EVALUATION OF *Bacillus licheniformis* STRAINS AND THEIR DOMINANT TYPE OF OIL RECOVERY MECHANISMS UNDER HYDROCARBON-RICH CONDITIONS

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ABSTRACT

Selection of potential bacteria and identification of their type of oil recovery mechanisms are keys to *in-situ* Microbial Enhanced Oil Recovery (MEOR) process. However, bacterial survivability, adaptability and functionality under oil reservoir environment has never been evaluated as a complete set for this process. As a consequence, mechanism of the potential bacteria to assist oil recovery became unpredictable and their performance are inconsistent under oil reservoir condition. Therefore, this study aimed to evaluate selected bacteria as MEOR agents based on their survivability, adaptability and functionality in hydrocarbon-rich conditions. The study was executed in the hydrocarbon-rich conditions to emulate the hydrocarbon contents of oil reservoir. Conducted analyses in batch experiments involved: 1) analysis of bacterial growth; 2) bacterial cell physiology and behaviour characterization; 3) *in-situ* monitoring of biofilm/ biofloc/ biosurfactant formation and 4) substrate utilization. The substrates were selected from different types of polycyclic aromatic hydrocarbons (PAHs: naphthalene and pyrene) with different concentrations (0.1 and 10 g/L). In addition, flow experiments utilising paraffin oil in porous media (micromodel and glass-bead packed column) were conducted with the purpose of validating the function of potential bacteria as MEOR agent in bacterial flooding test. Results from the experiments were statistically examined with single factorial, general and two-level (2^k) factorial design. Three strains of *Bacillus licheniformis* coded M1, Ta62bi and P6 were selected as potential MEOR agents. Results from batch experiments showed that strains M1 and Ta62bi acted as plugging agent whereas strain P6 functioned as emulsification-like agent in pyrene-rich medium. However, the flow experiments revealed only strains M1 and P6 consistently showed features similar to findings from batch experiments. The plugging effects of strain Ta62bi was most probably due to production of gas and not formation of biofilm. Nevertheless, these strains exhibited average oil recovery efficiency (%) of 31.2 ± 7.0 (Ta62bi), 34.8 ± 3.4 (M1) and 36.0 ± 5.7 (P6) from the remaining oil in column study. In conclusion, these selected bacteria were able to recover residual oil, but through different types of oil recovery mechanisms. Findings from this study have contributed to better understanding of bacterial application and to improving the evaluation strategy of potential bacteria selection for *in-situ* MEOR process.

ABSTRAK

Pemilihan bakteria berpotensi dan pengenalpastian mekanisma pungutan minyak pada skala makmal adalah kunci kepada proses Pungutan Minyak Berasaskan Mikrob (MEOR) secara *in-situ*. Walau bagaimanapun, daya hidup, penyesuaian dan kefungsian bakteria dalam keadaan telaga minyak tidak pernah dinilai sepenuhnya untuk proses ini. Akibatnya, mekanisma bakteria berpotensi dalam membantu pungutan minyak masih sukar dijangka dan prestasi mereka tidak tekal pada keadaan telaga minyak. Oleh itu, kajian ini bertujuan untuk menilai potensi bakteria sebagai agen MEOR berdasarkan kebolehan untuk terus hidup, menyesuaikan diri dan fungsi mereka pada keadaan hidrokarbon tinggi. Kajian ini dijalankan dalam keadaan hidrokarbon tinggi untuk meniru kandungan hidrokarbon telaga minyak. Analisis yang dijalankan dalam eksperimen sesekelompok melibatkan: 1) analisa pertumbuhan bakteria: 2) ciri-ciri fisiologi dan perilaku sel bakteria: 3) pemantauan pembentukkan biofilm/ bioflok/ biosurfaktan secara *in-situ* dan 4) penggunaan substrat. Substrat dipilih daripada dua jenis polisiklik aromatik hidrokarbons (PAHs: naftalin dan pyrene) dan berkepekatan yang berbeza (0.1 dan 10 g/L (w/v)). Eksperimen aliran pula menggunakan minyak parafin dalam media berliang (mikromodel dan turus padat bermanik kaca) yang bertujuan untuk mengesahkan fungsi bakteria yang berpotensi sebagai agen MEOR dalam ujian pembanjiran bakteria. Hasil kajian daripada eksperimen-eksperimen tersebut dianalisa secara statistik menggunakan reka bentuk faktor tunggal, umum dan dua-aras. Tiga strain *Bacillus licheniformis* berkod M1, Ta62bi and P6 telah dipilih sebagai agen MEOR yang berpotensi. Dapatan daripada eksperimen sesekelompok menunjukkan strain M1 dan Ta62bi sebagai agen plak manakala strain P6 berfungsi seperti agen emulsifikasi dalam medium kaya pyrene. Walaubagaimanapun eksperimen aliran membuktikan hanya strain M1 dan P6 secara konsisten menunjukkan ciri-ciri yang sama dengan dapatan daripada eksperimen sesekelompok. Manakala, kesan plak pada strain Ta62bi kemungkinan besar disebabkan oleh penghasilan gas dan bukan pembentukan biofilm. Sekurang-kurangnya, strain-strain ini menunjukkan purata kecekapan peningkatan (%) 31.2 ± 7.0 (Ta62bi), 34.8 ± 3.4 (M1) dan 36.0 ± 5.7 (P6) baki minyak terperangkap dalam kajian turus. Kesimpulannya, bakteria yang terpilih ini berkebolehan untuk memungut baki minyak terperangkap, tetapi menggunakan jenis mekanisma pemungutan minyak yang berlainan. Hasil kajian telah menyumbang kepada pemahaman aplikasi bakteria yang lebih baik dan menambahbaikkan strategi penilaian untuk pemilihan bakteria yang berpotensi dalam proses MEOR secara *in-situ.*

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LIST OF ABBREVIATIONS

AOR	-	Additional oil recovery
EOR	-	Enhanced Oil Recovery
MEOR	-	Microbial Enhanced Oil Recovery
EPS	-	Exopolysaccharides
CLSM	-	Confocal Laser Scanning Microscope
FESEM	-	Field Emission Scanning Microscope
IOR	-	Improved Oil Recovery
HPLC	-	High Performance Liquid Chromatography

LIST OF SYMBOLS

et al.	-	And others
sp.	-	Species
L	-	Liter
M	-	Molar
g/L	-	Gram per litre
mL	-	Mililitre
µm	-	Micrometre
°C	-	Degree of celcius
OD	-	Optical density
rpm	-	Rotation per minutes
w/v	-	Weight per volume
v/v	-	Volume per volume
Min	-	Minute (s)
h	-	Hour (s)
µ (h^{-1})	-	Specific growth rate (per hour)
∅	-	Porosity
V pore	-	Pore volume (PV)
V bulk	-	Bulk volume
r	-	Radius
h	-	High
K	-	Permeability (Darcy)
µ (cp)	-	Viscosity of fluid (centipoise)
L (cm)	-	Length (centimetre)
Q (cm^3/s)	-	Flow rate (cubic centimetres per second)
Δρ (atm)	-	Pressure different (atmospheres)
A (cm^2)	-	Cross sectional area (square centimetre)
Ki/Ko	-	Permeability ratio
Swi	-	Initial water saturation
Soi	-	Initial oil saturation
Sorwf	-	Residual oil saturation after water flooding

Sormt	-	Residual oil saturation after microbial treatment
Er	-	Oil recovery efficiency
NH_4NO_3	-	Ammonium nitrate
$CaCl_2$	-	Calcium chloride
$FeCl_3$	-	Ferric (III) Chloride
K_2HPO_4	-	Dipotassium phosphate
KH_2PO_4	-	Potassium diphosphate
$MgSO_4$	-	Magnesium sulphate
H_2SO_4	-	Sulphuric acid
NaOH	-	Sodium Hydroxide

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CHAPTER 1

INTRODUCTION

1.1 Research background

Microbial Enhanced Oil Recovery (MEOR) is one of bioprocess technology in petroleum industry for oil exploration and production (Singh *et al.,* 2012; Bachmann *et al.,* 2014). The technology involves stimulating indigenous reservoir microbes or injecting specially selected consortia of natural bacteria into the reservoir to produce specific metabolic events that lead to improved oil recovery (Sen, 2008). There are two ways where bacteria can be applied for oil recovery known as *ex-situ* and *in-situ* processes. Both processes involve bacterial metabolites such as surfactants, polysaccharides and other products that facilitate oil recovery. However, the *ex-situ* MEOR process requires more intensive capital and labour compared to *in-situ* MEOR process due to the dependency of the former process to the conventional fermentation techniques for the production of bacterial metabolites that were then are injected into the reservoir (Sarkar *et al.,* 1989; Volk and Hendry, 2010).

Alternatively, *in-situ* MEOR process is less expensive and allows flexible production conditions. The process involves direct use of nutrients and/ or bacteria that are injected either sequentially or simultaneously into the reservoir (Lazar *et al.,* 2007; Banat *et al.,* 2010; Gudiña *et al.,* 2012b). The application of *in-situ* MEOR is dependent on the certain activities of living microorganisms that gave effect to the reservoir environment and flow properties of oil, which facilitate the oil's transport (Sarkar *et al.,* 1989; Sen 2008; Halim *et al.,* 2017).

The physiological and behavioural properties of the bacteria play major role in affecting growth, metabolism and metabolite production within porous rock. Beneficial metabolites such as biofilms/ biosurfactants/ biopolymer could assist oil recovery particularly for problematic reservoir with heterogenous permeability issue.

Permeability of a reservoir relates the ability to transmit a particular fluid such as water though rock when other immiscible fluids such as oil are present in the reservoir. However, when the injected water is preferentially flows through high permeablity layers, residual oil remains unrecovered and trapped at the low permeablity layers, (Youssef *et al.,* 2009; Karambeigi *et al.,* 2013).

Strategies used to remedy the rock permeability problems have included the use of biopolymer and bacterial biomass (Lazar *et al.,* 2007). The biopolymer could increase the viscosity of flood water or act as biosurfactants which would decrease the interfacial tension between oil and water. As a result, it could increase the capillary number and improve oil recovery (Patel *et al.,* 2015). On the other hand, bacterial biomass (bacterial cells) able to form plugs, that would potentially aid in diversion of fluids into low permeable zones (Karambeigi *et al.,* 2013). This type of oil recovery mechanism is known as selective plugging mechanism or bacterial profile modification (Jeong *et al.,* 2018).

However, the current state of knowledge provides limited proofs and details on the parameters that affect the *in-situ* MEOR processes. Thus, it is impossible to elucidate clearly the function of bacteria and their mechanism in enhancing oil recovery (Maudgalya *et al.,* 2007; Brown, 2010; Rassenfoss, 2011; Head and Gray, 2016). This limitation is contributed from the difficulties to study the microbiological, physical and chemical aspects of the oil reservoir (Kaster *et al.,* 2012; Head and Gray, 2016; Klueglein *et al.,* 2016), thus, leading to the uncertainty and misinterpretation of data in describing the role of microbes in the system (Wolicka and Borkowski, 2012). As a result, the success of *in-situ* MEOR approach is not implicitly sustained and thoroughly studied (Brown, 2010; Head and Gray 2016).

This present study aims to evaluate potential bacteria as MEOR agents under oil reservoir conditions. The evaluation is mainly in selection of potential bacteria and identification of their type of oil recovery mechanism. The gained knowledge may improve understanding of bacterial applications for *in-situ* MEOR process and improvise evaluation strategy particularly at the laboratory scale.

1.2 Problem statement

The *in-situ* MEOR process starts from selection of potential bacteria at the laboratory scale (Gao, 2018). It was then followed by the proposal of bacterial dominant type of oil recovery mechanism based on batch and flow experiments (Gudiña *et al.,* 2013; Halim *et al.,* 2017; Couto *et al.,* 2018). However, the survivability, adaptability and functionality of the potential bacteria have not been fully revealed and proven under oil reservoir environment.

Several case studies have shown that the potential bacteria did survive under high temperature, wide range of pH, salinity concentrations and other extreme given conditions (Suthar *et al,* 2009; Nerurkar *et al.,* 2012; Halim *et al.,* 2017 and Coute *et al.,* 2018). However, the bacterial functionality to assist oil recovery is not completely demonstrated. In fact, only column study is implemented to represent flow experiment. In contrast, other case study have successfully showed the potential of selected bacteria to function as MEOR agent in column and micromodel studies (Lappan and Fogler, 1994; Steward and Fogler, 2001; Gandler *et al.,* 2006; Khan *et al.,* 2008) though the bacteria was not able to survive under extreme conditions.

From the many case studies reported in literatures, it can be concluded that the evaluation of *in-situ* MEOR process is not emphasized on bacterial physiology and behaviour though these properties significantly contribute to the adaptation of bacteria in oil reservoir conditions (Gandler *et al.,* 2006; Khan *et al.,* 2008; Nerurkar *et al.,* 2012; Halim *et al.,* 2017 and Coute *et al.,* 2018). As a consequence, the behaviour of selected bacterial is known to be unpredictable and their performances are inconsistent in assisting oil recovery under oil reservoir condition (Maudgalya *et al.,* 2007; Brown, 2010; Singh *et al.,* 2012). The situation is identified as one of the main reasons why MEOR method is not widely acceptable by the oil industry (Maudgalya *et al.,* 2007; Sen, 2008; Brown, 2010; Al-Sulaimani *et al.,* 2011).

This study was designed as an initial step to develop an understanding for an elucidation of the role of single bacteria in enhance oil recovery. It is hypothesized that oil reservoir conditions would induce the survived cells to activate their adaptation

1.4 Research objectives

Based on the research background and the problem statement, the research objectives for this study are:

(1) To select potential bacteria as Microbial Enhanced Oil Recovery (MEOR) agents based on their survivability and physiological characterizations under hydrocarbon-rich conditions

(2) To identify the dominant type of oil recovery mechanism by the selected bacteria in a chosen type and concentration of hydrocarbon-rich medium

(3) To validate the selected bacterial performance and their dominant type of oil recovery mechanism in bacterial flooding test

1.5 Research scope

This research primarily covers interaction of fluid, geological and biological part of oil reservoir that contribute to the achievement of research objectives. This was an attempt to mimic some of important reservoir condition in order to evaluate potential bacterial growth, physiology and behaviour for *in-situ* MEOR process. The condition were mainly developed by establishing hydrocarbon-rich conditions in batch and flow experiments.

Two phases of fluids used in this study: (1) Hydrocarbon (2) Aqueous phase. The aqueous phase was referring to type of medium used (Bushnell-Hass (BH) broth or brine solution). On the other hand, the hydrocarbon phase was referring to either polycyclic aromatic hydrocarbons (PAHs) or paraffin oil. The PAHs are parts of the aromatic chain in the paraffin oil. Both are constituent of hydrocarbons in oil reservoir (Wolicka *et al.,* 2010).

In this study, the PAHs would represent hydrocarbon models in order to select potential bacteria and to reveal their type of adaptation strategy. The PAHs were varied in term of type (naphthalene and pyrene) and concentrations (0.1 and 10 g/L (w/v)), respectively. Bushnell-Hass broth medium was used as aqueous phase to accompany the given type and concentration of PAH, respectively.

Another type of hydrocarbon was paraffin oil. It was combined with brine solution as aqueous phase for bacterial flooding test. In batch experiments, the brine solution was studied in two concentrations (2 % and 4 % (w/v)), respectively at different temperature (37 °C and 50 °C) in order to assess bacterial growth in paraffin oil. The batch experiments were carried out in flasks and simple flow cell at 37 °C under facultative aerobic condition, except that for the compatibility test in paraffin oil was carried out at 50 °C. The temperature of 37 °C and aeration condition was chosen according to the temperature commonly used in the study (Fareh, 2008 and Zulkefli, 2014) for growth of the selected bacteria. The outcome eventually determine the related parameters for flow experiments.

The flow experiments were carried out at room temperature (25 °C to 30 °C) except at shut-in phase, which was at 37 °C. The experiments were using paraffin oil and applying Cyclic Microbial Recovery (CMR) as a strategy for employed inocula injection in bacterial flooding test. No additional substrate was introduced in the inocula injection since the paraffin oil was acted as hydrocarbon phase as well as substrate.

A few procedures were applied to highlight bacterial cells' ability to taxis during the flow experiments. For instance, bacterial flooding phase and recovery phase was applied at low flow rate (0.33 ml/min). It was considered as approximately similar to liquid movement in oil reservoir (2 fts/day). Thus, the movements of bacterial cells were most probably due to their taxis ability, not by gravity force and given flow rate.

Additionally, heterogeneous permeability condition as geological condition of problematic oil reservoir was established in the apparatus of the flow experiments. The condition was conveyed in the construction of two types of porous media: (1) Glass-

research significance, research objectives and research scope. Finally it was concluded with thesis structure.

Chapter Two aimed to review and analyse strength and weakness of potential bacterial evaluation as MEOR agent. The review was initiated with research background which covered three main recovery phases of oil production. One of the oil production phase was tertiary phase that also known as Enhanced Oil Recovery (EOR). The description of EOR methods led to the research area, which was the *in-situ* MEOR process. Then, this chapter examined strength and weakness of theory, methodology and practice to evaluate potential MEOR agents at the laboratory scale. Besides, this chapter also assessed the impact of hydrocarbon-rich conditions to screen potential bacterial as MEOR agents. In the end, a theoretical framework and a related conceptual framework were constructed in order to formulate research operational framework.

Chapter Three comprised of operational framework and described the overall materials and methods for this study. The proposed research operational framework described in Chapter Three was implemented and discussed comprehensively in Chapter Four until Chapter Six. These chapters started with introduction section of their contents which includes the objective of each chapter. Then, the section was followed by explanations of research methodology that supported by flow of research activities according to the aim of each chapters.

Chapter Four presented selection phase of potential bacteria as MEOR agents under hydrocarbon-rich conditions. Chapter Five proposed dominant type of oil recovery mechanism by the selected bacterial agents in chosen hydrocarbon-rich medium. Chapter Six covered the validation phase of the selected bacterial performance and their dominant type of oil recovery in bacterial flooding test. Chapter Seven concluded the study and identifies the future work of research. The chapter drew the conclusions by describing the research outcomes in relation to the achievement of the research objectives. The chapter then examined the research limitation in term of theory, practice and methodology. Finally, the chapter presented the recommendations for future research.

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