Reduction of Surface Tension of Petroleum Using Hydrocarbon Degrading Bacterial Activity

Riuh Wardhani, Dirayah Rauf Husain* and Fuad Gani

Department of Biology, Faculty of Mathematics and Natural Science, Hasanuddin University

Hydrocarbon degrading bacteria produce biosurfactants, which facilitate the biodegradation process. This is the first step towards getting access to interactions between the bacteria’s hydrophilic surface and the hydrophobic surface of the hydrocarbons. Due to their amphipathic nature, biosurfactants facilitate this interaction. The focus of the study is to obtain hydrocarbon-degrading bacteria isolates and evaluate biosurfactant activity in reducing water surface tension. Hydrocarbon-degrading bacteria were isolated from contaminated marine sediment samples and consequently cultured on artificial seawater media with the addition of petroleum hydrocarbons as a carbon source. Surface tension reduction of biosurfactants was measured using a digital K20-EasyDyne tensiometer (KRÜSS: Hamburg, Germany). The study indicates that the isolates have biodegradation activity and reduced water surface tension by 22.14 mN/m, the data demonstrated that biosurfactant production was most effective on the third day of the exponential phase incubation. These studies demonstrate the effectiveness of hydrocarbon-degrading bacteria to produce biosurfactants as biodegradation agents to solve the problem of oil pollution.

**Keywords:** biodegradation; hydrocarbon; biosurfactant; surface tension

I. INTRODUCTION

Biosurfactants are known as amphipathic compounds obtained from plants and microorganisms. It is renowned as a promising alternative molecule for industrial (as a commercial and detergent material) and domestically because of its high biodegradability, low cytotoxicity, multifunction, ecologically friendly, and natural availability (Lawniczak et al., 2013; Akbari et al., 2018). Recently, biosurfactants have received attention because they are recognised as appropriate and ecologically friendly alternative materials for bioremediation technology (Elazzazy et al., 2015)

Biosurfactants promote the bioremediation of oil spills in the marine environment by improving the solubility of petroleum components and reducing the oil-water interface’s surface tension. Biosurfactants are useful as antimicrobial agents and immunomodulatory molecules (Fracchia et al., 2015). Microorganism-produced surfactants (biosurfactants) can be soluble in organic solvents (nonpolar) and water solvents (polar) and categorised based on their chemical structure and microbiological source. They include glycolipids, lipopeptides, proteins, phospholipids, polysaccharide-protein complexes, lipopolysaccharides, neutral lipids, and fatty acids (Desai & Banat, 1997). The biodegradation process of petroleum hydrocarbons can be carried out by microorganisms intracellularly and extracellularly. The degradation by intracellular method begins with the transfer of hydrocarbons into the cell. According to Rosenberg 1986, the mode of hydrocarbon transfer into the cell varies, which can be through direct contact between the cell of microorganisms and contact between bacteria with emulsified hydrocarbons through the help of biosurfactants (Rosenberg, 1991). In this case, biosurfactants promote the reduction of surface tension between water and oil, causing them to be transported into bacterial cells.

Surface tension is the most critical part of active tension agents and is the attraction between molecules in a liquid (Sobrinho et al., 2014). The interface described the
boundary between two liquids, whereas the surface described the boundary between the liquid and the surrounding air. A tensiometer is used to measure surface tension quantitatively. Most early analyses for identifying the presence of surfactants in the medium were based on these parameters. For distilled water, the surface tension of air/water is roughly 72 mN/m (or dynes/cm) (Sobrinho et al., 2014). The decrease of surface tension aims to facilitate the interaction of bacteria with petroleum to initiate the biodegradation process. This study aims to isolate hydrocarbon-degrading bacteria from tropical waters (Indonesia, particularly South Sulawesi) and evaluate the activity of biosurfactants in reducing water surface tension.

II. MATERIALS AND METHOD

A. Sampling

Samples of marine bacteria used were obtained from the sediment of the port of Paotere, Makassar, Indonesia, using a sediment core sampler. The territorial waters that show the existence of oil spills become the sampling point of marine bacteria. Sediment samples add into sterilised sample bottles.

B. Isolation of Hydrocarbon Degrading Bacteria

The sediment samples obtained were transferred into the artificial seawater medium (ASM), adding 1% of petroleum hydrocarbons as a carbon source (Chen et al., 2017). Petroleum hydrocarbons are the only source of carbon in the medium. Therefore only bacteria that can degrade hydrocarbons can grow in this medium.

A total of 3 gr of sediment samples were put into 100 mL ASM + 1% petroleum and then indicated on rotary shakers at 30 °C at a speed of 180 rpm for seven days. For seven days, periodic observations are made to visually determine the biodegradation process by observing the amount of petroleum and the level of turbidity of the medium.

C. Morphological Characterisation

The bacteria isolates that grown in isolation step purified with quadrant streak method. The purification was done 2-3 times to obtain a pure colony. Observations of cell morphology are made to determine the nature of gram bacteria. Furthermore, observations of colony morphology using stereo microscopes by observing shape, elevation, margin, and colour.

D. Growth Curves Measurement

Bacterial cultures that grow at the isolation stage are transferred to a fresh + 1% petroleum ASM. It aims to adapt bacteria to the media before measuring the growth curve. Growth curves are measured using spectrophotometers with the principle of turbidometry and using ASM as control. This measurement aims to find out the model of bacterial growth presented in various phases of growth (Zwietering et al., 1990). The data obtained is used as the basis for surface tension measurements.

E. Biosurfactant Extraction

The biosurfactant was precipitated overnight at 4°C after the cell-free supernatant was acidified to pH 2.0 with 6 M HCl. The precipitated biosurfactant was recovered by centrifugation (8000 rpm, 20 min) and redissolved in deionised water before extraction using chloroform-methanol (2:1, v/v) mixture. Evaporation of the organic phase yielded the pure biosurfactant (Varjani & Upasani, 2017).

F. Surface Tension Measurement

Biosurfactant surface tension measurements obtained from bacterial cultures of 1, 3, 4, and 6 days are performed using a digital K20-EasyDyne Tensiometer (KRÜSS: Hamburg, Germany) (Qazi et al., 2013). The effect of biosurfactant addition is measured in varying ways: 2 mL, 4 mL, and 6 mL. Biosurfactant surface tension values are obtained by averaging five measurements. The force required to draw the ring past the liquid membrane's surface is the parameter being tested. The value is represented in mN/m or dyne/cm units as the surface tension value.

III. RESULT AND DISCUSSION

Hydrocarbon degrading bacteria were successfully obtained from the sediments of the port of Paotere Makassar, Indonesia, and named HDBp (Hydrocarbon Degrading Bacteria paotere) isolate. The bacteria grow well in ALS
media that have added petroleum hydrocarbons. The observations showed that bacteria were slowly using hydrocarbons as a carbon source, mentioned by a reduction in the amount of petroleum and turbidity of the medium (Figure 1).

Figure 1. Biodegradation process of petroleum hydrocarbons: before biodegradation (left), after biodegradation (right)

The primary metabolic processes for the biodegradation of hydrocarbons have been identified (Atlas, 1981). The oxidation of substrates by the enzyme oxygenase, which requires molecular oxygen, is the initial step in the biodegradation of hydrocarbons by bacteria and fungi. After that, alkanes are transformed to carboxylic acids, which are then biodegraded by beta-oxidation (a crucial metabolic pathway for the synthesis of fatty acids from lipids, which ends in the generation of acetic acid, which subsequently enters the tricarboxylic acid cycle). Aromatic hydrocarbon rings are hydroxylated to generate diols, subsequently decomposed to form catechols, which are then degraded into tricarboxylic acid cycle intermediate chemicals. Fungi and bacteria, interestingly, create intermediates with differing stereochemistry. Trans-diol is produced by fungi, whereas cis-diol is nearly exclusively produced by bacteria (many trans-diols are potent carcinogens, whereas cis-diol is not biologically active). Biodegradation of aromatic hydrocarbons produces detoxification and no potential carcinogens because bacteria are the most common hydrocarbon decomposers in the marine environment. Complete hydrocarbon biodegradation (mineralisation) yields non-toxic end products like carbon dioxide and water, as well as cell biomass (mainly proteins) that can be safely incorporated into food webs (Atlas, 1995).

The observations under the microscope showed HDBp isolate is a gram-positive bacteria shaped with colony characteristics, namely circular shape, convex elevation, entire margin, and milky colour.

A. Growth Curve

Bacterial growth in ALS+1% petroleum media is measured by the growth curve shown in Figure 1. During 24 hours, bacteria adapt to the media and enter an exponential phase on day two and end on day 8. The stationary phase occurs on days 9 to 13 and ends with the death phase on days 14-15 (Figure 2).

The lag phase is the transition to an exponential phase where bacteria adapt to media conditions. In the exponential phase, bacteria divide twice as much as the previous amount. When the carbon source begins to run out, the bacteria are in a stationary phase. The number of bacteria dividing equals the number that dies. Ultimately, bacteria enter the death phase caused by the accumulation of toxic metabolites and depleted sources of nutrients in the media (Maier et al., 1982).

B. Biosurfactant Production

The phases formed on the results of the growth curve measurement become the basis of the active tension measurement. Figure 3 shows a significant decrease in surface tension after adding 2 mL, 4 mL, and 6 mL of biosurfactants. Biosurfactant production begins from the first 24 hours at the beginning of the exponential and
decreases significantly in the stationary phase until death. In line with Kumar, bacteria’s biosurfactant synthesis occurs from the exponential to stationary phases (Kumar et al., 2021). The data obtained showed that biosurfactant production was most effective at incubating the third day of the exponential phase. The number of biosurfactants added is also seen with variations in additions of 2 mL, 4 mL, and 6 mL. The data showed a reduction in maximum surface tension at the addition of 4 mL, seen in the addition of biosurfactants harvested from 3-day cultures experienced the highest decrease of 22.14 mN/m.

Biosurfactants are amphipathic compounds that have hydrophilic and hydrophobic structures. Biosurfactants lower surface tension effectively at specific concentrations called critical micelle concentration (CMC). When it passes through these concentrations, the biosurfactant will form a micelle structure in the water that causes its ability to reduce the surface tension and reach saturation point (Akbari et al., 2018; Alpandi et al., 2021). Surface tension tends to be stable and even increases in the addition of a 6 mL biosurfactant, if produced and applied in large quantities, this causes losses. Hence, it becomes essential to know the CMC of a biosurfactant. The application of biosurfactants is due to their amphipathic properties. Biosurfactant compounds are widely used in environmental bioremediation-based sectors, as well as agriculture and pharmaceuticals (cosmetics materials). Biosurfactants are also utilised as antibacterial and antibiotic agents because of their ability to inhibit pathogenic bacteria (Fakruddin, 2012; Kumar et al., 2021).

IV. CONCLUSION

Hydrocarbon-degrading bacteria absorb the hydrocarbon compound as a carbon source during biodegradation. An essential step in the degradation of petroleum hydrocarbons is how bacteria can contact the surface of petroleum. Bacteria can do this interaction in several ways. (1) microbial cells’ absorption of hydrocarbons in the water phase. (2) Microbial cells directly contact large hydrocarbon particles, and (3) Microbial cells interact with overlaid hydrocarbon particles. Cell hydrophobicity affects bacterial adhesion to petroleum hydrocarbons (Shi et al., 2019). In particular, high bacterial surface hydrophobicity is beneficial for adsorption between bacteria and petroleum hydrocarbons. Biosurfactant production was observed in media with and without petroleum in a scanning electron microscope (SEM), which showed that biosurfactant production is only in petroleum-containing mediums (Sharuddin et al., 2021); this proves the significance of the role of biosurfactants in the pathways of absorption or biodegradation of petroleum.

V. SUMMARY OF THE RESEARCH

Oil pollution in marine ecosystems can cause ecosystem imbalances due to the death of marine organisms (Blackburn et al., 2014; Fodrie et al., 2014). One method to eliminate oil spill in waters are to utilise marine microorganisms such as bacteria. Bacteria can produce biosurfactants that can reduce water surface tension so that they can initiate the biodegradation process (Atlas, 1981). The purpose of this study was to isolate hydrocarbon-degrading bacteria from tropical waters (Indonesia, particularly South Sulawesi) and evaluate the activity of
biosurfactants in reducing water surface tension. Surface tension is the tension between molecules in a liquid and is the most important component of active tension agents (Sobrinho et al., 2014).

The major conclusion of this study was that the bacteria can produce biosurfactants that lower the maximum surface tension in the stationary phase with a total reduction in surface tension of 22.14 mN/m. These findings are nearly equal to Planococcus sp. XW-1 from the Cold Marine Environment can reduce water surface tension by 26.8 mN/m (Guo et al., 2022). Biosurfactants play an important role in the bioremediation of oil spills in aquatic environments by increasing the solubility of petroleum components and effectively reducing the oil-air interface (Khan et al., 2014). The activity of biosurfactants from hydrocarbon-degrading bacteria in reducing surface tension in this study can be the basis for developing biosurfactants in the pharmaceutical and clinical fields such as their use as antibacterial agents.

VI. REFERENCES

Akbari, S et al. 2018, ‘Biosurfactants—a new frontier for social and environmental safety: a mini review’, Biotechnology Research and Innovation, Sociedade Brasileira de Biotecnologia, vol. 2, no. 1, pp. 81–90. doi: 10.1016/j.biorti.2018.09.001.

Alpandi, AH et al. 2021, ‘Critical micelle concentration, interfacial tension and wettability alteration study on the surface of paraffin oil-wet sandstone using saponin’, IOP Conference Series: Materials Science and Engineering, vol. 1153, no. 1, p. 012018. doi: 10.1088/1757-899X/1153/1/012018.

Atlas, RM 1981, ‘Microbial degradation of petroleum hydrocarbons: an environmental perspective’, Microbiological Reviews, vol. 45, no. 1, pp. 180–209. doi: 10.1128/mr.45.1.180-209.1981.

Atlas, RM 1995, ‘Petroleum biodegradation and oil spill bioremediation’, Marine Pollution Bulletin, vol. 31, no. 4–12, pp. 178–182. doi: 10.1016/0025-326X(95)00113-2.

Blackburn, M 2014, ‘Oil in our oceans: a review of the impacts of oil spills on marine invertebrates’ The Xerces Society for Invertebrate Conservation (January), pp. 1–160.

Chen, Q et al. 2017, ‘Study on the biodegradation of crude oil by the free and immobilized bacterial consortium in marine environment’, PLoS ONE, vol. 12, no. 3, pp. 1–23. doi: 10.1371/journal.pone.0174445.

Desai, JD & Banat, IM 1997, ‘Microbial production of surfactants and their commercial potential’, Microbiology and molecular biology reviews : MMBR, vol. 61, no. 1, pp. 47–64. doi: 10.1128/61.1.47-64.1997.

Elazzazy, AM, Abdelmoneim, TS & Almaghrabi, OA 2015, ‘Isolation and characterization of biosurfactant production under extreme environmental conditions by alkali-halo-thermophilic bacteria from Saudi Arabia’, Saudi Journal of Biological Sciences, King Saud University, vol. 22, no. 4, pp. 466–475. doi: 10.1016/j.sjbs.2014.11.018.

Fakruddin, Md 2012, ‘Biosurfactant: Production and Application’, Journal of Petroleum & Environmental Biotechnology, vol. 3, no. 4, pp. 1–5. doi: 10.4172/2157-7463.1000124.

Fracchia, L et al. 2015, ‘Potential therapeutic applications of microbial surface-active compounds’, AIMS Bioengineering, vol. 2, no. 3, pp. 144–162. doi: 10.3934/bioeng.2015.3.144.

Fodrie, F et al. 2014, ‘Integrating organismal and population responses of estuarine fishes in Macondo Spill Research’ BioScience, pp. 778–88.

Guo, P et al. 2022, ‘Isolation and characterization of a biosurfactant producing strain Planococcus sp. XW-1 from the cold marine environment’. Int. J. Environ. Res. Public Health, p. 782.

Khan, et al. 2014, ‘Perspectives on Using Biosurfactants in Food Industry’ Biosurfactants (November), pp. 306–23.

Kumar, A et al. 2021, ‘Microbial biosurfactant: A new frontier for sustainable agriculture and pharmaceutical industries’, Antioxidants, vol. 10, no. 9. doi: 10.3390/antiox10091472.

Ławniczak, Łukasz; Marecik, RCL 2013, ‘Ławniczak.pdf’, Appl Microbiol Biotechnol, vol. 93, no. 1, pp. 2327–2339.

Maier, Raina M Pepper, Ian L Gerba, CP 1982, ‘Environmental microbiology: biodegradation’, Philosophical Transactions of the Royal Society of London. B, Biological Sciences, vol. 297, no. 1088, pp. 575–597. doi: 10.1098/rstb.1982.0063.

Qazi, A. et al. 2013, ‘Yeast extract as the most preferable
substrate for optimized biosurfactant production by rhlB gene positive pseudomonas putida SOL-10 isolate’, Journal of Bioremediation & Biodegradation, vol. 04, no. 07. doi: 10.4172/2155-6199.1000204.

Rosenberg, M 1991, ‘Basic and applied aspects of microbial adhesion at the hydrocarbon: Water interface’, Critical Reviews in Microbiology, vol. 18, no. 2, pp. 159–173. doi: 10.3109/10408419109113512.

Sharuddin, SSN et al. 2021, ‘Potential bifunctional rhizobacteria from crude oil sludge for hydrocarbon degradation and biosurfactant production’, Process Safety and Environmental Protection, Elsevier, vol. 155, pp. 108–121. doi: 10.1016/j.psep.2021.09.013.

Shi, K et al. 2019, ‘Mechanism of degrading petroleum hydrocarbons by compound marine petroleum-degrading bacteria: surface adsorption, cell uptake, and biodegradation’, Energy and Fuels, vol. 33, no. 11, pp. 11373–11379. doi: 10.1021/acs.energyfuels.9b02306.

Sobrinho, HB et al. 2014, ‘Biosurfactants: classification, properties, and environmental applications, Biotechnology, vol. 11(January 2014), pp. 1–29.

Varjani, SJ & Upasani, VN 2017, ‘Critical review on biosurfactant analysis, purification, and characterization using rhamnolipid as a model biosurfactant’, Bioresource Technology, Elsevier Ltd, vol. 232, pp. 389–397. doi: 10.1016/j.biortech.2017.02.047.

Zwietering, MH et al. 1990, ‘Modeling of the bacterial growth curve’, Applied and Environmental Microbiology, vol. 56, no. 6, pp. 1875–1881. doi: 10.1128/aem.56.6.1875-1881.1990.