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OIL DISPLACEMENT PROPERTIES OF PRODUCED SURFACTIN: A COMPARATIVE STUDY

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ABSTRACT

Biosurfactants are amphiphilic compounds derived from plants and microorganisms, recognized for their biodegradability, low toxicity, and environmental compatibility. They find diverse applications in industries such as oil recovery, bioremediation, and hydrocarbon cleanup. Surfactin, a potent lipopeptide biosurfactant produced by *Bacillus subtilis*, was generated in this study using cassava wastewater as the fermentation medium, monitored via high performance liquid chromatography (HPLC). Approximately 600 mg L^{-1} of surfactant was produced. Subsequently, an oil displacement test assessed the efficacy of crude and purified surfactin against a synthetic surfactant sodium dodecyl sulfate (SDS) and a commercial biosurfactant (rhamnolipid). Both crude and purified surfactin demonstrated superior oil-dispersing capabilities compared to SDS and rhamnolipid, even at lower concentrations. These findings highlight biosurfactants, particularly surfactin, as promising alternatives to petroleum-derived surfactants due to their effectiveness and potential for direct application without costly downstream processes.

Keywords: Bioremediation. Cassava wastewater. Oil displacement. Oil recovery. Surfactin.

1 INTRODUCTION

Surfactants are chemical compounds characterized by amphiphilicity, featuring a hydrophobic tail and a hydrophilic head, enabling them to disperse in water and interface with air/water or oil/water boundaries. This property facilitates the solubilization of hydrophobic substances, rendering surfactants versatile across various industrial sectors [1,2]. Biosurfactants, distinguished by their structural diversity and functional capabilities, hold considerable promise in both industrial applications and environmental contexts, particularly in remediating contaminated environments and enhancing oil recovery [1,3].

The shift towards biosurfactants is driven by their eco-friendly profile, contrasting with synthetic surfactants known for their potential risks to public health and ecosystems. Biosurfactants offer multiple advantages, including reduced toxicity, enhanced biodegradability, favorable environmental compatibility, resilience under extreme conditions, and diverse biological activities [3,4]. Among these, surfactin emerges prominently as a potent biosurfactant produced by *Bacillus subtilis*, significantly lowering surface tension from 72 mN m^{-1} to 27 mN m^{-1}.[5]

However, its widespread application is hindered by the high costs associated with production and purification processes, primarily stemming from culture medium expenses and downstream processing. Efforts to mitigate these costs have explored alternative growth media, such as agricultural and industrial residues, with cassava wastewater proving particularly advantageous for surfactin production. Another strategy involves utilizing biosurfactants in their crude form without purification, thereby reducing costs [5-8].

Oil displacement capacity is an important parameter to be evaluated before applying biosurfactants as remediation agents or in enhanced oil recovery, as it indicates the quality and efficiency of the biosurfactant. This study investigates surfactin production by *B. subtilis* fermentation of cassava wastewater, evaluating both crude and purified forms for their ability to displace oil. Comparative assessments include synthetic surfactants and other commercial biosurfactants.

2 MATERIAL & METHODS

Surfactin production was carried out via fermentation of cassava wastewater using *B. subtilis* ATCC 6633, as described by [5]. Batch fermentation was conducted in Erlenmeyer flasks containing 200 mL of culture medium (cassava wastewater) with 7% (v/v) of inoculum. Incubation occurred for 72 hours at 150 rpm and 30°C. Post-incubation, the fermented broth was centrifuged at 10^4 g for 10 minutes to remove biomass. The surfactin concentration in the supernatant was analyzed using HPLC with LCMS-2020 model (Shimadzu Corp., Japan) equipped with a PDA detector (214 nm). The stationary phase used was Kromasil® C18 (100 Å, 300 mm × 4.6 mm i.d.), and the mobile phase comprised ultrapure water and 0.1% trifluoroacetic acid (v/v) in acetonitrile, with a total flow of 0.9 mL min^{-1} and an injection volume of 50 µL.

Surfactin purification employed an acid precipitation method. The sample pH was adjusted to 2 using 1.0 M HCl and allowed to decant for 24 hours. The samples were then centrifuged at 10^4 g for 10 minutes at 5°C. The precipitate was collected, neutralized with 1 M NaOH, and lyophilized. The solid was resuspended in tap water to the original volume, yielding purified surfactin. [9]

The oil displacement test was conducted according to [10]. In an 8 cm diameter Petri dish, 30 mL of distilled water was added, followed by 200 µL of crude oil to form an oil film on the surface. Then, 10 µL of surfactant solution was added under the oil surface, and the oil dispersion was measured by the halo diameter using ImageJ software [10]. Three surfactants were tested:

synthetic anionic surfactant sodium dodecyl sulfate (SDS), commercial rhamnolipid (Rh), and surfactin from both fermented raw broth and purified forms. Surfactant concentrations ranged from 25 to 600 mg L^{-1}. Each experiment was performed in triplicate.

3 RESULTS & DISCUSSION

Fermentation of cassava wastewater with B. subtilis yielded approximately 600 mg L^{-1} of surfactin, quantified using HPLC. Upon confirming surfactin production, crude surfactin (CS) underwent acid precipitation for purification, resulting in purified surfactin (PS), which was reconstituted in water to achieve a 600 mg L^{-1} surfactin solution. Subsequently, varying concentrations of CS, PS, Rhamnolipid, and SDS were evaluated for their efficacy in oil dispersion. Figure 1 illustrates their oil displacement capacities.

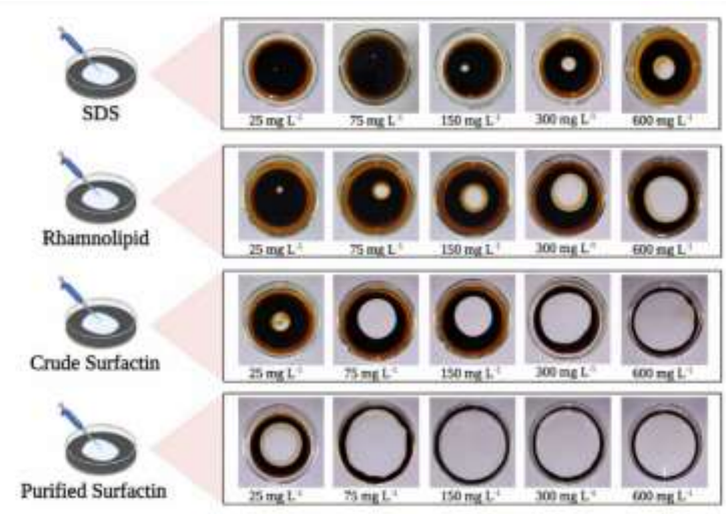

Figure 1 Images used to calculate oil dispersion in treatments with SDS, rhamnolipid, crude surfactin, and purified surfactin, at surfactant concentrations between 25 mg L^{-1} and 600 mg L^{-1}.

Figure 1 shows that purified surfactin (PS) achieved the highest oil dispersion, followed by crude surfactin (CS), rhamnolipid, and synthetic surfactant SDS. These qualitative observations are supported by ImageJ software analysis, which quantified the dispersion areas (Figure 2).

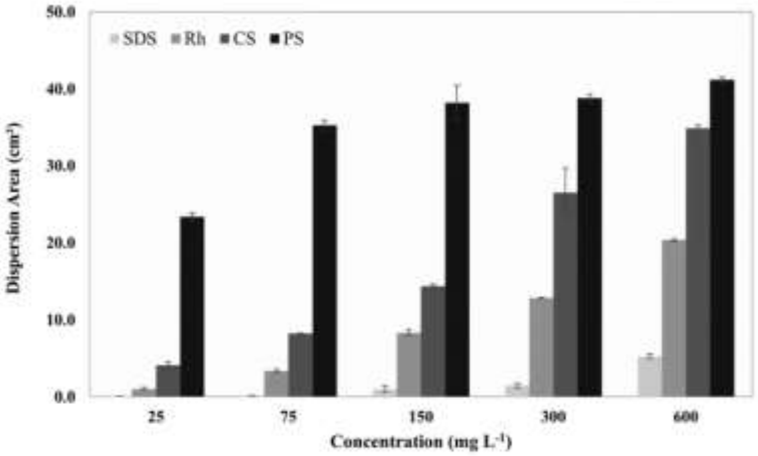

Figure 2 Oil dispersion by crude surfactin (CS), purified surfactin (PS), rhamnolipid (Rh), and sodium dodecyl sulfate (SDS) in the concentration range of 25 mg L^{-1} to 600 mg L^{-1}.

In Figure 2, biosurfactants demonstrate superior oil dispersal compared to SDS. At the highest tested concentration (600 mg L^{-1}), oil dispersion areas were: SDS, 5.2 cm²; Rh 20.04 cm²; CS 34.9 cm²; and PS 41.2 cm². Biosurfactants generally exhibit lower surface and interfacial tensions than synthetic surfactants [11], enhancing their oil dispersion efficiency.

Purified surfactin exhibits superior oil dispersion results, aligning with its reputation as a potent biosurfactant capable of reducing water surface tension from 72 mN m^{-1} to 27 mN m^{-1} [5, 11]. Even in its crude form, surfactin produced from *B. subtilis* ATCC 6633 fermentation of cassava wastewater showed higher oil removal capabilities than SDS and rhamnolipid.

This study addresses the challenge of optimizing biosurfactant production, particularly surfactin, to reduce costs and compete with synthetic dispersants. Utilizing cassava wastewater as a culture medium offers a cost-effective alternative for surfactin production, validated by recent research [5, 6, 8]. Purification processes typically incur substantial costs, comprising 70% to 80% of total production expenses [7,12]. Direct application of raw biosurfactants, such as CS, bypasses these costly steps, presenting an economically viable option for surfactin application [12]. This approach not only reduces production costs significantly but also underscores the potential of surfactin, whether crude or purified, for environmental applications like remediating oil-contaminated areas and enhancing oil recovery.

4 CONCLUSION

In conclusion, biosurfactants emerge as promising eco-friendly and efficient alternatives to synthetic surfactants for dispersant applications. This study utilized *B. subtilis* to produce surfactin from cassava wastewater, which was subsequently purified and evaluated for its oil dispersal capabilities. The findings demonstrated that both crude and purified surfactin were more effective in oil displacement compared to the synthetic surfactant SDS and standard rhamnolipid. Purified surfactin dispersed an area approximately 8 times larger than SDS and 2 times larger than Rh. Crude surfactin also exhibited substantial oil displacement, with purified surfactin dispersing only twice as much area as crude surfactin. The use of cassava wastewater as an alternative culture medium for surfactin production represents a cost-effective approach to mitigate production expenses. Given that downstream processes significantly contribute to overall production costs, the direct application of biosurfactants in their raw form can be economically advantageous by circumventing expensive purification steps. Further economic development of biosurfactants as environmentally friendly and efficient dispersants is crucial to mitigate the public health and environmental risks associated with synthetic commercial dispersants.

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