CHARACTERISATION OF BIOSURFACTANT PRODUCED BY HYDROCARBON-DEGRADING BACTERIA RESISTANT TO CADMIUM

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ABTRACT

Biosurfactant produced from bacteria is an alternative approach to bioremediate crude oil contaminated site which often contained heavy metal. However, there are limited studies about the production of biosurfactant in the presence of both hydrocarbon and heavy metal. Hence, the objective of this experiment was to study the effect of Cadmium (Cd) on biosurfactant production by bacteria exposed to crude oil hydrocarbon; and to characterise the biosurfactant in order to better understand its function when bacterial cells are exposed to crude oil and Cd. Three bacteria isolates (A, B and C) with known crude oil degradation activity were subjected to Cd toxicity test at 0.1 mg/L. Isolate A showed the highest growth ($p<0.05$) in the presence of Cd, thus most resistant to Cd, among the three tested isolates. Gram staining result showed that isolate A was Gram positive. The bacterial cells gave a positive absorbance reading of 0.99 ± 0.243 at OD625 nm for methylene blue assay and 58.4 % of cell adherence to crude oil in Bacterial Adhesion to Hydrocarbons (BATH) assay, suggesting hydrophobic cell surfaces due to potential secretion of biosurfactant. Biosurfactant production by isolate A in Bushnell-Haas media using crude oil as sole carbon source exposed to 0.1 mg/L Cd showed significant increased ($p<0.05$) of 29.6 % biosurfactant mass in dry weight compared to the control without Cd. Based on the Emulsifying Index (E24) study, the biosurfactant was able to formed the highest ($p<0.05$) emulsion layer with benzene (35.8 ± 1.43 %) followed by both hexane (5.0 ± 0.0 %) and crude oil (8.3 ± 3.36 %), at room temperature, thus showing good emulsion activities. However, the isolated biosurfactant was not thermal stable as it did not retained the hydrocarbon-emulsifying activity when exposed to temperature above 60°C. The isolated biosurfactant tested positive for the presence of sugar moiety using Dubois assay, with absorbance reading of 0.44 ± 0.032 at OD490 nm, but negative for protein using Biuret test. This suggested the biosurfactant produced by isolate A is from the glycolipid family. The presence of Cd significantly increased the secretion of biosurfactant suggested that isolate A employed the mechanism of biosynthesizing and secreting glycolipid biosurfactant to counter the toxicity due to Cd.

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LIST OF ABBREVIATION

BATH	Bacterial Adhesion to Hydrocarbons
BH	Bushnell-Haas
˚C	Celsius
Cd	Cadmium
$CdCl_2$	Cadmium Chloride
CFU/mL	Colony-Forming Units Per Milliliter
$CuSO_4$	Copper (II) Sulfate
EPA	Environmental Protection Agency
E24	Emulsifying Index
g/L	Grams Per Liter
h	Hour
L	Litre
M	Molarity
mg/L	Milligrams Per Liter
mins	Minutes
mL	Millilitre
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
nm	Nanometers
OD	Optical Density
PAH	Polycyclic Aromatic Hydrocarbon
ppm	Parts Per Million
ROS	Reactive Oxygen Species
rpm	Rounds Per Minute
SDS	Sodium Dodecyl Sulfate
TAE	Tris-Acetate-EDTA
v/v	Volume To Volume
w/v	Weight To Volume

CHAPTER 1

INTRODUCTION

One of the most important biological variables that determines the biodegradation of hydrocarbon compounds by microbes is the bioavailability of the hydrophobic components (substrates) to bacterial cells. This specifically refers to the ability of hydrophobic hydrocarbons to be solubilised and transported into the immediate vicinity of the cells (Aparna et al., 2011). The low water solubility of the majority of crude oil hydrocarbon compounds limit the capacity of microbes, which generally exist in aqueous phases, to have access and degrade these substrates. This 'bioavailability' is the rate-limiting step of metabolizing hydrophobic hydrocarbon during bioremediation (Cohen, 2002).

Microbial community that produces biosurfactants has a distinct advantage of increasing the emulsification of poorly water-soluble hydrocarbons and the bioavailability of such compounds for biodegradation (Cohen, 2002). Biosurfactants are usually found as extracellular products or as part of the cell membrane component (Sivakami, Saravana, & Premikishore, 2015). A variety of bacteria, yeast and fungi produced biosurfactant, which are amphiphilic in nature, consisting of hydrophobic and hydrophilic group (Das, & Chandran, 2011; Hamzah, Sabturani, & Radiman, 2013). The hydrophilic group consists of sugar or proteins whereas the hydrophobic group consists of fatty acid (Hamzah et al., 2013). Due to its amphiphilic properties, it can change the surface tension of the surrounding environment such as oil and water, allowing the two phases to mix together (Aparna, Srinikethan, & Smitha, 2011).

However, there are limited studies about the production of biosurfactant in the presence of both hydrocarbon and heavy metal. This is important because most crude oil contaminated sites contained mix pollutants, particularly heavy metals, which affects the efficiency of the microorganisms to biodegrade the hydrocarbons (Mielke et al. 2004). Whether the presence of heavy metals will enhance or reduce biosurfactant production, is yet to be verified.

Hence, the objective of this experiment is to study the effect of cadmium (Cd) on biosurfactant production by bacteria exposed to crude oil hydrocarbon; and to characterise the biosurfactant in order to better understand its function when bacterial cells are exposed to crude oil and Cd.

CHAPTER 2

LITERATURE REVIEW

2.1 CRUDE OIL

Crude oil exist naturally and served as raw materials for the production of gasoline, plastic, lubricant and industrial solvent (As'ad, Yeneneh, & Obanijesu, 2015). The principal element in crude oil is hydrocarbon which contribute to 90 % of its weight and the remaining 10 % are made up of sulphur, oxygen and traces amount of heavy metals (Salleh, Ghazali, Rahman, & Basri, 2003). Chrimas, Lima, and Menechini (2012) found the hydrocarbon present in the crude oil can be classified into 3 main group: aliphatic, asphaltenes, and aromatics as shown in Figure 2.1. The unique structure of hydrocarbon made them difficult to be degraded and remain persistent in the environment (Seker, Arakawa, Sekiguchi, & Ono, 2005). Due to the high structure complexity, some groups of hydrocarbons, particularly aromatics, are reported to exert high toxicity to living organisms when found in the environment (Das & Chandran, 2011).

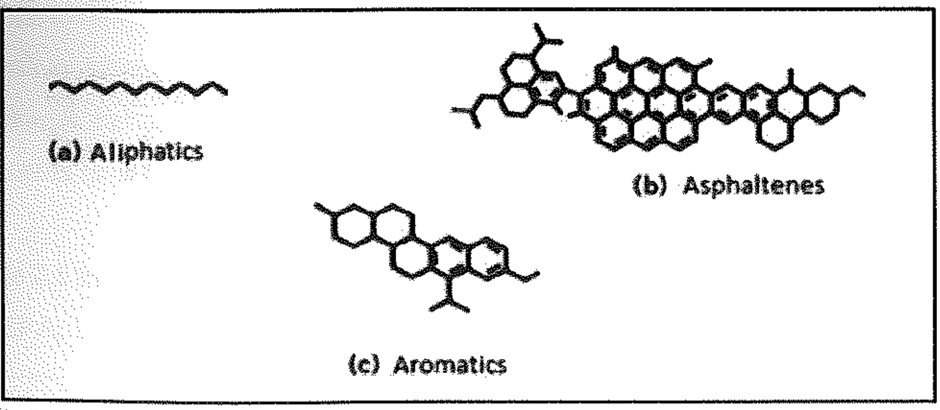

Figure 2.1 Schematic view of hydrocarbon for a) aliphatic b) asphaltenes c) aromatics (Chrimas et al., 2012).

Contamination of crude oil posed a serious hazardous risk towards aquatic life and the environment security. Some of the effect of crude oil contamination on ecological system are enumerated as follow:

1. Large area of oil spills that spread on the surface of aquatic environment can create an anaerobic environment below the water surface, resulting in the death of natural fauna and flora (Kimes, Callaghan, Suflita, & Morris, 2014).
2. Reproductive impairment in fish, bird and reptile (ITOPF, 2016).
3. Polycyclic aromatic hydrocarbons (PAHs), one of the major constituents found in the crude oil are carcinogenic (Yu, 2002; Rengarajan, Rajendran, Nandakumar, Lokeshmar, Rahendran, & Nishigaki, 2015).
4. PAHs induce the formation of Reactive Oxygen Species (ROS) which can cause tissue damage, leading to fatality (Fu, Xia, Hwang, Ray, & Yu, 2014).

2.2 CADMIUM IN HYDROCARBON EFFLUENT

Heavy metals are frequently found in the crude oil contamination sites (Mielke, Wang, Gonzales, Powell, Le, & Quach, 2004). According to United States Environmental Protection Agency (USEPA) (1998), different hydrocarbon-based industries such natural gas, petrochemical processing, and petroleum refining discharge were found to contain heavy metal, predominantly Cd as shown in Table 2.1. Due to the complexity of hydrocarbon-related industrial effluent co-contaminated by Cd, the treatment process of such wastewater before its release to the environment is most desirable (Olaniran, Adhika, & Balakrishna, 2013). One of the current technique adopted to treat mixed effluent is bioremediation (Bestawy, Hejin, Amer, & Kashmeri, 2014).

Table 2.1 Industrial fields with mixed pollutant containing cadmium hydrocarbons and (USEPA, 1998).

Industry Fields	Pollutant
Steel and Iron	Hydrocarbon oil, phenols, cadmium, acids,
Petrochemicals and refineries	Mineral oils, cadmium, phenols
Aircraft plating, finishing	Cadmium, hydrocarbon oil,
Mining	Acids, phenol, metals,

2.3 BIOREMEDIATION

Bioremediation is a process which uses microorganism to remove contaminants from the environment (USEPA, 2012). Numerous studies reported on the varieties of bacteria possessing the hydrocarbon degradation ability (Adams, Fufeyin, Okoro, & Ehinomen, 2015). Table 2.2 summarises some of the bioremediation strategies adopted to degrade crude oil waste.

Table 2.2 Utilisation of different bacteria to biodegrade crude oil hydrocarbons.

Type of Contaminant	Microorganism	Source of microorganisms	Reference
Crude Oil	*Acinetobacter baumannii* T30C	Soil contaminated by Tapis crude oil	Chang & Ibrahim, (2011)
Crude Oil	*Serratia* Sp BF40	Soil high in salinity and contaminated by crude oil	Wu, Xie, Li, & Yang, 2012
Crude oil from parking trucks, human activities and diesel	*Aspergillus terreus, Aspergillus carneus, Bacillus cereus, Bacillus subtilis, Gordonia rubripertincta, Kocuria rosea,*	Topical soil from crude oil contaminated sites	Díaz-Ramírez, Escalante-Espinosa, Schroeder, Fócil-Monterrubio, & Ramírez-Saad, 2013
Diesel from tank top accident	*Pseudomonas fluorescens*	Agricultural soil contaminated with diesel	Kuráň, Trögl, Nováková, & Dáňová,2014