SCREENING OF COPPER-RESISTING HYDROCARBON DEGRADING BACTERIA AND BIOSURFACTANT PRODUCTION

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ABSTRACT

Four bacterial isolates that were known to degrade hydrocarbons were screened for copper (Cu) resistance and biosurfactant production. Based on spectrophotometry, isolate D had the highest growth ($p<0.05$) followed by isolate A, isolate C and isolate B with the least growth. Biosurfactant screening assays confirmed that both isolates D and A produced biosurfactant. The dry-weight of biosurfactant obtained from partial purification suggested that there was a direct relationship between the number of cells present and the amount of biosurfactant produced. Gram staining showed that isolate D and isolate A are gram negative and gram positive respectively. The results showed that gram negative bacteria was able to show tolerance towards 20.0 mg/L Cu, possibly by secretion of biosurfactant to chelate Cu ions in reducing the metal toxicity.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATION

ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BATH	Bacterial Adhesion to Hydrocarbon
Ca	Calcium
CFU/mL	Colony-forming units per milliliter
Cu	Copper
$CuSO_4$	Copper Sulfate
EPS	Extracellular polymeric substances
L	Liter
LPS	Lipopolysaccharide
Mg	Magnesium
mg/L	Milligrams per liter
mL	Milliliters
nm	nanometers
OD	Optical density
PAH	Polycyclic aromatic hydrocarbon
ROS	Reactive oxygen species
rpm	Rounds per minute
T	Time
UV	Ultra Violet

CHAPTER 1

INTRODUCTION

Economic development has seen an increase in crude oil extraction and its application including fuelling vehicles, raw materials in numerous industries such as coke, metallurgy, cement and thermal power engineering (Rachwał, Magiera & Wawer, 2015). Eventhough this unavoidably contaminated the environment. Oil spills from tankers and non-tankers were identified as the major sources of crude oil release into the environment (ITOPF, 2016).

In recent years however, non-tanker spills which consist of industrial effluent has seen an increase in incidents over the past 45 years based on a report published by ITOPF in 2016. This report is significant because industrial effluents originated from hydrocarbon-based industries, contain additional pollutant such as heavy metals (Pérez, Cabrera, Gómez, Ábalos, & Cantero, 2010). In addition to this, a number of studies have also described that pollution of heavy metals occur in the soil of oil fields and industries. Carls, Fenn & Chaffey (1995) found that out of 18 of oil and gas drilling and production sites in their studies, 16 had elevated levels of heavy metals and petroleum hydrocarbons. Oil pollution was also reported accountable for increased levels of heavy metals in oil-contaminated soils (Fu, Cui, & Zang, 2014).

Bioremediation is gaining attention over conventional physicochemical techniques, as the favoured approach to clean up crude oil hydrocarbon contaminant because it is non-carcinogenic, and environmentally friendly (Scragg, 2005). Most importantly, bacteria are able to use crude oil as carbon source and degrading it completely to carbon dioxide and water (Hamer, 1997), as reported in numerous studies showing the success of microbial populations to degrade crude oil (Hamzah, Rabu, Azmy, & Yussoff, 2010; Sakthi Priya, Doble, & Sangwai, 2015; Sanil Kumar, Nair, Salas, Prashob Peter, & Ratheesh Kumar, 2016; Wong, Hamzah, Phan, & Abu Bakar, 2013).

Despite the numerous success reported for bioremediation, there are studies that have shown poor degradation results using microbes, mostly due to the presence heavy metals (Olaniran, Balgobind, & Pillay, 2013; Sandrin & Maier, 2003; Sokhn, De Leij, Hart, & Lynch, 2001). Heavy metals, including Cu exerts toxicity towards microbes by directly associating with microbial enzymes that are involved in metabolism or indirectly disrupt the microbial cells' plasma membrane, and thus impairing the metabolism and growth of microbial cells (Nies, 1999). This decreases the efficiency of bacteria to degrade crude oil.

To date, not many studies have reported on microbes that are Cu-resistant and degrades hydrocarbon. Thus, this research focused on:

i. Screening for Cu-resistant microbes among bacteria that have already been known to degrade crude oil
ii. Screening for biosurfactant production to suggest that it was part of Cu resistance mechanism employed by the best Cu-resistant isolates.

CHAPTER 2

LITERATURE REVIEW

2.1 CRUDE OIL CONTAMINATION

Crude oil, also known as petroleum is a composite mixture of hydrocarbons; aromatic, aliphatic and alicyclic and non-hydrocarbon compounds; oxygen, nitrogen, sulfur, and traces of metals (TOXNET NIH, 2016). Due to its extensive consumption in various industries, exploitation and transportation of crude oil largely contributed to crude oil contamination in soil and water (Coleman et al., 2003).

In 2015, the estimated world consumption of petroleum and other liquid fuels were 93.81 million barrels per day, an increment of 2.0% compared to 2014 (Eia.gov, 2016). Currently, in the first quarter of 2016, world consumption of petroleum has already exceeded the total consumption of petroleum in 2015, recording a total consumption of 94.22 million barrels per day, an increment of 0.4%. The U.S Energy Information Administration estimated that the worldwide consumption of petroleum and other liquid fuels will grow by 2.7% in 2017. Judging from the data projection of crude oil consumption increasing from year to year, it is unavoidable that oil spills incident will increase accordingly to sustain the need for crude oil hydrocarbons.

2.2 TOXICITY OF CRUDE OIL

Crude oil exerts its toxicity mainly due to the presence of polycyclic aromatic hydrocarbons (PAHs), which has been reported to be hazardous to living biota (Olsen et al., 2013). PAHs have been found to compromise the functionality of bacterial plasma membranes (Cerik et al., 2003). Accumulation of PAHs in the membrane lipids (phosphatidylcholine and phosphatidylethanolamine) of bacteria caused saturation of membrane fatty acids, which negatively affects the membranes fluidity and permeability (Salar et al., 2014). The membrane is a barrier that protects the cytoplasmic region of the cell where many important cell activities occur inside the cytoplasm and within the membrane itself. The accumulations of PAHs thus significantly disrupts the physiological functions of cells. It was also revealed that PAHs at low concentration (6 μg/L) can disrupt the specialized ion channel pores of the cardiac muscle cells of fish, leading to impaired heart beat (Block et al., 2014).

A case study conducted on marine biota in Alaska after the disastrous Exxon Valdez oil spill in 1989 showed bioaccumulation of PAHs in clams causing retarded growth, six years after the oil spill incident (Fukuyama, Shigenaka, & Hoff, 2000). This highlighted the toxic effect of crude oil persisting in the environment for many years. Table 1 summarises some of the deleterious effect of hydrocarbons on biota. Thus, effective strategies to remove crude oil hydrocarbons from the contaminated environment becomes an important agenda.

Table 1 Summary of the toxic effects of hydrocarbons on living organisms

Organism	Hydrocarbons	Toxic Effect	References
Zooplankton	Benzene , pyrene	Mortality and impeded reproductive abilities	Olsen et al., 2013
Human	Benzene	Reduce in red blood cells count (haematoxicity)	Koh, Jeon, Lee & Ryu, 2015
	PAH	Increased mortality among ovarian cancer patients	García-Pérez et al., 2015
Fish	PAH	Disrupted specialized ion channel pores of the cardiac muscle cells of fish, leading to impaired heart beat	Block et al., 2014

2.3 BIOREMEDIATION OF CRUDE OIL

Owing to the huge amount of chemicals used in the physicochemical methods to cleanup crude oil hydrocarbons, which further pollutes the environment (Contreras et al., 2008), the alternative method of using microbes for environmental cleaning up has thus been intensively studied. Bioremediation of crude oil using microbes to degrade hydrocarbons have been widely reported as shown in Table 2. Although bioremediation are widely applied in real environmetal scenarios, the success varies according to various reports. Recent studies by Wong et al. (2013) suggested that the presence of heavy metals could be one of the main factors contributing to the inconsistent results reported. In the report, cadmium at 0.1 mg/L significantly inhibit crude oil degradation by 25% in mixed bacterial population.

Table 2 Biodegradation of crude oil hydrocarbons using bacteria

Hydrocarbon	Microorganisms added	Initial total petroleum hydrocarbon (TPH) (mg/kg)	TPH reduction (%)	References
Motor oil	*Bacillus* sp., *Pseudomonas* sp.	40 000	65%	Abdulsalam et al.,2009
Diesel	*Rhodococcus* sp. EH 831	30 000	>50%	Eun Hee et al., 2011
Crude oil	*Acinetobacter albaumannii* T30 C	4200	43%	Chang et al., 2011
Diesel TPH	*Candida* SK 21	16 300	83%	Fan at al., 2013
Diesel	*Pseudomonas fluorescence*	>5000	>60%	Kuran et al., 2014

Source: Adapted from Adams, Fufeyin, Okoro & Ehinomen (2015)