Potential Biosurfactant Producer And Bioemulsifier Isolated From Petroleum Sludge

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ABSTRACT

Biosurfactant are gaining much interest in diverse industrial applications mainly due to their advantages such as structural diversity, low toxicity, greater biodegradability, ability to function in wide ranges of pH, temperature and salinity as well as greater selectivity. In this study, hydrocarbon-degrading bacteria were isolated from petroleum sludge and the isolates were screened for biosurfactant production and biodegradation capability. All of the bacterial isolates were grown in minimal media, Bushnell Haas broth supplemented with 1% (v/v) crude oil as carbon source and further identified as *Achromobacter sp*., *Pseudomonas sp.* and *Citrobacter sp*. by 16S rDNA gene sequencing analysis. The biosurfactants activity were determined by emulsification index (E24) and surface tension measurement. The range of emulsification index produced by all isolates were between 50.83% to 72.81% and the range of surface tension measurements for all isolates that exhibited were between 45.67 mN/m to 18.92 mN/m. In this study, *Pseudomonas sp*. P15 was selected as the highly potential biosurfactant producer due to its ability to reduce surface tension as low as 18.92 mN/m with high emulsification activity (67.41%). Interestingly, *Pseudomonas sp*. P15 showed high percentages of TPH degradation up to 85.1% in 12-days incubation. Therefore, our study proposed that *Pseudomonas sp.* P15 has the potential to be used as bioemulsifier and biosurfactant producer.

Keywords: Biosurfactant, biosurfactant-producing bacteria, petroleum sludge, surface tension, bioemulsifier

BACKGROUND

Biosurfactant are amphipathic molecules that have similar properties as chemically-made surfactants (Figure 1). Nowadays, biosurfactant are widely used in petroleum industry to **enhance the remediation** or **augmentation process**. Despite on reducing environmental pollutions, Biosurfactant have specialty to **enhance oil recovery** throughout oil refining process including in **reservoir, pipeline** and **oil spill** (Liu et al. 2012). On the other hand, Biosurfactant help in **increasing of crude oil production** simultaneously.

By passing years, many researchers become more interested the application of biosurfactant which have many advantages for petrochemical industry (Figure 2). In previous study, some common biosurfactant producing bacteria which have been isolated including *Pseudomonas aeruginosa* (Yan et al. 2012), *Bacillus cereus* (Pereira et al. 2013) *Acinetobacter sp*. (Hamzah et al. 2010) and etc.

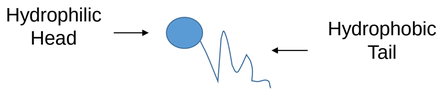


Figure 1: Biosurfactant consist of hydrophilic & hydrophobic end.

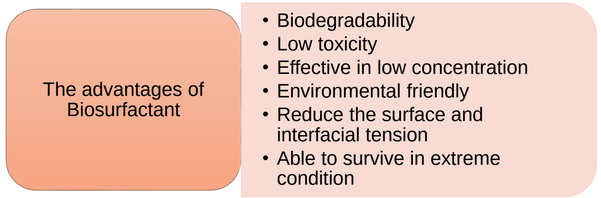
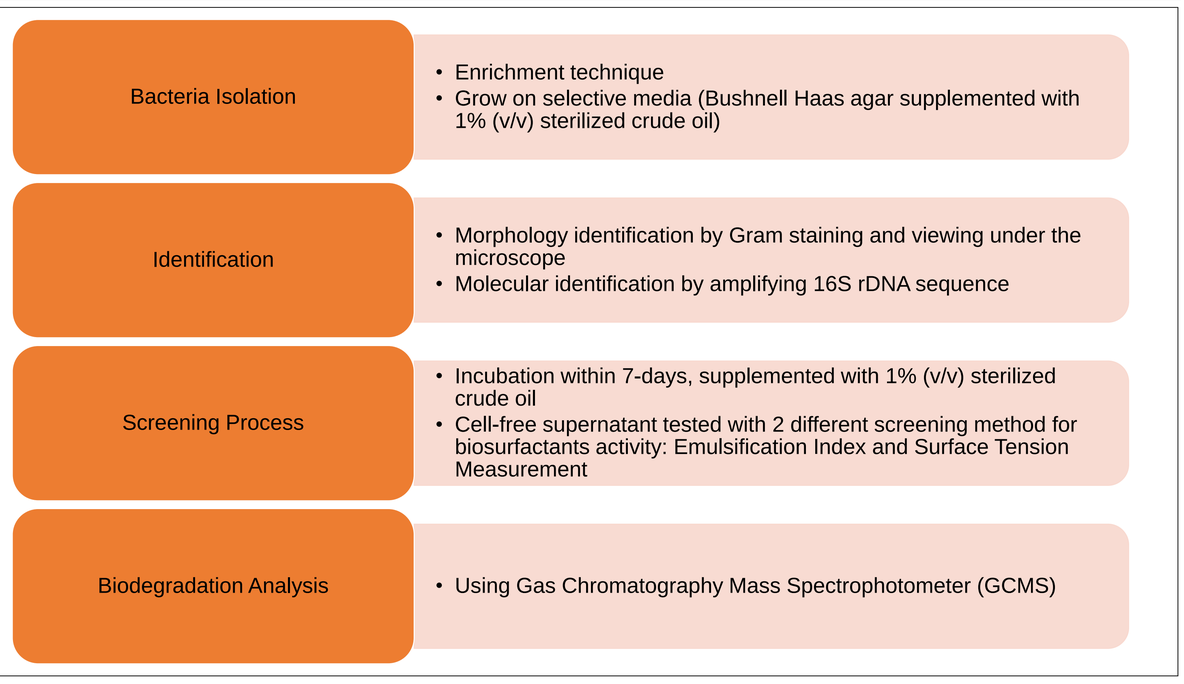


Figure 2: The advantages of Biosurfactant that make these compound can be an alternative to the chemically-made surfactants.

METHODOLOGY



RESEARCH FINDINGS

Bacteria Isolation, Identification & Screening Process

- **26 bacterial isolates** were successfully isolated from the petroleum sludge sample using selective media; Bushnell Haas supplemented with sterilized crude oil agar (Figure 3: A) .
- Most of the isolates are Gram negative bacteria. Based on the screening process, the isolates with potential to produce bioemulsifier and biosurfactant are **identified** as *Pseudomonas sp.* (P15, P16, P17), *Acromobacter sp.* (P12, P24) and *Citrobacter sp.* (P21) (Table 1).
- All isolates **screened** for potential biosurfactant producer. Biosurfactant activity of all isolates analysed using Emulsification Index (E24) and Surface Tension Measurement (Figure 4). The isolates that have positive result in both methods are considered as potential producer

(A)

(B)

Figure 3: A) Petroleum Sludge collected from Petrochemical Industry. B) Surface tension measurement using tensiometer.

Table 1: Isolates identification using Gram staining reaction and 16S rDNA sequencing.

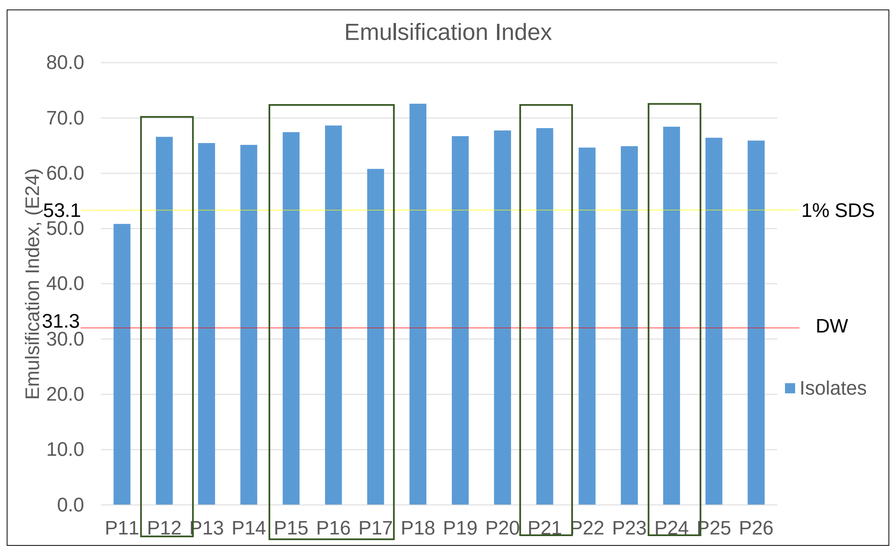
Isolates	Gram Staining Reaction	Percentages of similarity	16S rDna Sequencing
P12	Gram Negative	99.73%	*Achromobacter sp.*
P15	Gram Negative	98.29%	*Pseudomonas sp.*
P16	Gram Negative	99.9%	*Pseudomonas sp.*
P17	Gram Positive	99.31%	*Pseudomonas sp.*
P21	Gram Positive	99.37%	*Citrobacter sp.*
P24	Gram Negative	99.9%	*Achromobacter sp.*

Biodegradation Analysis

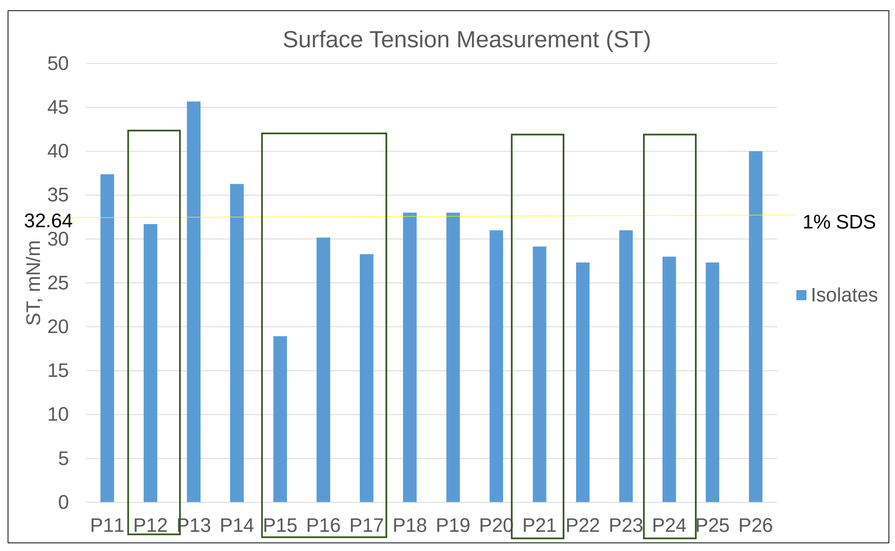
- P15 was selected to be further evaluated for its ability to degrade total petroleum hydrocarbon (TPH). Almost 86% of total petroleum hydrocarbon was found to be degraded by P15 after 12 days of incubation (Table 2).

Table 2: The percentages of TPH degradation in three day interval.

Incubation days	% TPH degradation
3	-27.2%
6	73.1%
9	78.2%
12	85.1%



(A)



(B)

Figure 4: The biosurfactants activity produced by isolates analysed using Emulsification Index (A) and Surface Tension Measurement (B). Both methods using same positive and negative controls. (+) control: 1% (w/v) Sodium Dodecylsulphate (SDS) solution, (-) control: sterilized distilled water (DW). A) The isolates with higher emulsification index compared to 1% SDS could be considered as potential bioemulsifier producers. (B) The isolates with lower surface tension measurement compared to 1% SDS could be considered as potential biosurfactant producers. The surface tension of negative control is 56.04 mN/m.

CONCLUSION

- From 26 isolates, P15 was selected as a highly potential biosurfactant producer due to its ability to reduce surface tension as low as 18.92 mN/m with high emulsification activity (67.41%).
- In further analysis, P15 showed effectively increases in percentages of TPH degradation. Almost 85% TPH has been degraded in this course of 12 days by P15. Effective TPH biodegradation by P15 might be aided by the biosurfactant produced by it.
- Therefore, P15 has the high potential to produce biosurfactant that can be used in enhancing oil recovery and bioremediation of petroleum contaminated sites.
- Future work includes extraction and purification of biosurfactant from these biosurfactant producing bacteria and evaluate their effectiveness in enhancing oil recovery.

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