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Reduction of Surface Tension of Petroleum Using Hydrocarbon Degrading Bacterial Activity

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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, multi-function, ecologically friendly, and natural availability (Ławniczak *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

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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).

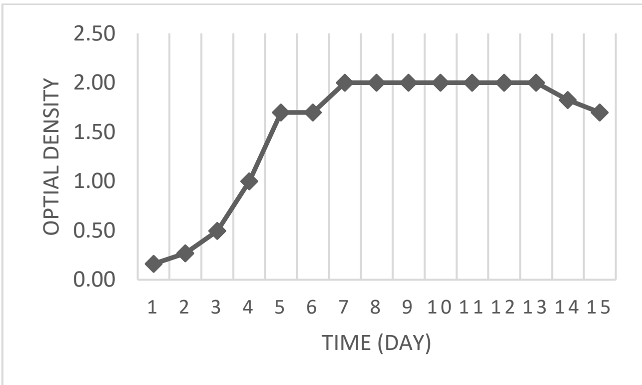

Figure 2. Growth curve of HDBp bacteria

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 antibiofilm agents because of their ability to inhibit pathogenic bacteria (Fakruddin, 2012; Kumar *et al.*, 2021).

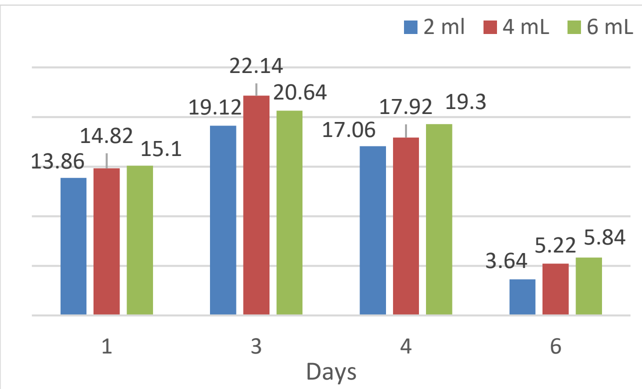

Figure 3. Reducing surface tension

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.

IV. CONCLUSION

Hydrocarbon-degrading bacteria have been isolated using ASM + 1% petroleum media. The turbidity of the media is an indicator of the growth of hydrocarbon-degrading bacteria. Biosurfactant production was most effective on the third day of culture age (exponential phase). The bacteria can also produce biosurfactants that lower the maximum surface tension in the exponential phase with a total surface tension reduction of 22.14 mN/m. These results show the potential of bacterial isolates from the port of paotere, Indonesia, as biodegradation agents and biosurfactant production.

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 methods 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.

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