Journal of Molecular Liquids 243 (2017) 706–713

Contents lists available at ScienceDirect

Journal of Molecular Liquids

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

An anticorrosive study on potential bioactive compound produced by *Pseudomonas aeruginosa* TBH2 against the biocorrosive bacterial biofilm on copper metal

Jayaraman Narenkumar [a,*], Kuppusamy Sathishkumar [a], Raja Kumaresan Sarankumar [a], Kadarkarai Murugan [a,b], Aruliah Rajasekar [a,*]

[a] Environmental Molecular Microbiology Research Laboratory, Department of Biotechnology, Thiruvalluvar University, Serkkadu, Vellore 632 115, Tamil Nadu, India
[b] Division of Entomology, Department of Zoology, School of Life Sciences, Bharathiar University, Coimbatore 641 046, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 1 July 2017
Received in revised form 15 August 2017
Accepted 20 August 2017
Available online 24 August 2017

Keywords:
Anticorrosion
Bioactive compound
Microbial influenced corrosion
Pyocyanin
Pseudomonas aeruginosa

ABSTRACT

In recent years, the control of MIC using green based chemical (bioactive) compounds were found to show promising results on this context, the present study evaluated the anti-corrosive role of pyocyanin bioactive compound produced by *Pseudomonas aeruginosa* TBH2 biofilm forms there *Bacillus* on the surface of copper metal CW024A (Cu). UV–Vis spectrophotometer and Gas chromatography–mass spectrometry (GC–MS) confirm that *Pseudomonas aeruginosa* TBH2 identified as potential production of bioactive compound (pyocyanin) was about 3.6 g/L. The activity of pyocyanin against the corrosive bacterial strain *Bacillus* sp. EN2, EN3 and EN9 on Cu and its control by pyocyanin were evaluated by weight loss (WL), surface analysis (X-ray diffraction spectroscopy (XRD)) and electrochemical studies (ES). The WL study showed a significant reduction about 0.002 ± 0.001 g of Cu when compared to biotic (0.007 ± 0.002 g) and abiotic system (0.003 ± 0.001 g). Biocorrosion system with pyocyanin was found to exhibit 72% corrosion inhibition efficiency. The results of XRD and ES evidenced the role of pyocyanin towards inhibition of the bacterial biofilm on Cu metal surface. Thus the present study has gained importance in reporting on a promising an alternative chemical and green biocide option to replace.

1. Introduction

Microbial influenced corrosion (MIC) is defined as electrochemical process where microbes accelerate the corrosion of the metal [1–3]. The degree of the severity of MIC depends on salinity of the re-circulating water used in cooling water system (CWS). In general CWS operates with a high amount of used water rather than fresh water [4]. This promotes biofilm formation, precipitation of calcium carbonate and biocorrosion process. Among various metals copper is one of the widely used metal elements in the cooling tower material due to its excellent insulation properties and high rate of conductivity [5]. To overcome the process, few commercially available and highly expensive green based biocide and inhibitors are generally employed namely polyphosphates, zinc sulphate, azoles and nanoparticles [6,7] to control MIC in CWS.

Microbes are capable of producing a variety of extra cellular pigments possessing significant properties including anticorrosive abilities. The present study evaluated the anti-corrosion property of the bioactive compound produced by *Pseudomonas* sp. A group of *Pseudomonas* sp. producing higher amount of water soluble, blue green pyocyanin pigments [8] and their application in various industries such as pharmacological, control of phyto-pathogens, bioprocess and downstream process etc. [9–11]. Its application in the other fields such as biosensors, agriculture, medicine, environmental and microbial fuel cell has also been reported [10,12]. Pyocyanin is used as an effective antibiotic against a number of microorganisms such as, *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermis* and *Saccharomyces cerevisiae* etc., [13]. In the present study, we have explored this antibacterial property of pyocyanin produced by *Pseudomonas* sp. TBH2 in controlling biocorrosive bacterial biofilm formed by *Bacillus* sp.

This study gains importance as production of pyocyanin from the indigenous bacteria is not an expensive process, moreover, it is environment friendly. The anticorrosion role towards the

* Corresponding authors.
E-mail addresses: narencherry77@gmail.com (J. Narenkumar), rajasekargood@gmail.com, rajasekar.aruliah@gmail.com (A. Rajasekar).

http://dx.doi.org/10.1016/j.molliq.2017.08.075
0167-7322/

control of MIC on Cu metal surface in a CWS was supported with various characterization techniques. The commercially available pyocyanin cost 82 EUR/5 μg [8]. Hence, the present study will truly reveal a cheap and eco-friendly alternative to severe corrosion problem.

2. Materials and methods

2.1. Bacteria

B. thuringiensis EN2, *T. aidingensis* EN3 and *B. oleronius* EN9 were isolated from biofilm sample of cooling water system as reported earlier in our previous study [14]. The anticorrosive *Pseudomonas aeruginosa* TBH2 strain was isolated from the produced water in an oil reservoir, Karaikal, Tamil Nadu (latitude: 10.6694° N/S and longitude: 79.3155°) and identified by 16S rDNA gene sequence as described earlier [15]. These strains were recovered from glycerol stock and sub-cultured in the sterile nutrient agar (NA) plates (Himedia, Mumbai, India) and incubated at 37 °C for 24 h and used for present study. The nucleoside sequence of EN2, EN3, EN9 and TBH2 were deposited in NCBI under the accession number of KR183873, KR183874, KR183880 and KU708864 respectively.

2.2. Optimization of culture conditions for the production of bioactive compound

The production medium for pyocyanin was optimized using various factors such as pH (5, 6, 7, 8, 9 and 10), carbon sources (Glucose, lactose, starch, fructose, paddy and ground net waste) and temperature (10, 20, 30, 40 and 50° C) [16]. The initial concentration of 1.4 × 10^4 CFU/mL of TBH2 strain was used for pyocyanin production. Overnight grown culture of TBH2 was diluted in 1:100 ratio with fresh prepared sterile nutrient broth (NB) and incubated at 37 °C for 24 h. After incubation period, the culture was centrifuged at 10,000 rpm for 10 min and the supernatant was separated and 4.5 mL of chloroform was added to the mixture until a blue coloured layer was formed. This was further transferred into a new test tube and 1.5 mL of 0.2 M hydrochloric acid was added to it (colour changing to pink was noted). The end product in the supernatant was harvested by centrifuging at 10,000 rpm for 2 min and biomass was collected. Each experiment was carried out in triplicate. The production of Pyocyanin was further confirmed by GCMS and UV–Vis spectrophotometer.

2.3. Optimization of bioactive compound by response surface methodology

Based on one factor at a time, optimization studies of pH, temperature, carbon source (1% glucose) and time were studied as key parameters and optimized by response surface methodology (RSM). The RSM experiments were designed using design expert software version 6.0.10. A full factorial central composite design (CCD) was used. The experiment design was presented in Table 1. Based on the coded factor and actual factor, the final equations were derived [17].

$$\text{Biomass} = +1.48 - 0.34 * A + 0.000 * B + 0.26 * C + 0.25 * D - 0.73 * A^2 - 0.43D^2 + 0.26 * A * B - 0.012 * A * C + 0.012 * A * D - 0.012 * B * C - 0.012 * C * D$$

A- pH
B- Temperature
C- Time (hours)
D- Carbone source percentage

Table 1
Data of response surface methodology parameters for optimization and experimental design based on design expert.

Run order	pH	Temperature (°C)	Time (h)	Glucose (%)
1	8	10	5	5
2	7	10	5	1
3	7.5	35	14.5	3
4	8	60	24	1
5	7	60	24	5
6	7.5	35	24	3
7	8	10	24	5
8	7.5	35	14.5	1
9	7.5	35	5	3
10	8	35	14.5	3
11	7	60	5	5
12	7	35	14.5	3
13	7	10	24	1
14	7.5	60	14.5	3
15	7.5	10	14.5	3
16	7.5	35	14.5	5
17	8	60	5	1

$$\text{Biomass} = -154.85640 + 42.62339 * \text{pH}\ 0.15674 * \text{Temperature} + 0.050921 * \text{Time} + 0.69173 * \text{Carbon source} - 2.93585 * \text{pH}^2 - 0.10849 * \text{Carbon source}^2 + 0.021000 * \text{pH} * \text{Temperature} - 2.63158E-003 * \text{pH} * \text{Time} + 0.012500 * \text{pH} * \text{Carbon source} - 5.26316E-005 * \text{Temperature} * \text{Time} - 6.57895E-004 * \text{Time} * \text{Carbon source}$$

2.4. Assessment of antimicrobial properties of pyocyanin

2.4.1. Agar-well diffusion assay

This assay is performed to evaluate the antibacterial activity of pyocyanin against corrosive bacterial biofilm on MHA plates prepared at five different concentrations (10, 20, 30, 40 and 50 ppm) of pyocyanin. Three biocorrosive bacterial strains (EN2, EN3 and EN9) were spread plated evenly throughout the surface of sterile MHA agar plates. The wells were cut on the MHA agar by sterile glass borer and different concentrations of pyocyanin were dispensed into the respective well. Tetracycline (10 μg) was used as positive control due to its broad spectrum of antimicrobial activity and sterile distilled water was used as negative control. The zone of the inhibition formed was measured in millimetres (mm). The experiment was carried out in triplicate.

2.4.2. Biofilm formation assay

Biofilm assay was carried out as described by O'Tool et al. [18]. Overnight grown culture (EN2, EN3 and EN9) in NB medium were diluted in 1:20 ratio with fresh NB medium. Different concentrations of (10, 20, 30 ppm) the bioactive compound were added to microtitre wells followed by addition of 100 μL cultures into a 96-well polystyrene microtiter plate. The plate was incubated at room temperature for 24 h. The culture was discarded and washed with phosphate buffered saline (137 mM NaCl, 2.7 mM KCl, 10 mM NaHPO, 2 mM KHPO, pH 7.2). 120 μL of crystal violet solution was added and incubated at 37 °C for 20 min. At the end of the incubation 125 μL of acetic acid was added and incubated again for 15 min at 37 °C. The obtained result was studied using UV–visible spectrophotometer at 600 nm [18].

2.4.3. Analysis of extracellular polymeric substance (EPS)

Extracellular polymeric substance (EPS) extraction produced from mixed consortium (EN2, EN3 and EN9) was adopted from Padmavathi et al. [19]. EPS extraction was evaluated with and without bioactive compound (pyocyanin) at a concentration of 20 ppm. Extracted EPS was analyzed for protein and carbohydrate analysis by Lowry's and phenol/sulphuric acid method respectively [20].