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Investigation in effect of different culture medium on the anti-corrosive performance of bacterial biopolymer

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ABSTRACT

According to enormous defects of corrosion, ruining near 1–5% GNP, many methods are applied to reduce these disadvantages. One of the novelist methods to encounter this destructive phenomenon is using environmental-friendly bacterial biopolymer coating. The effective anticorrosive ability of this coating has been proved. It is shown that changing the biopolymer production medium can affect this property. In this paper, Chryseobacterium Indologenes MUT.2 made biopolymer has been studied under different culture medium constituents. The biopolymers produced under different conditions, visually tested in VNSS solution and salt spray test. The samples with more anti corrosive property have been analyzed in more accurate EIS test to achieve the most resistant one. It has been showed that the biopolymer produced in molasses and sodium nitrite, as carbon and nitrogen sources respectively, has the highest anti corrosive ability.

1. Introduction

Corrosion, *i.e.* destruction or deterioration of a material because of reaction with its environment, is one of the most hazardous industrial phenomena. In fact, corrosion protection and treatment consume more than 20% of a typical industrial budget. In 1960, The National Institute of Standards and Technology (NIST) evaluated that the cost of corrosion for the United States is over $300 billion [1]. Currently, there are three primary methods of corrosion control: reducing metal oxidation (*e.g.* anodic or cathodic protection), decreasing corrosion of corrosive media (*e.g.* inhibitors), and isolating metal from the corrosive environment via protective films (*e.g.* organic coating). Although most inhibitors are harmful and can contaminate the global environment, Green biopolymers along with polyamide compounds, polyacrylic acid, polymeric materials, and cellulosic polymers can act as a promising inhibitor. Table 1 shows some applicable typical approaches of corrosion protection and their advantageous/disadvantageous features.

Biopolymers are classified as a promising green corrosion inhibitor. As pointed in Table 1, biopolymer inhibitors are generally ecofriendly and low-cost. Indeed, the cost and reachability of biopolymer and biopolymer production medium have the main role on total production cost of biopolymer [6]. So, the garden soil extracted Chryseobacterium Indologenes MUT.2 bacteria [7] and waste, low cost materials (molasses and cheese whey) earned here as best medium sources can lead to cheap, acceptable industrial green inhibitor. The easy-to-reach bacteria, low cost, ecofriendly biopolymer with high corrosion resistance is the major criteria of authors bacterial choose.

Various classes of organic inhibitors, either as herbal or animal extracted form (*e.g.* starch, chitosan) or bacterial made biopolymer, are successfully used as corrosion inhibitors till date [8–11]. Literature survey reveals that only limited number of works have been carried out for the corrosion protection of carbon steel in VNSS and saline medium using bacterial made biopolymer [5,7].

Finkenstadt et al. [12] expressed that the anticorrosion properties of bacterial biopolymers were strain-specific. Hence, considering the differences between the bacterial strains [12,13] and also in the metal type, the culture media and in the experimental procedures, it is not possible to make a comparison amongst different bacteria. So, the amount of coating corrosion resistance (R_t) will vary drastically in different conditions. Up to this fact, the only factor to compare the corrosion resistance of biopolymer-generating bacteria (BGB) is corrosion resistance ratio (Eq. 1) (the amount of the corrosion resistance enhancement by adding the biopolymer).

$$\text{C.R.r} = \frac{R_{coated}}{R_{uncoated}} \tag{1}$$

where, *Rcoat* and *Runcoat* respectively are corrosion resistance of biopolymer coated and without biopolymer coated metal surface. Table 2 represents the comparative amount of corrosion resistance

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Table 1
Brief comparison of some typical corrosion protection methods.

Corrosion protection method	Disadvantage	Environmental hazards	Total cost	Ref.
Anodic/Cathodic protection	Severely vulnerable to MIC	–	Highly expensive	[2]
Organic and inorganic coatings	Subjected to abrasion and short time re-applying	Some organic hazard disposal	Expensive due to several re-applying	[3]
Chemical coating	Restricted to microbial degradation	Biocides	Normal	[4]
Typical corrosion inhibitor	Severe environmental concern	Chromates, nitrates, and molybdates	Normal	[2]
New green corrosion inhibitor	Ongoing functional optimization	–	Almost cheap (varied according to its production medium and strain type)	[5]

ratio under different conditions and biopolymers and their type of resistance measurement.

C. indologenes MUT.2 produced biopolymer proved to increase the corrosion resistance ratio up to nearly 4 times [7], is so promising to further detailed studies.

Composition of the culture medium and other fermentation conditions such as pH, temperature, oxygen concentration and agitation greatly affect the quantity and quality of bacterial produced biopolymers [18,19]. Biopolymers chemical and physical characteristics are highly related to their cultivation conditions [6,20]. Fermentation conditions effects on the biopolymers production amount are subjected to significant research works [21–23], while their roles on the biopolymer performance are less studied [24,25]. So, the influence of changing the culture medium on the biopolymer performance has been studied here.

In this paper, data on the effect of C. indologenes MUT.2 bacterial biopolymer cultivated on different media are presented and discussed. To precisely differentiate the produced biopolymers anticorrosive property, some comprehensive qualitative and quantitative tests were carried out. The SEM surface analysis was applied to prove the biopolymer formed coating on metal surface.

2. Materials and methods

2.1. Strain and inoculum preparation

The Chryseobacterium indologenes MUT.2, prepared by Biotechnology Research Centre, Tehran, Iran, was applied. The strain MUT.2 was identified via Sequence analysis of 16S rRNA gene. The sequence is accessible at gene bank database (NCBI) under JN831444. Chryseobacterium was a gram negative, catalase-positive, nonmotile, oxidase-positive, non-glucose-fermenting bacterium, indole-positive [7].

The LB-agar medium which used to strain maintenance and growth, had been composed of following ingredients: sodium chloride 5.0 g/l, yeast extract 5.0 g/l, tryptone 10.0 g/l, and agar 15.0 g/l. LB-agar was autoclaved at 121 °C for 15 min. After growing for 24 h at 30 °C, the culture was kept in 4 °C refrigerator [26].

LB-broth with composition of sodium chloride 0.5% (w/v), tryptone 1.0% (w/v), and yeast extract 0.5% (w/v) [27] was prepared as the inoculum culture. The inoculum medium sterilization process was carried out in 121 °C for 15 min. 100 ml of prepared LB-broth was incubated with strain cells in 30 °C for 12 h. the orbital shaker (Tecnal mod.TE-424, Tehran, Iran) with 160 rpm was applied for incubation. Cell concentration of 10^7CFU m/l^{-1} was acceptable point of cell growth confirmed with periodic monitoring by Perkin-Elmer model Lambda 20.

2.2. Culture medium

The optimized values of strain cultivation medium consist of six constituents [28]: sucrose (21 g/l), glutamic acid (20 g/l), K_2HPO_4 (6 g/l), NaH_2PO_4 (7 g/l), NH_4CL (0.7 g/l), and $MgSO_4$ (0.5 g/l). To obtain the desirable biopolymers, one factor experimental design with substituting the carbon and nitrogen sources and constant mineral salt was considered. Sucrose, glycerol, molasses, cheese whey and corn steep liquor (CSL) as well as glutamic acid, sodium glutamate and sodium nitrite were selected as the carbon and nitrogen sources, respectively. Calculating the required gram of C and N elements in culture medium from sucrose and glutamic acid, other sources optimum amount were obtained by their molecular weight and density.

After mixing the culture components, due to presence of several salts, the medium pH tended toward the acidic side adjusted to 7.1 by gradually adding 10 molar NaOH solution. Finally, the process of sterilization was carried out in 121 °C for 15 min.

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