China.
** Corresponding author. Key Laboratory of Marine Environmental Corrosion and Bio-fouling, Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao, 266071, China.
E-mail addresses: wujiajia@qdio.ac.cn (J. Wu), zhangdun@qdio.ac.cn (D. Zhang).

https://doi.org/10.1016/j.ibiod.2017.11.027
Received 5 April 2017; Received in revised form 25 September 2017; Accepted 29 November 2017

2014). Except being utilized alone, D-amino acids could also be used as enhancers to other biocides and antimicrobials to inhibit SRB to form biofilms. Xu et al. (2012, 2014) showed that biocide tetrakis hydroxymethyl phosphonium sulfate (THPS) could inhibit biofilm formation of *Desulfovibrio vulgaris* significantly when combined with D-tyrosine (D-Tyr) or D-methionine (D-Met). For example, when 50 ppm THPS was used alone, the number of sessile cells was more than 10^4 cells cm^{-2}. However, it reduced to less than 10^2 cells cm^{-2} when 50 ppm THPS was blended with 1 ppm D-Tyr (Xu et al., 2012, 2014). Jia et al. (2017) also found that 1 ppm D-Tyr and 50 ppm D-Met individually could significantly enhance the efficacy of 10 ppm alkyldimethylbenzylammonium chloride (ADBAC) against the *D. vulgaris* biofilm on carbon steel coupons (Jia et al., 2017). But D-Tyr or D-Met alone had no effect. It maybe because the effect of D-amino acids is strain-specific, i. e., a strain responds distinctly to treatments with different D-amino acids or a given D-amino acid cannot inhibit biofilm formation of all strains (Kolodkin-Gal et al., 2010; Hochbaum et al., 2011; Ramón-Peréza et al., 2014; Li et al., 2016). For example, D-Tyr, D-Leu, D-Trp, and D-Met could inhibit biofilm formation by *B. subtilis*, whereas D-isomers of other amino acids, such as D-Phe, had no effect (Kolodkin-Gal et al., 2010). However, D-Leu, D-Trp, and D-Met had no inhibitory effect on the biofilm formation of *S. aureus*. The most effective D-amino acids in inhibiting its biofilm formation were D-Phe, D-proline, and D-Tyr (Hochbaum et al., 2011).

Since D-amino acids can inhibit bacterial biofilm formation, is there one kind of D-amino acids which can influence the corrosion activity of SRB, and how does it work? In this study, a nonsporing SRB strain *Desulfovibrio* sp. was utilized. Corrosion of Q235 carbon steel caused by *D*. sp. in the absence or presence of D-Phe was analyzed through weight loss analysis, scanning electron microscopy (SEM) and 3-D digital microscope. And possible mechanisms by which D-Phe affected the corrosion activity of *D*. sp. were investigated by studying its effects on culture environment including pH and dissolved oxygen concentration, biofilm formation, and H_2S metabolism.

2. Experimental

2.1. Microorganism cultivation

The strain used in this study, *D*. sp., was isolated from marine sludge collected from the Bohai Sea of China. The strain was cultured at 30 °C in a modified Postgate's medium containing K_2HPO_4 0.50 g, NH_4Cl 1.00 g, $CaCl_2$ 0.10 g, $MgSO_4$ 2.00 g, Na_2SO_4 0.50 g, yeast extract 1.00 g, and sodium lactate 4.00 mL per litre of natural seawater. The autoclaved media were kept for a week before use. It was to make sure that the media were air-saturated and not contaminated. Before being inoculated into new medium, the seed culture was washed with sterilized natural seawater three times to eliminate the influence of bacterial metabolites on the following experiment.

2.2. Preparation of amino acids

D-Phe and L-Phe were purchased from Aladdin Reagent (Shanghai, China). Specification of these amino acids is 99%. Each amino acid was prepared as a concentrated stock solution (150 mM) in distilled water, and filter sterilized through a 0.22 μm syringe filter prior to use. The concentration of amino acids used in this study was 10 mM.

2.3. Weight loss and corrosion morphology analysis

Q235 carbon steel was used in the study. The elemental composition (wt %) of it is 0.180 C, 0.220 Si, 0.600 Mn, 0.020 S, 0.016 P, and balance Fe. The size of coupons was 10 mm × 10 mm × 3 mm, and they were prepared and autoclaved as previously described (Wu et al., 2016). Sterilized coupons were fixed by autoclaved hooks, and immerged in a 125 mL vial containing 100 mL medium perpendicularly. One millilitre of *D*. sp. seed culture or sterilized natural seawater was inoculated into the medium. Then D-Phe, or L-Phe, or equal volume of distilled water was added into the above bacteria media. The initial concentration of bacteria was about 10^3 cells ml^{-1}. And then vials were covered with rubber stoppers and sealed with silicon rubber. All the vials were kept statically in a thermostatic incubator at 30 °C, and there was no aeration during the whole experiment period. After 7 days of immersion, coupons were taken out and cleaned with the Clark's solution (ASTM Standard G1-03, 2003). And then they were rinsed with ethanol, dried with N_2, and weighed. The corrosion rate was assessed from the weight loss divided by the surface area of the coupon. The final results were averaged from three systems with six coupons. Surface morphologies of coupons were observed with SEM (JSM-6700F, JEOL Ltd., Tokyo, Japan) after being weighted. Optical images were obtained using a digital microscope system (HIROX KH-8700, Hirox Europe Ltd., Tokyo, Japan) to illustrate the 3-D topology of the surface of coupons. The number of pits was calculated from fifty-four images captured randomly from three different coupons submerged in cultures with or without D-Phe.

2.4. Enumeration of bacterium

One millilitre of bacteria culture was sampled after coupons were taken out and the vial was shaken thoroughly in the clean bench. The number of active bacteria in it was estimated using the most probable number (MPN) method according to ASTM D4412-84 (ASTM D4412-84, 2009). To measure the average cell density in the biofilm, coupons were taken out, rinsed with sterilized seawater gently, and immerged in 2 mL sterilized seawater in a 5 mL centrifuge tube. Cells were harvested by sonication for 15 min in an ultrasonic cleaner followed by mixing for 15 s using a vortex mixer. The detached cell number was measured by the MPN method.

2.5. Analysis of dissolved oxygen content and pH

Dissolved oxygen (DO) concentration and pH were monitored with a DO meter (Thermo Orion 5-Star, Thermo Scientific Inc., Massachusetts, USA) and a pH meter (PHS-3C; INESA Scientific Instruments Co., Ltd; Shanghai, China), respectively. The bacteria culture was shaken thoroughly to eliminate possible gradients of DO and pH, and then the DO and pH meters were introduced and located in the middle of the culture immediately after the removal of rubber stoppers.

2.6. Characterizations of biofilm morphology

Samples covered with biofilms were removed from the hook gently and exposed to 2.5% glutaraldehyde for 2 h to immobilize the biofilm. Then they were dehydrated successively with an ethanol gradient (at 30%, 50%, 70%, 90%, and 100% for 15 min each). After being dehydrated, samples were transferred into a critical point dryer (HCP-2; Hitachi Koki Co., Ltd.; Tokyo, Japan). In the chamber, coupons were exposed to CO_2 for 1.5 h at 20 °C before the temperature was raised to 31.8 °C at which liquid CO_2 vaporized without surface tension effects. The dried coupons were sputter-coated with gold and subjected to SEM analysis.

For CLSM analysis, coupons covered with biofilms were treated with 2.5% glutaraldehyde for 2 h, rinsed with PBS gently, and stained with 4', 6-diamidino-2-phenylindole (DAPI) for 15 min. Z-stack 3-D images of the biofilms were taken using a Carl Zeiss LSM 710 confocal laser scanning microscopy (CZ Microimaging GmbH, Jena, Germany). To calculate the thickness of biofilms, at least thirteen 3-D images from coupons submerged in cultures with or without D-Phe were captured randomly.