Microbiol. Biotechnol. Lett. (2023), 51(4), 425–431
http://dx.doi.org/10.48022/mbl.2308.08009
pISSN 1598-642X eISSN 2234-7305

MBL
Microbiology and Biotechnology Letters

Bioflocculant and Anticorrosive Activities of the Biopolymer Produced by *Lysinibacillus macroides*

Kartik Andharia, Prateek Shilpkar*, and Mayur Shah

Department of Microbiology, Gujarat Vidyapith, Ashram Road, Ahmedabad, Gujarat, India

Received: August 22, 2023 / Revised: September 13, 2023 / Accepted: September 19, 2023

The biopolymer produced by *Lysinibacillus macroides* can be useful as an emulsifying agent, flocculating agent, anti-oxidative agent, and anti-corrosive agent. The present work focused on the functional activities of this biopolymer such as bioflocculant and anti-corrosion. The results of the study for the biopolymer as a bioflocculating agent reveal that the biopolymer gave 72.3% flocculating activity against activated carbon at a concentration of 5 mg/l. In the optimization study, the highest bioflocculant activity was obtained in the presence of $CaCl_2$ mineral at 15% biopolymer concentration, 7.0 pH, and 40℃ temperature. The biopolymer was also studied for its anti-corrosive nature and observed that it was able to prevent corrosion of steel paper clips in a 6% $CaCl_2$ solution at a concentration of 0.5% after 7 days.

Keywords: **Anticorrosion, bioflocculation, biopolymer, *Lysinibacillus macroides***

Introduction

Bioflocculation is used in mining industries, wastewater treatment, etc. In the presence of divalent minerals, biopolymers are able to form bonds with kaolin or activated charcoal, which eventually reduces the turbidity of the solution. Up to a certain level, the flocculation activity was correlated with the biopolymer concentration directly [1]. They recorded a maximum flocculation activity of 88.35% at 40 mg l^{-1} concentration of the biopolymer from *Bacillus cereus*. Nouha *et al.* [2] also reported similar flocculation activity of 89.8% at 1.8 g l^{-1} concentration of the biopolymer from *Cloacibacterium normanense*. Kumar *et al.* [3] reported the biosurfactant activities of the biopolymer produced by *Planococcus maitriensis*. The biopolymer having flocculating and biosorption capacity has been produced from *Pseudoalteromonas* strain SM9913, isolated from the deep-sea sediments of the Yellow Sea, China [4, 5]. The bio-adhesive properties of the biopolymer from *Vibrio alginolyticus* have also been investigated [6].

The challenging and serious issue of metal corrosion is faced by industries worldwide. Most of the bacteria produce biofilms by attaching to the surface of the materials, which can help to inhibit metal corrosion by preventing cathodic and/or anodic reactions and ultimately changing the electrochemistry. The biopolymers produced by *Staphylococcus aureus* and *Lactobacillus plantarum* have been reported to produce an anticorrosive effect on steel [7]. Gaikwad *et al.* [8] have reported similar results on steel metal plates for the pullulan produced by *Aureobasidium pullulans*. Glucan secreted by *Lb. reuteri* SK24.003 was reported to produce an anticorrosive effect on steel paper clips in a solution of 0.5% biopolymer concentration [9].

Materials and Methods

Microorganism and growth media

Lysinibacillus macroides, isolated from the rhizo-

***Corresponding author**
Phone: +91-9427408880
E-mail: prateek@gujaratvidyapith.org

spheric soil, were taken for this study. Modified Yeast Extract (MYE) medium containing (g l^{-1}) sucrose, 30; yeast extract, 1; KH_2PO_4, 1; and $MgSO_4$, 0.5; was used for the biopolymer production [10].

Biopolymer production and extraction

100 ml of MYE medium was inoculated with 10% v/v inoculum and incubated at room temperature for 96 h on a rotary shaker (120 rpm) [11]. After incubation, 5% w/v of trichloroacetic acid (TCA) was added to the medium and kept in agitation for 30 min at room temperature to precipitate proteins [12–15]. Precipitated proteins were separated by centrifugation (10,000 rpm) and recovered supernatant. Three volumes of ice-cold ethanol were added to the supernatant and kept overnight at 4℃. Precipitated biopolymer was collected by centrifugation as above, given three washes with ice-cold ethanol, dried (65℃), and weighed [10].

Bioflocculant activity

The bioflocculant activity of the biopolymer was measured by modifying the method used earlier by Mandal [16]. A stock solution of activated carbon was prepared by suspending 5 g l^{-1} of activated carbon in distilled water and the pH of the suspension was adjusted to 7. A solution of biopolymer (5 mg l^{-1}) and 1% (w/v) calcium chloride solution was also prepared. Stock solution (9 ml), biopolymer solution (0.1 ml), and calcium chloride solution (0.9 ml) were added to a glass test tube. The solutions were gently mixed by stirring for 2 min and allowed to settle down for 5 min at room temperature. The OD of the clarified upper solution was measured at 550 nm with a spectrophotometer (UV-spectrophotometer-1800, 240V, Shimadzu, Japan). A control was prepared by using 0.1 ml distilled water instead of a biopolymer solution. The flocculating activity was calculated according to the following equation:

$$\text{Flocculating activity (\%)} = [(B - A) / B] \times 100$$

Where A and B are the OD of the sample and control, respectively, at 550 nm.

Effect of biopolymer concentration, various minerals, pH, and temperature on flocculating activity

The bioflocculant activity is significantly affected by various factors like biopolymer concentration, minerals, pH, and temperature. The effect of these parameters was investigated in this study (Table 1). Flocculating activities for each of the experimental sets were estimated as described above.

Table 1. Effect of various parameters affecting the flocculating activity.

Parameter	Variable
Biopolymer concentration (mg l^{-1})	1, 5, 10, 15, 20 and 25
Mineral (1% w/v)	Calcium chloride, Magnesium chloride, Manganese chloride, Zinc sulphate, Copper sulphate, Cobalt chloride, Ferrous chloride, Nickel chloride, Sodium chloride, and Potassium chloride
pH	3, 4, 5, 6, 7, 8, 9 and 10
Temperature (℃)	10, 20, 30, 40, 50, 60, 70, 80, 90 and 100

Anticorrosion effect

To study the anticorrosion effect, the biopolymer sample was dissolved in a calcium chloride solution (6% w/v) at different concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5%. The solutions were stirred until the biopolymer was completely dissolved. Then the solutions were transferred to the glass test tubes. A steel metal plate was added to each of the tubes and kept in the open for 7 days. A control was prepared without adding a biopolymer sample [9].

Results

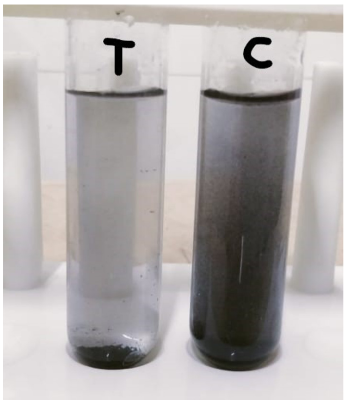
Bioflocculant activity

In the present study, the biopolymer of *L. macroides* was tested for bioflocculant activity. The biopolymer at a concentration of 5 mg l^{-1} showed 72.3% flocculating activity against activated carbon suspension as calculated by the equation mentioned in the materials and methods (Fig. 1).

Effect of biopolymer concentrations on the flocculating activity

The effect of lower or higher concentrations of biopolymer on the flocculating activity was investigated.

http://dx.doi.org/10.48022/mbl.2308.08009



T=Test; C=Control

Fig. 1. Bioflocculant activity shown by biopolymer.

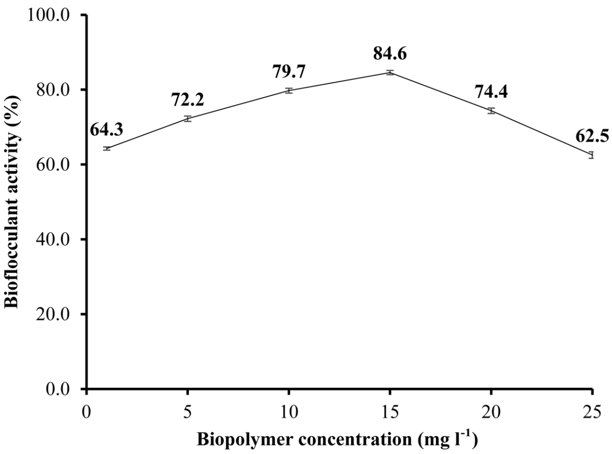


Fig. 2. Effect of biopolymer concentration on bioflocculant activity.

Different concentrations of biopolymer (1, 5, 10, 15, 20, and 25 mg l^{-1}) were studied in this work to obtain optimum concentration. The data shown in Fig. 2 revealed the effect of biopolymer concentrations on the flocculating rate. It was noticed that the flocculating activity was observed to increase with an increase in the biopolymer concentration by up to 15%. Then it slowed down at 20 and 25% concentrations. The highest flocculating activity of 84.6% was observed at 15 mg l^{-1} of biopolymer concentration followed by 10 mg l^{-1} (79.7%). The flocculant activity at other biopolymer concentrations of 1, 5, and 20 mg l^{-1} was found 64.3, 72.2, and 74.4%, respectively. The flocculant activity at a higher biopolymer concentration of 20 mg l^{-1} was observed lesser than the activity of

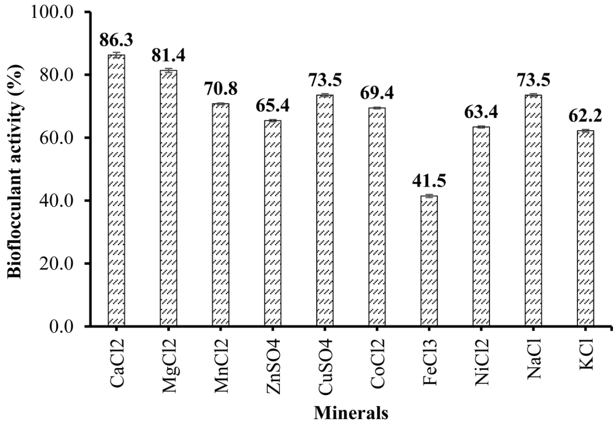


Fig. 3. Bioflocculant activity in the presence of various minerals.

a lower biopolymer concentration of 10 mg l^{-1}, but more than the activity found for a biopolymer concentration of 5%. The least flocculant activity was observed at a biopolymer concentration of 25 mg l^{-1} (62.5%).

Effect of various minerals on the flocculating activity

The effect of various minerals (1% w/v) on the flocculating activity was shown in Fig. 3. The results revealed that the highest flocculating activity of 86.3% was observed with $CaCl_2$ followed by $MgCl_2$ (81.4%). The biopolymer gave the least flocculating activity of 41.5% with $FeCl_3$. Comparatively good flocculating activities of 70.8, 65.4, 73.5, 69.4, 63.6, 73.5 and 62.2% were observed for other minerals such as $MnCl_2$, $ZnSO_4$, $CuSO_4$, $CoCl_2$, $NiCl_2$, NaCl and KCl, respectively. Furthermore, with divalent minerals, higher flocculating activities were noticed compared to monovalent minerals.

Effect of various pH on the flocculating activity

The effect of different pH (3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0) on the flocculating activity was presented in Fig. 4. The increase in flocculating activity was noticed at increasing pH up to 7.0 and then it decreased. The results revealed that the highest flocculating activity of 86.6% was observed at neutral pH of 7.0. At acidic pH of 3.0, 4.0, 5.0, and 6.0, the flocculating activity was obtained at 73.4, 75.3, 79.1, and 81.2%, respectively. At alkaline pH of 8.0, 9.0, and 10.0, a reduction in flocculating activity was observed (83.1, 78.5, and 74.2%, respectively) compared to pH 7.0.

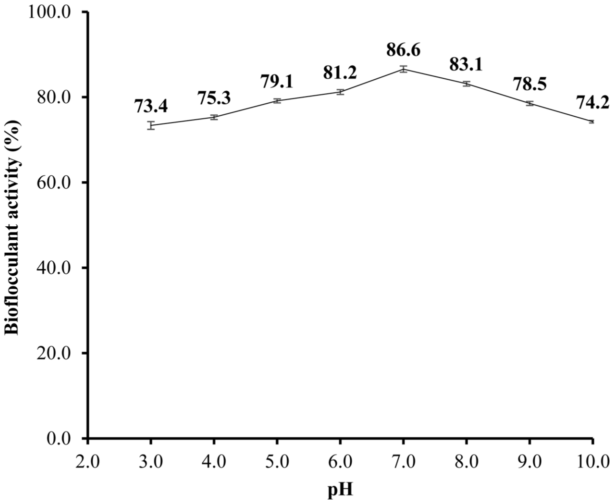


Fig. 4. pH affecting the bioflocculant activity.

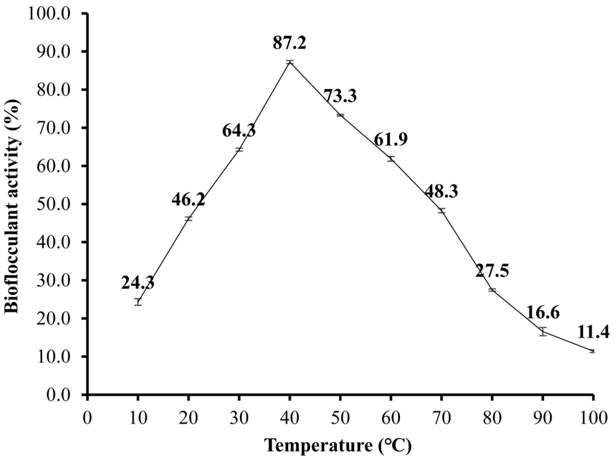


Fig. 5. Biofloculating activity at different temperatures.

Effect of temperature on the flocculating activity

The effect of different temperatures (10, 20, 30, 40, 50, 60, 70, 80, 90, and 100℃) on the flocculating activity was presented in Fig. 5. The increase in flocculating activity was observed at increasing temperatures up to 40℃ and then it decreased. The results revealed that the highest flocculating activity of 87.2% was observed at 40℃ followed by 50℃ (73.3%). It was noticed that the flocculating activity was comparatively lower at lower temperatures of 10, 20, and 30℃ (24.3, 46.2, and 64.3%, respectively). It was also observed that the biopolymer was thermally unstable at more than 40℃ up to 100. It is showing flocculating activity of 61.9, 48.3, 27.5, and 16.6% at the higher temperatures of 60, 70, 80, and 90℃, respectively. At 100℃, it was reduced as low as 11.4%.

Anticorrosion effect

Different concentrations of biopolymers (0.1, 0.2, 0.3, 0.4, and 0.5%) and a 6% (w/v) solution of calcium chloride were taken in this study. The $CaCl_2$ solution was clear and the paper clip was of smooth surface and silver in colour. After incubation for 7 days at room temperature, it was observed that the solution containing 0.5% biopolymer showed maximum protection against corrosion. All other tubes of concentrations 0.1, 0.2, 0.3, and 0.4% were observed highly corroded. The anti-corrosion effect of the biopolymer (at 0.5% concentration) on a steel metal paper clip was shown in Fig. 6. As illustrated in the figure, it was visually observed that the paper clip kept in $CaCl_2$ solution with 0.5% biopolymer lesser amount of black rust than the control, which indicated

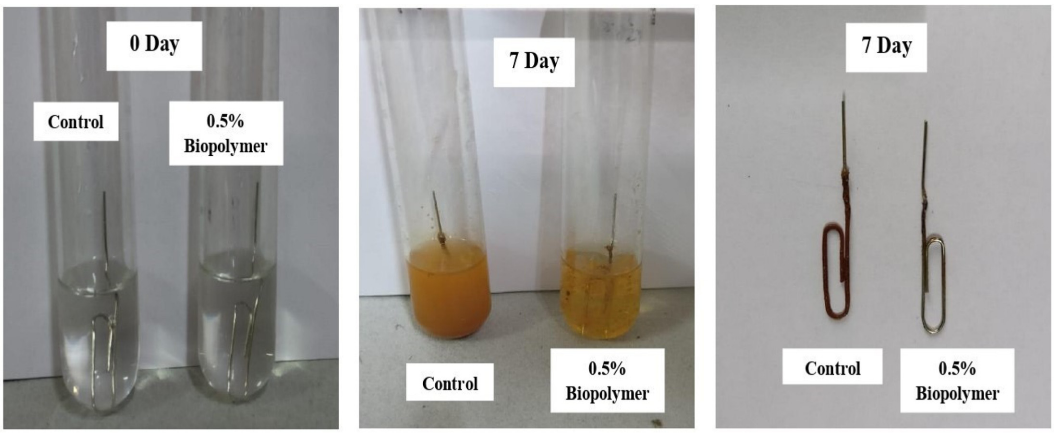


Fig. 6. Anticorrosive effect of biopolymer on a steel paper clip.

that the layer of biopolymer on the steel metal clip inhibits its direct contact with a salt solution with metal by significantly altering the redox potential of ferric ion to ferrous ion. In this manner, it indirectly protected the clip from corrosion. Further studies need to quantify the amount of corrosion in control as well as in test paper clips to understand the anticorrosive properties of biopolymers in detail.

Discussion

Presently, there is an increasing demand for biodegradable and renewable bioflocculant instead of chemical flocculants [16]. In the process of flocculation, bioflocculant molecules attach with activated carbon molecules and create electrical zeta potential, which can be neutralized by $CaCl_2$, form flocks, and decrease solubility. In this manner, the biopolymer functions as a bridge in the flocculation generation [17]. In the present study, we have obtained 72.3% flocculating activity at a biopolymer concentration of 5 mg l^{-1} against activated carbon, which is in accordance with the results published by Shukla [17].

However, an increase or decrease in the biopolymer concentration showed lower flocculating rates. When the concentration of biopolymer is not sufficient, the bridging process gets affected and ultimately reduces the flocculation [18]. Some researchers described the relationship between the biopolymer concentration and flocculation rate earlier. Results showed that at lower concentrations of biopolymer, ineffective bridging caused poor flocculation, while at higher concentrations, the excessive presence of negatively charged biopolymer caused incomplete dispersion of excessive biopolymer leading to poor stability of flocculation [18]. We obtained the highest flocculating rate of 84.6% at a biopolymer concentration of 15 mg l^{-1}. Yim *et al.* [19] recorded a maximum flocculation of 90.5 ± 1.1% from the biopolymer produced by a micro-alga, *Gyrodinium impudicum* KG03.

Minerals are able to stimulate or suppress the flocculating activity [20]. Wang *et al.* [21] reported two different mechanisms for flocculating activity: (1) minerals are responsible for the stabilization and neutralization of the charge functional group in flocculants, and (2) the addition of minerals increases the ionic strength of the suspension and decreasing the electrostatic forces of the suspended particles. The Ca^{+2} ions enhanced the formation of compact and large flocs in the present study due to the increased interface charges and increased bond formation between carbon and flocculant [22]. We observed an 86.3% flocculation rate in the presence of 1% $CaCl_2$ which is in agreement with the flocculating activity of 83.63% obtained by marine bioflocculant-producing *Bacillus* species [23].

The effect of different pH on the flocculating activity was investigated in the present study and observed maximum flocculation rate at neutral pH (7.0). Acidic pH inhibits ionization of the COOH group present in the bioflocculant. This prevents the bridging action and could be one of the reasons for the loss of flocculation stability. In neutral or alkaline pH, COOH is ionized to COO^- and H^+, which is the reason for increasing the flocculating activity [24]. Our results of 86.6% flocculating rate are in accordance with the 94% flocculating rate at pH 7.0 reported by the biopolymer of *B. cereus* MSI021 [25].

The results shown in Fig. 5 indicated that the bioflocculant produced by the biopolymer of *L. macroides* was thermally unstable beyond the temperature of 40℃. This instability might be due to the polymer chain breakdown of the biopolymer and the inability to form a bridge with the activated carbon particles [20]. In contrast to our findings, some research work published that flocculants rich in carbohydrate components have a higher thermal resistance property than those of protein and nucleic acids [26]. We obtained the highest flocculating activity at 40℃ (87.2%). Shukla [17] also reported maximum flocculation activity (79.42%) at the same temperature. Sajayan *et al.* [25] also reported a heat-labile bioflocculant from *B. cereus* MSI021 with a maximum flocculation rate at a temperature of 30℃.

Corrosion of steel starts with the anodic oxidation of iron (Fe) to ferric ion (Fe^{+2}). Ferric ions are further oxidized to ferrous ions (Fe^{+3}), which enhances the conversion rate of Fe to Fe^{+2}. Therefore, the metal-biopolymer system is generated. This system can reduce the amount of electron acceptor sites at the surface of the metal and inhibit corrosion [27]. A visual inspection of the tubes reveals that the ferrous oxide layer was formed over the metal in the control tube, whereas no layer or a very small layer was found on the metal of the tube containing biopolymer. Our result explored that the biopolymer produced from *L. macroides* formed a barrier layer over the metal surface and significantly inhibited the process of corrosion. We observed corrosion protection of steel metal clips at 0.5% biopolymer concentration in the 6% $CaCl_2$ solution. These results compare well to those of Miao *et al.* [9], who documented that a thin black rust layer on the paper clip surface was inhibited by the biopolymer of *L. reuteri* SK24.003 after 7 days. According to them, the lower pH of magnesium chloride significantly alters the redox potential of Fe^{+2}/Fe^{+3} and affects it more than calcium chloride. Jayaraman *et al.* [28] observed that the biopolymer from *Pseudomonas fragi* was more efficient than the biopolymer of *Escherichia coli* DH5α in inhibiting corrosion.

Conclusion

The biopolymer at a concentration of 5 mg l^{-1} showed 72.3% flocculating activity against activated carbon suspension. During the study, the highest bioflocculant activity was observed at 15% biopolymer concentration, 7.0 pH, and 40℃ temperature in the presence of $CaCl_2$ mineral. The biopolymer was able to prevent corrosion of steel paper clips at a concentration of 0.5% after 7 days in a 6% $CaCl_2$ solution.

Acknowledgments

The authors are thankful to excel industries limited for providing a laboratory facility for research work and financial support.

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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