

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

FAREH NUNIZAWATI BT. DAUD @ ABDULLAH

UNIVERSITI TEKNOLOGI MALAYSIA

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

FAREH NUNIZAWATI BT. DAUD @ ABDULLAH

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Doctor of Philosophy (Bioscience)

Faculty of Science
Universiti Teknologi Malaysia

AUGUST 2019

ACKNOWLEDGEMENT

Alhamdulillah, first of all, I deeply indebted to Dr. Adibah bt. Yahya, my main supervisor, through her enormous support and guidance. Alhamdulillah, I also would like to acknowledge my co-supervisors, Prof. Emeritus Dr. Ahmad Kamal b. Idris and Dr. Arifah bt. Bahar whom roles have given me valuable supervision to branch out application of bacteria in Microbial Enhanced Oil Recovery (MEOR) and strengthen the research outcome with Design of Experiments (DOE), respectively.

Alhamdulillah, my thanks also goes to Puan Fatimah bt. Harun, Encik Hairul Akmal b. Jawahir, Puan Zaleha bt. Jaafar, Encik Yusnizam b. Samsudin, Encik Roslan b. Jas and Encik Zulkifle b. Nasir from Department of Biosciences, Faculty of Science and School of Chemical and Energy Engineering, Faculty of Engineering, UTM, for sharing their knowledge and guidance. Alhamdulillah, my next appreciation goes to UTM-ZAMALAH for strong financial support throughout my undertakings.

Alhamdulillah, thank you, my friends and colleagues: Dr. Nur Azaliah bt. Abu Bakar, Dr. Siti Nor Zawani bt. Ahmmad, Dr. Norzieana bt. Khairuddin, Dr. Munirah bt. Tharek, Dr. Syarifah Saidah bt. Syed Omar, Puan Lily Suhana Ayoub, Puan Maznah bt. Ismail, Puan Izni Izzati bt. Mohamad, Puan Noor Laily bt. Mohamad, Encik Hishamuddin b. Wahab, Encik Mohd. Fahmi b. Muhammad, Dr. Hanif b. Md. Nor, Dr. Roslan b. Ikubar, Dr. Lam Chi Yong, Dr. Nur Balqis bt. Azman, Dr. Ivy Bay, Encik Zulkefflizan bin Jamaludin, Puan Sri Yusrina bt. Saliken and all postgraduates students from Department of Biosciences, Faculty of Science. Every single of you is very important in giving colours to my PhD journey in varies ways.

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 bakteri 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 kefungsiannya 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 pembentukan 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 pembersihan 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*.

TABLE OF CONTENTS

	TITLE	PAGE
	DECLARATION	ii
	DEDICATION	iii
	ACKNOWLEDGEMENT	iv
	ABSTRACT	v
	ABSTRAK	vi
	TABLE OF CONTENTS	vii
	LIST OF TABLES	xiii
	LIST OF FIGURES	xv
	LIST OF ABBREVIATIONS	xxi
	LIST OF SYMBOLS	xxii
	LIST OF APPENDICES	xxiv
CHAPTER 1	INTRODUCTION	1
1.1	Research background	1
1.2	Problem statement	3
1.3	Research significance	4
1.4	Research objectives	5
1.5	Research scope	5
1.6	Thesis organization	7
CHAPTER 2	LITERATURE REVIEW	9
2.1	Introduction	9
2.2	Microbial Enhanced Oil Recovery (MEOR) as alternative to other Enhanced Oil Recovery (EOR) methods	9
2.2.1	Microbial Enhanced Oil Recovery (MEOR) as bioprocess technology in petroleum industry	12

2.3	Basic concepts of <i>in-situ</i> Microbial Enhanced Oil Recovery (MEOR) process	14
2.3.1	Type of injection strategy for <i>in-situ</i> Microbial Enhanced Oil Recovery (MEOR) process	17
2.4	Evaluation of potential bacteria for <i>in-situ</i> MEOR process	20
2.4.1	Strength and weakness in evaluation of potential bacteria for <i>in-situ</i> MEOR process at the laboratory scale	21
2.5	Impact of hydrocarbon content on screening of potential bacteria as MEOR agents	25
2.5.1	Polycyclic aromatic hydrocarbons (PAHs) as bacterial substrate	25
2.5.2	Bioavailability process for hydrocarbon uptake	28
2.5.3	Bacterial adaptation strategies under hydrocarbon-rich conditions	30
2.6	Concluding remarks	37
CHAPTER 3	MATERIALS AND METHODS	43
3.1	Introduction	43
3.2	Bacteria for experiments	45
3.3	Bacterial maintenance, storage and inocula preparations	46
3.3.1	Bacterial maintenance and storage	47
3.3.2	Inoculum preparation in nutrient broth (NB) medium	47
3.3.3	Inoculum preparation in Bushnell-Haas (BH) broth medium	48
3.3.4	Inoculum preparation in brine solution	49
3.4	Media preparation	50
3.4.1	Nutrient broth (NB) and nutrient agar (NA) medium	50
3.4.2	Bushnell-Hass (BH) broth medium	51
3.4.3	Bushnell-Hass (BH) broth medium containing polycyclic aromatic hydrocarbon (PAH)	51
3.4.4	Brine solution containing paraffin oil	52

3.5	Stock solution preparation	52
3.5.1	Polycyclic aromatic hydrocarbons (PAHs) in solution	53
3.5.2	Paraffin oil	53
3.5.3	Dyed paraffin oil	53
3.5.4	Brine solution	53
3.5.5	Tris-borate-EDTA (TBE) buffer	54
3.5.6	Phenol solution	54
3.6	Molecular identification of bacteria	54
3.6.1	Chromosomal DNA extraction	55
3.6.2	Polymerase Chain Reaction (PCR) amplification of 16S rRNA gene sequences	55
3.6.3	Purification of PCR amplification of 16S rRNA gene sequences	56
3.6.4	Agarose gel DNA analyses	57
3.6.5	Sequence analyses and phylogenetic tree assembly	57
3.7	Analytical methods	58
3.7.1	Determination of bacterial growth	58
3.7.2	Cell hydrophobicity	59
3.7.3	Cell autoaggregation	60
3.7.4	Exopolysaccharides (EPS) assay	60
3.7.5	Emulsification index (E ₂₄)	61
3.7.6	Modified capillary chemotaxis analysis	61
3.7.7	Quantitative analysis of pyrene	63
	3.7.7.1 Determination of pyrene consumption	64
3.8	Microscopy analyses	64
3.8.1	Field Emission Scanning Electron Microscope (FESEM)	65
3.8.2	Phase contrast inverted microscope	65
3.8.3	Confocal Laser Scanning Microscope (CLSM)	66
3.9	<i>In-situ</i> monitoring of bacterial growth and adaptation strategy using simple flow cell	67

3.10	Validation of selected bacterial performance and their dominant type of oil recovery mechanisms using saturated porous media	68
3.10.1	Glass-bead packed column construction	69
3.10.2	Bacterial flooding test using glass-bead packed column	70
3.10.3	Micromodel construction	73
3.10.4	Bacterial flooding test using micromodel	74
3.11	Statistical analyses	75

CHAPTER 4	SELECTION OF POTENTIAL BACTERIA AS MICROBIAL ENHANCED OIL RECOVERY AGENT BY MEANS OF SURVIVABILITY AND PHYSIOLOGICAL CHARACTERISTICS UNDER HYDROCARBON-RICH CONDITIONS	79
4.1	Introduction	79
4.2	Research methodology	80
4.2.1	Culture maintenance	81
4.2.2	Inoculum preparation	81
4.2.3	Analyses for bacterial survivability and physiological characterizations under hydrocarbon-rich conditions	82
4.3	Results and discussion	83
4.3.1	The effects of naphthalene and pyrene amendment on growth and survival of eight bacterial strains	83
4.3.2	The effects of naphthalene and pyrene, respectively on the physiological characteristics of five survived bacteria	98
4.3.3	Selection of potential bacteria and hydrocarbon model for <i>in-situ</i> MEOR study	104
4.3.4	Molecular identification of three potential bacteria as Microbial Enhanced Oil Recovery (MEOR) agents	108
4.4	Conclusion	111

CHAPTER 5	IDENTIFICATION OF DOMINANT TYPE OF OIL RECOVERY MECHANISMS BY THE SELECTED STRAINS OF <i>Bacillus licheniformis</i> IN PYRENE-RICH MEDIUM	113
5.1	Introduction	113
5.2	Research methodology	114
	5.2.1 Culture maintenance	114
	5.2.2 Inoculum preparation	115
	5.2.3 Analyses for bacterial physiology, chemotaxis, pyrene consumption, cellular morphology and microscopic <i>in-situ</i> monitoring	115
5.3	Results and discussion	118
	5.3.1 Formation of biofilm as adaptation strategy of selected strains of <i>Bacillus licheniformis</i> in pyrene-rich medium	118
	5.3.2 <i>In-situ</i> monitoring of biofilm/ biofloc formation by three selected strains	121
	5.3.2.1 <i>In-situ</i> monitoring of <i>Bacillus licheniformis</i> strain M1 grown in pyrene-rich medium	123
	5.3.2.2 <i>In-situ</i> monitoring of <i>Bacillus licheniformis</i> strain Ta62bi grown in pyrene-rich medium	127
	5.3.2.3 <i>In-situ</i> monitoring of <i>Bacillus licheniformis</i> strain P6 grown in pyrene-rich medium	132
	5.3.3 Bacterial adherence to hydrocarbon and pyrene consumption	136
	5.3.3.1 <i>Bacillus licheniformis</i> strain M1	139
	5.3.3.2 <i>Bacillus licheniformis</i> strain Ta62bi	141
	5.3.3.3 <i>Bacillus licheniformis</i> strain P6	143
	5.3.4 Proposed type of oil recovery mechanism	145
	5.3.4.1 <i>Bacillus licheniformis</i> strain M1	147
	5.3.4.2 <i>Bacillus licheniformis</i> strain Ta62bi	148
	5.3.4.3 <i>Bacillus licheniformis</i> strain P6	150
5.3.5	Conclusion	152

CHAPTER 6	VALIDATIONS OF THE SELECTED BACTERIAL PERFORMANCE AND THEIR DOMINANT TYPE OF OIL RECOVERY MECHANISMS IN BACTERIAL FLOODING TEST	153
6.1	Introduction	153
6.2	Research methodology	154
6.2.1	Culture maintenance	154
6.2.2	Inoculum preparation	155
6.2.3	Analyses in batch experiments	155
6.2.4	Analyses in flow experiments	156
6.3	Results and discussion	159
6.3.1	Growth compatibility of three selected bacteria in brine solution containing paraffin oil	159
6.3.2	Performance of three selected bacteria as potential MEOR agents in glass-bead packed column	163
6.3.3	Investigation of MEOR mechanisms by three selected bacteria at pore scale using micromodel	167
6.4	Conclusion	176
CHAPTER 7	GENERAL CONCLUSIONS AND FUTURE WORK	179
7.1	General conclusions	179
7.2	Future work	183
REFERENCES		185
Appendices A-D		201-267

LIST OF TABLES

TABLE NO.	TITLE	PAGE
Table 2.1	Examples of injection strategies for <i>in-situ</i> Microbial Enhanced Oil Recovery (MEOR)	18
Table 2.2	The comparison of biofilm and biofloc formations for selective plugging mechanism	34
Table 3.1	General information of eight bacterial strains used in this study	45
Table 3.2	Composition of the nutrient broth (NB) premix (Merck KGaA)	51
Table 3.3	Composition of the Bushnell-Hass (BH) broth medium	51
Table 4.1	Effects of different types and concentrations of PAHs on population rate (h^{-1}) and viability cell counts (CFU/mL $\times 10^3$) of eight bacterial strains	85
Table 4.2	Time involved for population rate calculation and sampling point for maximum viable cells of bacterial strain M1 under different concentrations of naphthalene	91
Table 4.3	Time involved for population rate calculation and sampling point for maximum viable cell counts of bacterial strain M1 under different concentrations of pyrene	91
Table 5.1	Transformation of bacterial cell morphology upon growth in low nutrient medium supplemented with 10 g/L (w/v) pyrene	120
Table 5.2	Type of cellular morphology and location of biofilm at 72 hours incubation in pyrene-rich medium by selected bacterial strains	122
Table 5.3	<i>In-situ</i> monitoring of cellular morphology and development of biofilm of <i>Bacillus licheniformis</i> strain M1	126
Table 5.4	<i>In-situ</i> monitoring of cellular morphology and development of biofilm of <i>Bacillus licheniformis</i> strain Ta62bi	131
Table 5.5	<i>In-situ</i> monitoring of cellular morphology and development of biofilm of <i>Bacillus licheniformis</i> strain P6	136

Table 5.6	Maximum rate of pyrene consumption and maximum chemotaxis index by three selected bacteria	138
Table 6.1	Phases and conditions for bacterial flooding test using glass-bead packed column and micromodel	157
Table 6.2	Summary of the recommended level of brine concentration and temperatures for population rate of three selected bacteria	161
Table 6.3	Summary of column characteristics and results of bacterial flooding test by three selected bacteria	164
Table 6.4	Comparison of Additional Oil Recovery (AOR) from column study using different <i>Bacillus</i> isolates	166
Table 6.5	Microscopic visualization at pore scale level after bacterial flooding phase by three selected bacteria	174
Table 6.6	Modification of batch and flow experiments to complement each other under hydrocarbon-rich conditions	176

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
Figure 2.1	Classification of Enhanced Oil Recovery (EOR) methods (Adapted from Thomas, 2008)	11
Figure 2.2	Microbial Enhanced Oil Recovery (MEOR) and biorefining approaches in petroleum industry (Adapted from Singh <i>et al.</i> , 2012)	13
Figure 2.3	Interconnections of three main parameters that affect microbial propagation and their activities for <i>in-situ</i> MEOR process (Adapted from Sarkar <i>et al.</i> , 1989; Ward <i>et al.</i> , 2009)	15
Figure 2.4	Evaluation of potential MEOR agents from laboratory scale to field trials (Adapted from Shennan and Vance, 1987; Gandler <i>et al.</i> , 2006; Khan <i>et al.</i> , 2008; Gudina <i>et al.</i> , 2013; Halim <i>et al.</i> , 2017; Couto <i>et al.</i> , 2018)	21
Figure 2.5	Chemical structures, physical and toxicological characteristic of polycyclic aromatic hydrocarbons (PAHs). The symbols are: (DA) DNA adducts, (SCE) sister chromatid exchange, (CA) chromosomal aberrations. (Ames) <i>Salmonella typhimurium</i> reversion assay, (UDS) unscheduled DNA synthesis, (-) not genotoxic (Adapted from Cerniglia, 1992; Bamforth and Singleton, 2005)	26
Figure 2.6	Pathways for the microbial catabolism of polycyclic aromatic hydrocarbons (PAHs) (Adapted from Cerniglia, 1992; Haritash and Kaushik 2009)	27
Figure 2.7	Bioavailability process for hydrocarbon uptake into bacterial cell (Adapted from Hua and Wang, 2014; Murínová and Dercová, 2014)	28
Figure 2.8	Prediction of dominant type of oil recovery mechanism by potential bacteria as MEOR agents based on their adaptation strategy under hydrocarbon-rich conditions (Adapted from Gray <i>et al.</i> , 2008; Harner <i>et al.</i> , 2011; Klueglein <i>et al.</i> , 2016; Geetha <i>et al.</i> , 2018; Jeong <i>et al.</i> , 2018)	31
Figure 2.9	Bacterial approaches in term of physiological, behavioural and metabolite production that may pave away biofilm/ biofloc formation by using hydrocarbon as substrate (Adapted from Semple <i>et al.</i> , 2007; Grimaud, 2010; Satputra <i>et al.</i> , 2010; Wolicka and Borkowski, 2012)	33

Figure 2.10	Microscopic illusion of selective plugging mechanism (Adapted from Suthar <i>et al.</i> , 2009; Klueglein <i>et al.</i> , 2016; Jeong <i>et al.</i> , 2018)	35
Figure 2.11	Microscopic illusion of oil recovery mechanism using biosurfactant/ bioemulsifier (Adapted from Sen, 2008; Banat <i>et al.</i> , 2010; Halim <i>et al.</i> , 2017; Geetha <i>et al.</i> , 2018)	36
Figure 2.12	Constructed theoretical framework to evaluate potential <i>in-situ</i> MEOR agents in this study	38
Figure 2.13	Constructed conceptual framework to identify bacterial dominant type of oil recovery mechanism under hydrocarbon-rich conditions in this study	40
Figure 3.1	Three phases of operational framework to evaluate potential bacteria as MEOR agents under hydrocarbon-rich conditions	44
Figure 3.2	Flow of culture maintenance, storage, inoculum preparation, enrichment and selective media used in this study	46
Figure 3.3	Spread plating and colony counting procedures (Adapted from Madigan <i>et al.</i> , 2012)	58
Figure 3.4	Schematic diagram for syringe-needle chemotaxis chamber (Adapted from Mazumder <i>et al.</i> , 1999; Jain and Pandey, 2010)	62
Figure 3.5	Schematic diagram of simple flow cell (Adapted from Rodriques <i>et al.</i> , 2005)	68
Figure 3.6	Dimension of glass-beads packed column	69
Figure 3.7	Bacterial flooding test set-up using glass-bead packed column in this study	70
Figure 3.8	Heterogeneous pattern on number of micromodel in this study	73
Figure 3.9	Bacterial flooding test set-up using micromodel in this study	74
Figure 4.1	Flow of research activities for the selection of potential bacteria as MEOR agent under hydrocarbon-rich conditions	83
Figure 4.2	Growth profiles of bacterial strain M1 in Bushnell-Hass (BH) broth within: (a) 72 hours (b) 120 hours in naphthalene-rich medium (c) within 120 hours in pyrene-rich medium	87

Figure 4.3	Population rate and maximum viable cells in Bushnell-Hass (BH) broth supplemented with different concentrations of (a) naphthalene; (b) pyrene by bacterial strain M1	89
Figure 4.4	Growth of bacterial strain Ta62bi in Bushnell-Hass (BH) broth supplemented with different concentrations and type of PAHs (a) Growth profiles and (b) Population rate and viable cells at 72 hours	92
Figure 4.5	Growth of bacterial strain P6 in Bushnell-Hass (BH) broth supplemented with different concentrations and type of PAHs (a) Growth profiles and (b) Population rate and viable cells at 72 hours	94
Figure 4.6	Growth profiles of bacterial strain (a) RT and (b) RU in Bushnell-Hass (BH) broth supplemented with different type and concentrations of PAHs	96
Figure 4.7	Population rate and viable cells at 72 hours of bacterial strain (a) RT and (b) RU in medium supplemented with different concentrations and type of PAHs	97
Figure 4.8	Cell hydrophobicity, cell autoaggregation and exopolysaccharides (EPS) production of bacterial strain (a) M1, (b) Ta62bi and (c) P6 in Bushnell-Hass (BH) broth supplemented with different types and concentrations of PAHs	101
Figure 4.9	Cell hydrophobicity, cell autoaggregation and exopolysaccharides (EPS) production of bacterial strain (a) RT and (b) RU in Bushnell-Hass (BH) broth supplemented with different concentrations and type of PAHs	103
Figure 4.10	Formation of cells by selected bacterial strain (a) M1 and (b) P6 formed look-like floc of cells while (c) Ta62bi's cells covered under layer of polymer-like structure after 72 hours of incubation in pyrene-rich medium. All magnifications were shown at 2500 X except for bacterial strain Ta62bi (10000 X)	106
Figure 4.11	Knowledge gaps between effects of given polycyclic aromatic hydrocarbons (PAHs) on bacterial adherence to hydrocarbon approaches and predicted role of biofilm/biofloc formation under hydrocarbon-rich conditions	107
Figure 4.12	A determination of 1500 bp of Polymerase Chain Reaction (PCR) product of selected bacterial 16S ribosomal RNA gene on agarose gel using VC 1 kb ladder (Vivantis Technologies Sdn. Bhd.)	109

- Figure 4.13 Phylogenetic location of bacterial strains coded M1, Ta62bi and P6 among *Bacillus licheniformis* strains. *A. acidiphilius* was used as an outgroup. The scale bar indicates the number of substitution per nucleotide position 110
- Figure 5.1 Flow of research activities for the identification of dominant types of oil recovery mechanisms by the selected bacteria in pyrene-rich medium 117
- Figure 5.2 Scanning electron micrograph showing transformation of cellular morphology after 24 hours in nutrient broth (NB) medium by *Bacillus licheniformis* strain (a) M1, (c) Ta62bi and (d) P6; and after 72 hours grown in Bushnell-Hass (BH) broth medium containing 10 g/L (w/v) pyrene by *Bacillus licheniformis* strain (b) M1, (e) Ta62bi and (f) P6. The observation were carried out under 30000 X magnification, respectively 119
- Figure 5.3 *In-situ* monitoring of bacterial strain M1 using phase contrast inverted microscope. Scale bars represents length equivalent to 10 μm . Observations were carried out with the total magnification of 400 X 124
- Figure 5.4 *In-situ* monitoring of bacterial strain M1 using CLSM with the BacLight LIVE/DEAD viability stains. Pyrene crystals appear as fluorescent blue object while the bacteria cells appear as either green (intact cytoplasmic membrane) or red (damaged cytoplasmic membrane) fluorescent objects. Scale bars represent length equivalent to 50 μm , except for 0 hour and 24 hours (100 μm). Magnifications: a) 200 X at 0 and 24 hour; b) 400 X at 48 and 72 hours 125
- Figure 5.5 *In-situ* monitoring of bacterial strain Ta62bi using phase contrast inverted microscope. Scale bars represents length equivalent to 10 μm . Observations were carried out at total magnification of 400 X 128
- Figure 5.6 *In-situ* monitoring of bacterial strain Ta62bi using CLSM with the BacLight LIVE/DEAD viability stains. Pyrene crystals appear as fluorescent blue object while the bacteria cells appear as either green (intact cytoplasmic membrane) or red (damaged cytoplasmic membrane) fluorescent objects. Scale bars represent length equivalent to 50 μm ; except for 0 hour (100 μm). Magnifications: 200 X at 0 hours while 400 X at 24, 48 and 72 hours 130
- Figure 5.7 *In-situ* monitoring of bacterial strain P6 using phase contrast inverted microscope. Scale bars represents length equivalent to 10 μm . Observations were carried out at total magnification of 400 X 133

- Figure 5.8 *In-situ* monitoring of bacterial strain P6 using CLSM with the BacLight LIVE/DEAD viability stains. Pyrene crystals appear as fluorescent blue object while the bacteria cells appear as either green (intact cytoplasmic membrane) or red (damaged cytoplasmic membrane) fluorescent objects. Scale bars represent length equivalent to 50 μm , except for 0 hour (100 μm). Magnifications: 200 X at 0 hour while 400 X at 24, 48 and 72 hours 135
- Figure 5.9 Relationships between growth of bacterial strain M1 and (a) exopolysaccharides (EPS) production; (b) Consumption of soluble pyrene in aqueous phase; (c) Chemotaxis indexes during growth in BH broth medium containing 10 g/L (w/v) of pyrene 140
- Figure 5.10 Relationships between growth of bacterial strain Ta62bi and (a) EPS production; (b) Consumption of soluble pyrene in aqueous phase; (c) Chemotaxis indexes during growth in BH broth medium containing 10 g/L (w/v) of pyrene 142
- Figure 5.11 Relationships between growth of bacterial strain P6 and (a) EPS production; (b) Consumption of soluble pyrene in aqueous phase; (c) Chemotaxis indexes during growth in BH broth medium containing 10 g/L (w/v) of pyrene 144
- Figure 5.12 Conceptual model to identify dominant type of oil recovery mechanism by selected strains of *Bacillus licheniformis* in pyrene-rich medium 146
- Figure 5.13 Proposed selective plugging mechanism of *Bacillus licheniformis* strain M1 in BH broth medium containing pyrene (10 g/L). The pyrene crystal is in blue while the cells, are either in green fluorescent for healthy cells or in red fluorescent for damaged membrane cells 148
- Figure 5.14 Proposed plugging mechanism of *Bacillus licheniformis* strain Ta62bi in BH broth medium containing pyrene (10 g/L). The pyrene crystal is in blue while the cells, are either in green for intact or in red for damaged cytoplasmic membrane. The yellow colour is illustrated as smear of polymer-like structure 149
- Figure 5.15 Proposed emulsification-like mechanism by *Bacillus licheniformis* strain P6 studied in BH broth medium containing pyrene (10 g/L). The pyrene crystal is in blue while the cells, are either in green for intact or in red for damaged cytoplasmic membrane. The orange fiber is illustrated as polymer-like structure 150

Figure 6.1	Flow of research activities for the validation of selected bacterial performance and their proposed type of oil recovery in porous media	158
Figure 6.2	Growth profile of bacterial strain (a) M1, (b) P6 and (c) Ta62bi in brine solution supplemented with paraffin oil at different concentration of brine (2 % and 4 % (w/v)) and temperatures (37 °C and 50 °C)	160
Figure 6.3	Observations on static test tubes of selected bacterial strain (a) M1, (b) Ta62bi and (c) P6 in brine solution supplemented with paraffin oil at different concentration of brine (2 % (w/v) to 4 % (w/v)) and temperatures (37 °C to 50 °C), within 48 to 96 hours of incubation	162
Figure 6.4	Reduction of permeability ratio by bacterial strain (a) M1, (b) Ta62bi and (c) P6 with constant permeability ratio	167
Figure 6.5	Microscopic visualization according to phases: (a) Water saturation, (b) Oil flooding and (c) Water flooding for coordinate of 4 while (d) Water saturation, (e) Oil flooding and (f) Water flooding for coordinate of 6. Scale bars represents length equivalent to 100 μm. Observations were carried out at the total magnification of 40 X. Blue arrow represents direction of fluids' flow	168
Figure 6.6	Microscopic visualization of bacterial strain according to phases: (a) Bacterial flooding, (b) Shut-in, (c) Recovery by bacterial strain M1 while (d) Bacterial flooding, (e) Shut-in and (f) Recovery by bacterial strain Ta62bi. Scale bars represents length equivalent to 100 μm. Observations were carried out at the total magnification of 40 X. Blue arrow represents direction of solution flow	170
Figure 6.7	Microscopic visualization of bacterial strain P6 according to phases: (a) Bacterial flooding, (b) Shut-in, (c) Recovery. Scale bars represents length equivalent to 100 μm. Observations were carried out at the total magnification of 40 X. Blue arrow represents direction of solution flow	172
Figure 6.8	Summary of outcomes from flow and batch experiments under hydrocarbon-rich conditions	174
Figure 7.1	Roadmap of research contributions by improvising available strategy to evaluate potential <i>in-situ</i> MEOR agents at laboratory scale	181

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

<i>et al.</i>	-	And others
sp.	-	Species
L	-	Liter
M	-	Molar
g/L	-	Gram per litre
mL	-	Mililitre
μm	-	Micrometre
$^{\circ}\text{C}$	-	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)
\emptyset	-	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)
Δp (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

Sor _{mt}	-	Residual oil saturation after microbial treatment
Er	-	Oil recovery efficiency
NH ₄ NO ₃	-	Ammonium nitrate
CaCl ₂	-	Calcium chloride
FeCl ₃	-	Ferric (III) Chloride
K ₂ HPO ₄	-	Dipotassium phosphate
KH ₂ PO ₄	-	Potassium diphosphate
MgSO ₄	-	Magnesium sulphate
H ₂ SO ₄	-	Sulphuric acid
NaOH	-	Sodium Hydroxide

LIST OF APPENDICES

APPENDIX	TITLE	PAGE
Appendix A1	Standard curve of exopolysaccharide (EPS)	201
Appendix A2	Capillary chemotaxis analysis	202
Appendix A3	Standard curve of pyrene	203
Appendix A4	Preparation for microorganism grown in liquid culture (HMDS method)	204
Appendix A5	Stock solution of viability kit (L7012) and its protocol of application	205
Appendix A6	Simple flow cell	206
Appendix A7	Construction and operation of glass-bead packed column	207
Appendix A8	Porous media analyses and calculations	211
Appendix A9	Construction and operation of micromodel	216
Appendix A10	Statistical model	220
Appendix B1	ANOVA for general factorial design of a) population rate and b) viable cells at 72 hours under different type and concentration of PAHs on by three selected bacteria	222
Appendix B2	ANOVA for single factorial design of a) population rate and b) viable cells at 72 hours under different type and concentration of PAHs by three selected bacteria	225
Appendix B3	ANOVA for single factorial design of a) population rate and b) maximum viable cells under different concentrations of naphthalene and pyrene, respectively by bacterial strain M1	229
Appendix B4	ANOVA for general factorial design of a) cell hydrophobicity; b) exopolysaccharides (EPS) production and c) cell autoaggregation under different type and concentration of PAHs by five selected bacteria	233
Appendix B5	ANOVA for single factorial design of a) cell hydrophobicity; b) exopolysaccharides (EPS) production and c) cell autoaggregation under different type and concentration of PAHs by five selected bacteria	241
Appendix B6	Partial sequence of 16S ribosomal RNA gene of three selected bacteria	249

Appendix B7	Clustal W dendrogram and multiple sequence alignment of three selected bacteria	252
Appendix C1	Examples of HPLC Chromatogram of insoluble pyrene	255
Appendix C2	Estimation of biofilm thickness by three selected bacteria in pyrene-rich medium	256
Appendix D1	ANOVA for two-level (2^k) factorial design of population rate under different concentration of dyed paraffin, brine and temperatures by bacterial strain M1 (between 48 to 72 hours)	267
Appendix D2	ANOVA for two-level (2^k) factorial design of a) population rate and b) maximum viable cell counts under different concentration of brine and temperatures by three selected bacteria	268
Appendix D3	Prediction of the best combination of brine concentration and temperatures based on population rate and maximum viable cells by three selected bacteria	271

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 through rock when other immiscible fluids such as oil are present in the reservoir. However, when the injected water is preferentially flows through high permeability layers, residual oil remains unrecovered and trapped at the low permeability 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 Couto *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 Couto *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.

REFERENCES

- Abbasnezhad, H., Gray, M. and Foght, J. M. (2011) Influence of adhesion on aerobic biodegradation and bioremediation of liquid hydrocarbons. *Applied Microbiology and Biotechnology*. 92(4), 653-675.
- Adav, S.S. and Lee, D. J. (2008) Single-culture aerobic granules with *Acinetobacter calcoaceticus*. *Applied Microbiology and Biotechnology*. 78, 551-557.
- Afrapoli, M. S., Alipour, S. and Torsaeter, O. (2011) Fundamental study of pore scale mechanisms in microbial improved oil recovery processes. *Transport in Porous Media*. 90, 949–964.
- Afrapoli, M. S., Crescente, C., Li, S., Alipour, S. and Torsaeter, O. (2012) Simulation study of displacement mechanisms in microbial improved oil recovery experiments. *SPE EOR Conference at Oil and Gas West Asia*. 16–18 April. Muscat, Oman. 1-11.
- Ahn, Y., Sanseverino, J. and Sayler, G. S. (1999) Analyses of polycyclic aromatic hydrocarbon-degrading bacteria isolated from contaminated soils, *Biodegradation*. 10, 149-157.
- Aladasani, A. and Bai, B. (2010) Recent developments and updated screening criteria of enhanced oil recovery techniques. *CPS/SPE International Oil & Gas Conference and Exhibition*. 8-10 June. Beijing, China, 1-24.
- Al-Sulaimani, H., Joshi, S., Al-Wahaibi, Y., Al-Bahry, S., Elshafie, A. and Al-Bemani, A. (2011) Microbial biotechnology for enhancing oil recovery: Current developments and future prospects. *Biotechnology, Bioinformatics and Bioengineering*. 1(2), 147-158.
- Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*. 25(17), 3389–3402.
- Alkan, H. H., Biegel, E., Krüger, M., Sitte, J., Kögler, F., Bültemeier, H., Beier, K., McInerney, M. J., Herold, A. and Hatscher, S. (2014) An integrated MEOR project; workflow to develop a pilot in a German field. *SPE Improved Oil Recovery Symposium*. 12-16 April. Tulsa, Oklahoma, USA, 1-14.

- Alkan, H. H., Klueglein, N., Mahler, E., Kögler, F., Beier, K., Jelinek, W Herold, A., Hatscher, S. and Leonhardt, B. (2016) An integrated German MEOR project, update : risk management and huff n puff design. *SPE Improved Oil Recovery Conference*. 11-13 April. Tulsa, Oklahoma, USA, 1-21.
- Almeida, D. G. D., Silva, R. D. C. F. S. D., Juliana M. Luna, J. M., Raquel D. Rufino, R. D., Santos, V. A., Banat, I. M. and Sarubbo, L. A. (2016) Biosurfactants : promising molecules for petroleum biotechnology advances. *Frontiers in Microbiology*. 7, 1-14.
- Almeida, P. F., Moreira, R. S., Almeida, R. C. C., Guimarães, A. K., Carvalho, A. S., Quintella, C., Esperidiã, M. C.A. and Taft, C. A. (2004) Selection and application of microorganisms to improve oil recovery. *Engineering in Life Sciences*, 4(4), 319–325.
- Amy, P. S. and Morita, R. Y. (1983) Starvation-survival patterns of sixteen freshly isolated open- ocean bacteria. *Applied and Environmental Microbiology*. 45(3), 1109–1115.
- Andrews, J. M. (2001) Determination of minimum inhibitory concentrations. *The Journal of Antimicrobial Chemotherapy*. 48(Suppl 1), 5–16.
- Armstrong, R. T. and Wildenschild, D. (2012) Investigating the pore-scale mechanisms of microbial enhanced oil recovery. *Journal of Petroleum Science and Engineering*. 94-95, 155–164.
- Bachmann, R. T., Johnson, A. C. and Edyvean, R. G. J. (2014) Biotechnology in the petroleum industry: An overview. *International Biodeterioration & Biodegradation*. 86, 225-237.
- Bae, J. H., Chambers, K. T. and Lee, H. O. (1996) Microbial profile modification with spores. *SPE Reservoir Engineering*. 11(3), 163-167.
- Bamforth, S. M. and Singleton, I. (2005) Review bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. *Journal of Chemical Technology and Biotechnology*. 80, 723–736.
- Banat, I. M. (1995) Biosurfactants production and possible uses in microbial enhanced oil recovery and oil pollution remediation: a review. *Bioresource Technology*. 51, 1–12.

- Banat, I. M., Franzetti, A., Gandolfi, I., Bestetti, G., Martinotti, M. G., Fracchia, L., Smyth, T. J. and Marchant, R. (2010) Microbial biosurfactants production, applications and future potential. *Applied Microbiology and Biotechnology*, 87, 427–444.
- Bao, M., Liu, T., Chen, Z., Guo, L., Jiang, G., Li, Y. and Li, X. (2013) A laboratory study for assessing microbial enhanced oil recovery. *Energy Sources*, 35, 2141-2148.
- Behlülçil, K and Mehmetoğlu, M. T. (2002) Bacteria for improvement of oil recovery : A Laboratory Study. *Energy Sources*. 24, 413–421.
- Berney, M., Hammes, F., Bosshard, F., Weilenmann, H. U. and Egli, T. (2007) Assessment and interpretation of bacterial viability by using the LIVE/ DEAD BacLight kit in combination with flow cytometry. 73(10), 3283–3290.
- Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S. and Escaleira, L. A. (2008) Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta*. 76, 965-977.
- Bordoloi, N. K. and Konwar, B. K. (2009) Bacterial biosurfactant in enhancing solubility and metabolism of petroleum hydrocarbons. *Journal of Hazardous Materials*. 170, 495–505.
- Bouchez, M., Blanchetl, D. and Vandecasteele, J. P. (1997) An interfacial uptake mechanism for the degradation of pyrene by a *Rhodococcus* strain. *Microbiology*. 143, 1087–1093.
- Brown, L. R. (2010) Microbial enhanced oil recovery (MEOR). *Current Opinion in Microbiology*. 13, 1–5.
- Bryant, R. S. and Douglas, J. (1988) Evaluation of microbial system in porous medium for EOR. *SPE Reservoir Engineering*. 3, 489-495.
- Bryant, R. S. and Burchfield, T. E. (1989) Review of microbial technology for improving oil recovery. *SPE Reservoir Engineering*. 4(2), 151-154.
- Bryant, S. L. and Lockhart, T. P. (2002) Reservoir engineering analysis of microbial enhanced oil recovery. *SPE Reservoir Evaluation & Engineering*. 5(5), 365-374.
- Bubela, B. (1989) Geobiology and microbiologically enhanced oil recovery, in Donaldson, E. C., Chilingarian, G. V. and Yen, T.F. (eds.) *Developments in Petroleum Science: Microbial Enhanced Oil Recovery*. Elsevier Science Publishers, pp. 75-97.

- Bushnell, L. D. and Haas, H. F. (1941) The utilization of certain hydrocarbons by microorganisms. *Journal of Bacteriology*. 41(5), 653–673.
- Busscher, H. J. and Van der Mei, H. C. (2006) Microbial adhesion in flow displacement systems. *Clinical Microbiology Reviews*. 19(1), 127-141.
- Cerniglia, C. E. (1992) Biodegradation of polycyclic aromatic hydrocarbons. *Biodegradation*. 3, 351–368.
- Chakraborty, S., Mukherji, S. and Mukherji, S. (2010) Surface hydrophobicity of petroleum hydrocarbon degrading *Burkholderia* strains and their interactions with NAPLs and surfaces. *Colloids and Surfaces B: Biointerfaces*. 78, 101–108.
- Chang, W. N., Liu, C. W. and Liu, H. S. (2009) Hydrophobic cell surface and bioflocculation behavior of *Rhodococcus erythropolis*. *Process Biochemistry*. 44, 955–962.
- Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T. J., Higgins, D. G. and Thompson, D. (2003) Multiple sequence alignment with the clustal series of programs. *Nucleic Acids Research*. 31(13), 3497–3500.
- Chojnacka, K. (2010) Biosorption and bioaccumulation – the prospects for practical applications. *Environment International*. 36, 299–307.
- Cooper, D. G. and Goldenberg, B. G. (1987) Surface-active agents from two *Bacillus* species. *Applied and Environmental Microbiology*. 53(2), 224–229.
- Couto, M. R., Gudiña, E. J., Ferreira, D., Teixeira, J. A., Rodrigues, L. R., Soares, L. P. and Ribeiro, M. T. (2018) Characterization of a biopolymer produced by *Arthrobacter viscosus* CECT 908 for application in microbial enhanced oil recovery. *SPE EOR Conference at Oil and Gas West Asia*. 26-28 March. Muscat, Oman, 1-12.
- Crescente, C., Rekdal, A., Abraiz, A., Torsaeter, O., Hultmann, L., Stroem, A., Rasmussen, K. and Kowalewski, E. (2008) A pore level study of MIOR displacement mechanisms in glass. *2008 SPE/DOE Improved Oil Recovery Symposium*. 19–23 April. Tulsa, Oklahoma, U.S.A, 1-20.
- Dandie, C. E., Thomas, S. M., Bentham, R. H. and McClure, N. C. (2004) Physiological characterization of *Mycobacterium* sp. strain 1B isolated from a bacterial culture able to degrade high-molecular-weight polycyclic aromatic hydrocarbons. *Journal of Applied Microbiology*. 97, 246–255.

- Das, K. and Mukherjee, A. K. (2007) Differential utilization of pyrene as the sole source of carbon by *Bacillus subtilis* and *Pseudomonas aeruginosa* strains: role of biosurfactants in enhancing bioavailability. *Journal of Applied Microbiology*. 102(1), 195–203.
- Dastgheib, S. M. M., Amoozegar, M. A., Elahi, E., Asad, S. and Banat, I. M. (2008) Bioemulsifier production by a halothermophilic *Bacillus* strain with potential applications in microbially enhanced oil recovery. *Biotechnology Letters*. 30(2), 263–270.
- Datta, P., Tiwari, P. and Pandey, L. M. (2018) Isolation and characterization of biosurfactant producing and oil degrading *Bacillus subtilis* MG495086 from formation water of Assam oil reservoir and its suitability for enhanced oil recovery. *Bioresource Technology*. 270, 439–448.
- Deary, M. E., Ekumankama, C. E. and Cummings, S. P. (2016) Development of a novel kinetic model for the analysis of PAH biodegradation in the presence of lead and cadmium co-contaminants. *Journal of Hazardous Materials*. 307, 240-252.
- Deziel, E., Paquette, G., Villemur, R., Lepine, F. and Bisailon, J. G. (1996) Biosurfactant production by a soil *Pseudomonas* strain growing on polycyclic aromatic hydrocarbons. *Applied and Environmental Microbiology*. 62(6), 1908–1912.
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A and Smith, F. (1956) Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*. 28(3), 350–356.
- Egli, T. (2015) Microbial growth and physiology: a call for better craftsmanship. *Frontiers in Microbiology*. 6, 1–12.
- Eriksson, M., Dalhammar, G. and Mohn, W. W. (2002) Bacterial growth and biofilm production on pyrene. *FEMS Microbiology Ecology*. 40, 21–27.
- Fareh Nunizawati binti Daud (2008) Selection of biosurfactant-producing bacteria and optimization of biosurfactant production by *Bacillus* sp. B160. Master Thesis. Universiti Teknologi Malaysia, Skudai.
- Folmsbee, M., Duncan, K., Han, S. O., Nagle, D., Jennings, E. and McInerney, M. (2006) Re-identification of the halotolerant, biosurfactant-producing *Bacillus licheniformis* strain JF-2 as *Bacillus mojavensis* strain JF-2. *Systematic and Applied Microbiology*. 29, 645–649.

- Gandler, G. L., Gbosi, A., Bryant, S.L. and Britton, L.N. (2006) Mechanistic understanding of microbial plugging for improved sweep efficiency. *SPE/DOE symposium on Improved Oil Recovery*. 22-26 April. Tulsa, Oklahoma. U.S.A, 1-8.
- Gao, C. (2018) Experiences of microbial enhanced oil recovery in Chinese oil field. *Journal of Petroleum Science and Engineering*. 166, 55-62.
- Garrett, T. R., Bhakoo, M. and Zhang, Z. (2008) Bacterial adhesion and biofilms on surfaces. *Progress in Natural Science*. 18, 1049–1056.
- Gaskin, S. and Bentham, R. (2005) Comparison of enrichment methods for the isolation of pyrene-degrading bacteria. *International Biodeterioration & Biodegradation*. 56, 80–85.
- Geetha, S. J., Banat, I. M. and Joshi, S. J. (2018) Biosurfactants: production and potential applications in microbial enhanced oil recovery (MEOR). *Biocatalysis and Agricultural Biotechnology*. 14, 23–32.
- Gray, M. R., Yeung, A., Foght, J. M. and Yarranton, H. W. (2008) Potential microbial enhanced oil recovery processes: a critical analysis. *SPE Annual Technical Conference and Exhibition*. 21–24 September. Denver, Colorado, USA, 1-25.
- Grimaud, R. (2010) Biofilm development at interfaces between hydrophobic organic compounds and water, in Timmis, K. N (ed.) *Handbook of Hydrocarbon and Lipid Microbiology*. Berlin Heidelberg: Springer-Verlag, pp. 1491-1499.
- Groudeva V.I., Ivanova, I.A., Groudev, S.N., and Uzunov, G.C. (1993) Enhanced oil recovery by stimulating the activity of the indigenous microflora of oil reservoirs. *Biohydrometal. Technol. Proc. Int. Biohydrometalurgical Symp.* 2, 349-356.
- Gudiña, E. J., Pereira, J. F. B., Rodrigues, L. R., Coutinho, Teixeira, J. A. (2012a) Isolation and study of microorganisms from oil samples for application in microbial enhanced oil recovery. *International Biodeterioration & Biodegradation*, 68, 56–64.
- Gudiña, E. J., Rodrigues, L. R., Teixeira, J. A., Pereira, J. F., Coutinho, J. A. and Soares, L. P. (2012b) Biosurfactant producing microorganisms and its application to enhance oil recovery at lab scale. *SPE EOR Conference at Oil and Gas West Asia*. 16–18 April. Muscat, Oman, 1-8.

- Gudiña, E. J., Pereira, J. F. B., Costab, R., Coutinho, J. A. P., Teixeira, J. A. and Rodrigues, L. R. (2013) Biosurfactant-producing and oil-degrading *Bacillus subtilis* strains enhance oil recovery in laboratory sand-pack columns. *Journal of Hazardous Materials*. 261, 106–113.
- Halim, A., Shapiro, A., Lantz, A. E. and Nielsen, S. M. (2014) Experimental study of bacterial penetration into chalk rock : mechanisms and effect on permeability. *Transport in Porous Media*. 101, 1–15.
- Halim, A. Y., Pedersen, D. S., Nielsen, S. M. and Lantz, A. E. (2015) Profiling of indigenous microbial community dynamics and metabolic activity during enrichment in molasses-supplemented crude oil-brine mixtures for improved understanding of microbial enhanced oil recovery. *Applied Biochemistry and Biotechnology*. 176, 1012–1028.
- Halim, A. Y., Nielsen, S. M., Nielsen, K. F. and Lantz, A. E. (2017) Towards the understanding of microbial metabolism in relation to microbial enhanced oil recovery. *Journal of Petroleum Science and Engineering*. 149, 151–160.
- Hamme, J. D. V., Singh, A. and Ward, O. P. (2003) Recent advances in petroleum microbiology. *Microbiology and Molecular Biology Reviews*. 67(4), 503–549.
- Haritash, A. K. and Kaushik, C. P. (2009) Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. *Journal of Hazardous Materials*. 169, 1–15.
- Harner, N. K., Richardson, T. L., Thompson, K. A., Best, R. J., Best, A S. and Trevors, J. T. (2011) Microbial processes in the Athabasca oil sands and their potential applications in microbial enhanced oil recovery. *Journal of Industrial Microbiology and Biotechnology*. 38, 1761–1775.
- Head, I. M. and Gray, N. D. (2016) Microbial Biotechnology 2020; microbiology of fossil fuel resources. *Microbial Biotechnology*. 9, 626–634.
- Head, I. M., Jones, D. M. and Larter, S. R. (2003) Biological activity in the deep subsurface and the origin of heavy oil. *Nature*. 426, 344–352.
- Höök, M., Hirsch, R. and Aleklett, K. (2009) Giant oil field decline rates and their influence on world oil production. *Energy Policy*, 37, 2262–2272.
- Hua, F. and Wang, H. Q. (2014) Uptake and trans-membrane transport of petroleum hydrocarbons by microorganisms. *Biotechnology & Biotechnological Equipment*. 28, 165-175.

- Hunter, R. D., Ekunwe, S. I. N., Dodor, D. E, Hwang, H. M and Ekunwe, L, (2005) *Bacillus subtilis* is potential degrader of pyrene and benzo[a]pyrene. *International Journal of Environmental Research and Public Health*. 2(2), 267–271.
- Jain R. K. and Pandey, J. (2010) Chemotactic responses, in Timmis, K. N. (ed.) *Handbook of Hydrocarbon and Lipid Microbiology*. Berlin Heidelberg: Springer-Verlag, pp. 3933-3955.
- Javaheri, M., Jenneman, G. E., McInerney, M. J. and Knapp, R. M. (1985) Anaerobic production of a biosurfactant by *Bacillus licheniformis* JF-2. *Applied and Environmental Microbiology*. 50(3), 698–700.
- Jeong, M. S., Hong, E. and Lee, K. S. (2018) Promising biotechnology on selective plugging and wettability alteration for enhanced oil recovery. *Advances in Biotechnology & Microbiology*. 9(2), 1-3.
- Johnsen, A. R. and Karlson, U. (2004) Evaluation of bacterial strategies to promote the bioavailability of polycyclic aromatic hydrocarbons. *Applied Microbiology and Biotechnology*. 63, 452–459.
- Johnsen, A. R., Wick, L. Y. and Harms, H. (2005) Principles of microbial PAH-degradation in soil. *Environmental Pollution*. 133, 71–84.
- Jones, D. S., Adair, C. G., Mawhinney, W. M. and Gorman, S. P. (1996) Standardisation and comparison of methods employed for microbial cell surface hydrophobicity and charge determination. *International Journal of Pharmaceutics*. 131, 83–89.
- Joshi, S. J., Al-Wahaibi, Y. M., Al-Bahry, S. N., Elshafie, A. E., Al-Bemani, A. S., Al-Bahri, A. and Al-Mandhari, M. S. (2016) Production, characterization, and application of *Bacillus licheniformis* W16 biosurfactant in enhancing oil recovery. *Frontiers in Microbiology*. 7, 1–14.
- Kallimanis, A., Frillingos, S., Drainas, C. and Koukkou, A. I. (2007) Taxonomic identification, phenanthrene uptake activity, and membrane lipid alterations of the PAH degrading *Arthrobacter* sp. strain Sphe3. *Applied Microbiology and Biotechnology*. 76, 709–717.
- Kamari, A., Moeini, F., Moghadam, M. J. S., Mohammadi, S. A. H. and Sarapardeh, A. H. (2016) Modeling the permeability of heterogeneous oil reservoirs using a robust method. *Geosciences Journal*. 20(2), 259–271.

- Karambeigi, M. S., Schaffie, M. and Fazaelpoor, M. H. (2013) Improvement of water flooding efficiency using mixed culture of microorganisms in heterogeneous micro-models. *Petroleum Science and Technology*. 31(9), 923-931.
- Kaster, K. M., Hiorth, A., Eilertsen, G. K., Boccadoro, K., Lohne, A., Berland, H., Stavland, A. and Brakstad, O. G. (2012) Mechanisms involved in microbially enhanced oil recovery. *Transport in Porous Media*. 91, 59–79.
- Khachatoorian, R., Petrisor, I. G., Kwan, C. C. and Yen, T. F. (2003) Biopolymer plugging effect: laboratory-pressurized pumping flow studies. *Journal of Petroleum Science and Engineering*. 38, 13–21.
- Khan, H. A., Gbosi, A., Britton, L. N and Bryant, S. L. (2008) Mechanistic model of microbe growth in heterogeneous porous media. *SPE/DOE Improved Oil Recovery Symposium*. 19–23 April. Tulsa, Oklahoma, U.S.A, 19–23.
- Khanna, G. P., Goyal, D and Khanna, S. (2011) Pyrene biodegradation by *Bacillus* spp. isolated from coal tar-contaminated soil. *Bioremediation Journal*, 15(1), 12–25.
- Khire, J. M. (2010) Bacterial biosurfactants and their role in microbial enhanced oil recovery (MEOR), in Sen, R. (ed.) *Biosurfactant*. Landes Bioscience and Springer Science+Business Media, pp. 146-157.
- Kim, S. J., Kweon, O., Jones, R. C., Ricky D., Edmondson, R. D., Freeman, J. P. and Cerniglia, C. E. (2007) Complete and integrated pyrene degradation pathway in *Mycobacterium vanbaalenii* PYR-1 based on systems biology. *Journal of Bacteriology*. 189(2), 464–472.
- Klueglein, N., Kögler, F., Adaktylou, I. J., Wuestner, M. L., Mahler, E., Scholz, J. and Alkan, H. (2016) Understanding selective plugging and biofilm formation of a halophilic bacterial community for MEOR application. *SPE Improved Oil Recovery Conference*. 11–13 April. Tulsa, Oklahoma, USA, 1-15.
- Lacal, J., Martínez, F. M., Darías, J. A. R., Duque, E., Matilla, M., Segura, A., Calvo, J. J. O., Sánchez, C. J., Krell, T. and Ramos. J. L. (2011) Bacterial chemotaxis towards aromatic hydrocarbons in *Pseudomonas*. *Environmental Microbiology*. 13(7), 1733–1744.
- Lappan, R. E. and Fogler, H. S. (1992) Effects of bacterial polysaccharides production on formation damage. *SPE Production Engineering*. 7(2), 167–171.

- Lappan, R. E. and Fogler, H. S. (1994) *Leuconostoc mesenteroides* growth kinetics with application to bacterial profile modification. *Biotechnology and Bioengineering*. 43, 865–873.
- Lazar, I., Petrisor, I. G. and Yen, T. F. (2007) Microbial enhanced oil recovery (MEOR). *Petroleum Science and Technology*. 25(11), 1353–1366.
- Ledolter, J. and Hogg, R. V. (2010) Applied Statistical for Engineers and Physical Scientists. Upper Saddle River, N.J: Pearson Education, Inc.
- Li, J., Liu, J., Trefry, M. G., Park, J., Liu, K., Haq, B., Johnston, C. D. and Volk, H. (2011) Interactions of microbial-enhanced oil recovery processes. *Transport in Porous Media*. 77–104.
- Liu, Y., Yang, S. F., Li, Y., Xu, H., Qin, L. and Tay, J. W. (2004a) The influence of cell and substratum surface hydrophobicities on microbial attachment. *Journal of Biotechnology*. 110, 251–256.
- Liu, Y., Yang, S. F., Tay, J. H., Liu, Q. S., Qin, L. and Li, Y. (2004b) Cell hydrophobicity is a triggering force of biogranulation. *Enzyme and Microbial Technology*. 34, 371–379.
- Luo, Y. R., Tian, Y., Huang, X., Yan, C. L., Hong, H. S., Lin, G. H. and Zheng, T. L. (2009) Analysis of community structure of a microbial consortium capable of degrading benzo (a) pyrene by DGGE. *Marine Pollution Bulletin*. 58, 1159–1163.
- Madigan, M. T., Martinko, J. M., Dunlop, P. V. and Clark, D. P. (2009) *Brock: Biology of microorganisms*. 12th edn. Upper Sadder River, NJ: Pearson Prentice Hall, pp. 153-155.
- Marghmaleki, A. N., Kord, S., Hashemi, A. and Motamedi, H. (2018) Experimental investigation of efficiency of MEOR process in a carbonate oil reservoir using *Alcaligenes faecalis*: Impact of interfacial tension reduction and wettability alteration mechanisms. *Fuel*. 232, 27–35.
- Marx, R. B. and Aitken, M. D. (2000) Bacterial chemotaxis enhances naphthalene degradation in a heterogeneous aqueous system', *Environmental Science and Technology*. 34, 3379–3383.
- Maudgalya, S., McInerney, M. J., Knapp, R. M., Nagle, D. and Fomsbee, M. J. (2005) Tertiary oil recovery with microbial biosurfactant treatment of low-permeability berea sandstone cores. *SPE Production and Operations Symposium*. 17 -19 April. Oklahoma City, U.S.A, 1-7.

- Maudgalya, S., Knapp, R. M and McInerney, M. J. (2007) Microbial enhanced oil recovery technologies: a review of the past, present and future. *SPE Production and Operation Symposium*. 31 March–3 April. Oklahoma City, U.S.A, 1-11.
- Mazumder, R., Phelps, T. J., Krieg, N. R. and Benoit, R. E. (1999) Determining chemotactic responses by two subsurface microaerophiles using a simplified capillary assay method. *Journal of Microbiological Methods*. 37, 255–263.
- Mnif, S., Chamkha, M., Labat, M. and Sayadi, S. (2011) Simultaneous hydrocarbon biodegradation and biosurfactant production by oilfield-selected bacteria. *Journal of Applied Microbiology*. 111, 525–536.
- Molina, M. C, Gonzalez, N., Bautista, L. F., Sanz, R., Simarro, R., Sanchez, I. and Sanz, J. L. (2009) Isolation and genetic identification of PAH degrading bacteria from a microbial consortium. *Biodegradation*. 20, 789-800.
- Murínová, S. and Dercová, K. (2014) Response mechanisms of bacterial degraders to environmental contaminants on the level of cell walls and cytoplasmic membrane. *International Journal of Microbiology*. 2014, 1-16.
- Nerurkar, A. S., Suthar, H. G. and Desai, A. J. (2012) Biosystem development for microbial enhanced oil recovery (MEOR), in Satyanarayana, T., Johri, B. N. and Prakash, A. (eds.) *Microorganisms in Sustainable Agriculture and Biotechnology*. Springer Science+Business Media B.V, pp. 711-737.
- Nigam, P., Banat, I. M. and Marchant, R. (1998) Degradation of naphthelene by bacterial cultures. *Environment International*. 24 (5/6), 671–677.
- Palmer, J., Flint, S and Brooks, J. (2007) Bacterial cell attachment, the beginning of a biofilm. *Journal of Industrial Microbiology and Biotechnology*. 34, 577–588.
- Pandey, G and Jain, R. K. (2002) Minireviews: bacterial chemotaxis toward environmental pollutants: role in bioremediation. *Applied and Environmental Microbiology*. 68(12), 5789–5795.
- Patel J., Borgohain, S., Kumar, M., Rangarajan, V., Somasundaran, P. and Sen, R. (2015) Recent developments in microbial enhanced oil recovery. *Renewable and Sustainable Energy Reviews*. 52, 1539–1558.
- Peihui, H., Fengrong, S. and Mei, S. (2001) Microbial EOR laboratory studies on the microorganisms using petroleum hydrocarbon as a sole carbon source. *SPE Asia Pacific Improved Oil Recovery Conference*. 8–9 October. Kuala Lumpur, Malaysia, 8–11.

- Prakash, O., Nimonkar, Y. and Shouche, Y. S. (2013) Practice and prospects of microbial preservation. *FEMS Microbiology Letters*. 339, 1-9.
- Rassenfoss, S. (2011) From bacteria to barrels : microbiology having an impact on oil fields. *Journal of Petroleum Technology*. 32, 32–39.
- Rodrigues, A. C., Wuertz, S., Brito, A. G. and Melo, L. F. (2005) Fluorene and phenanthrene uptake by *Pseudomonas putida* ATCC 17514: kinetics and physiological aspects. *Biotechnology and Bioengineering*. 90 (3), 281–289.
- Rosenberg, M. and Rosenberg, E. (1985) Bacterial adherence at the hydrocarbon-water interface. *Oil & Petrochemical Pollution*. 2, 155–162.
- Rosenberg, M. and Rosenberg, E. (1981) Role of adherence in growth of *Acinetobacter calcoaceticus* RAG-1 on hexadecane. *Journal of Bacteriology*. 148(1), 51–57.
- Safdel, M., Anbaz, M. A., Daryasafar, A. and Jamialahmadi, M. (2018) Microbial enhanced oil recovery, a critical review on worldwide implemented field trials in different countries. *Renewable and Sustainable Energy Reviews*. 74, 159–172.
- Saitou, N. and Nei, M. (1987) The neighbor-joining method : a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*. 4(4), 406–425.
- Sarkar, A. K., Goursaud, J. C., Sharma, M. M and Georgiou, G. (1989) A critical evaluation of MEOR processes. *In-situ*, 13(4), 207–238.
- Satpute, S. K., Banat, I. M., Dhakephalkar, P. K., Banpurkar, A. G. and Chopade, B. A. (2010) Biosurfactants, bioemulsifiers and exopolysaccharides from marine microorganisms. *Biotechnology Advances*. 28, 436–450.
- Segovia, G. C., Huerta, V. A. and Gutierrez, G. C. (2009) Improving MEOR performance by a selection methodology in mature oilfields', *Latin American and Caribbean Petroleum Engineering Conference*. 31 May-3 June. Cartagena, Colombia, 1-13.
- Semple, K. T. Doick, K. J., Wick, L. Y and Harms, H. (2007) Microbial interactions with organic contaminants in soil: definitions, processes and measurement. *Environmental Pollution*. 150, 166–176.
- Sen, R. (2008) Biotechnology in petroleum recovery: the microbial EOR', *Progress in Energy and Combustion Science*. 34, 714–724.

- Sheehy, A. J. (1990) Field studies of microbial EOR. SPE/DOE Seventh Symposium on Enhanced Oil Recovery. 22–25 April. Tulsa, Oklahoma, 785-790.
- Shennan, J. L. and Vance, I (1987) Microbial enhanced oil recovery techniques and offshore oil production, in Hill, E. C., Shennan, J. L. and Watkinson, R. J (eds.) *Microbial Problems in the Offshore Oil industry*. Chichester: Wiley, pp. 73-91.
- Shibulal, B., Al-Bahry, S. N., Al-Wahaibi, Y. M., Elshafie, A. E., Al-Bemani, A. S. and Joshi, S. J. (2014) Microbial enhanced heavy oil recovery by the aid of inhabitant spore-forming bacteria: an insight review. *The Scientific World Journal*, 1-12.
- Siegert, M., Sitte, J, Galushko, A. and Krüger, M. (2013) Starting up microbial enhanced oil recovery, in Schippers A., Glombitza F., Sand W. (eds) *Geobiotechnology II. Advances in Biochemical Engineering/Biotechnology*. Berlin, Heidelberg: Springer-Verlag. 142, pp. 1-94.
- Sikkema, J., De Bont, J. A., and Poolman, B. (1995) Mechanisms of membrane toxicity of hydrocarbons. *Microbiological Reviews*. 59(2), 201–222.
- Singh, A., Singh, B., and Ward, O., A. (2012) Potential application of bioprocess technology in petroleum industry', *Biodegradation*. 23, 865–880.
- Soudmand-asli, A., Ayatollahi, S. S., Mohabatkar, H., Zareie, M and Shariatpanahi, S. F. (2007) The *in-situ* microbial enhanced oil recovery in fractured porous media. *Journal of Petroleum Science and Engineering*. 58, 161–172.
- Stewart, T. L. and Fogler, H. S. (2001) Biomass plug development and propagation in porous media. *Biotechnology and Bioengineering*. 72(3), 353-363.
- Stewart, T. L. and Kim, D. S. (2004) Modeling of biomass-plug development and propagation in porous media. *Biochemical Engineering Journal*. 17, 107–119.
- Stewart, T. L. and Fogler, H. S (2002) Pore-scale investigation of biomass plug development and propagation in porous media. *Biotechnology and Bioengineering*. 77(5), 577–588.
- Suthar, H., Hingurao, K., Desai, A. and Nerurkar, A. (2009) Selective plugging strategy based microbial enhanced oil recovery using *Bacillus licheniformis* TT33. *Journal of Microbiology and Biotechnology*. 19(10), 1230–1237.
- Suthar, H., K., Desai, A and Nerurkar, A., H. (2008) Evaluation of bioemulsifier mediated enhanced oil recovery using sand pack column. *Journal of Microbiological Methods*. 75, 225–230.

- Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013) MEGA6 : Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution*. 30(12), 2725–2729.
- Thomas, S. (2008) Enhanced oil recovery – an overview. *Oil & Gas Science and Technology*. 63(1), 9–19.
- Thompson, J. D., Higgins, D. G. and Gibson, T. J. (1994) CLUSTAL W : improving the sensitivity of progressive multiple sequence alignment through sequence weighting , position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*. 22(22), 4673-4680.
- Toledo, F. L., Calvo, C., Rodelas, B. and Lopez, J. G. (2006) Selection and identification of bacteria isolated from waste crude oil with polycyclic aromatic hydrocarbons removal capacities. *Systematic and Applied Microbiology*. 29, 244–252.
- Town, K., Sheehy, A. J. and Govreau, B. R. (2010) MEOR success in southern Saskatchewan. *SPE Reservoir Evaluation & Engineering*. 13(5), 773-781.
- Volk, H. and Hendry, P (2010) Tertiary oil recovery: fundamental approaches and principles of microbiologically enhanced oil recovery, in Timmis, K. N. (ed.) *Handbook of Hydrocarbon and Lipid Microbiology*. Berlin Heidelberg: Springer-Verlag, pp. 2727-2738.
- Wammer, K. H. and Peters, C. A. (2005) Polycyclic aromatic hydrocarbon biodegradation rates: a structure-based study. *Environmental Science and Technology*. 39, 2571–2578.
- Ward, O. P., Singh, A., Van Hamme, J. D., Voordouw, G. (2009) Petroleum microbiology, in Schaechter, M. (ed.) *Encyclopedia of Microbiology*. 3rd edn. Amsterdam: Elsevier, pp 443–456.
- Winters, Y. D., Lowenstein, T. K. and Timofeeff, M. N. (2015) Starvation-survival in haloarchaea. *Life*. 5, 1587–1609.
- Wolf, B. F. and Fogler, H. S. (2001) Alteration of the growth rate and lag time of *Leuconostoc mesenteroides* NRRL-B523. *Biotechnology and Bioengineering*. 72(6), 603-610.
- Wolf, B. F. and Fogler, H. S. (2005) Growth of *Leuconostoc mesenteroides* NRRL-B523 in an alkaline medium: suboptimal pH growth inhibition of a lactic acid bacterium. *Biotechnology and Bioengineering*. 89(1), 96–101.

- Wolfaardt, G. M., Lawrence, J. R., Headley, J. V., Robarts, R. D. and Caldwell, D. E. (1994) Microbial exopolymers provide a mechanism for bioaccumulation of contaminants. *Microbial Ecology*. 27, 279–291.
- Wolicka, D. and Borkowski, A. (2012) Microorganisms and Crude Oil, in Zerón, L. R. (ed.) *Introduction to Enhanced Oil Recovery (EOR) Processes and Bioremediation of Oil-Contaminated Sites*. InTech, pp. 113-142.
- Wolicka, D., Borkowski, A. and Dobrzyński D. (2010) Interactions between microorganisms, crude oil and formation waters. *Geomicrobiology Journal*. 27, 43–52.
- Yakimov, M. M., Amro, M. M., Bock, M., Boseker, K., Fredrickson, H. L., Kessel, D. G and Timmis, K. N. (1997) The potential of *Bacillus licheniformis* strains for *in-situ* enhanced oil recovery. *Journal of Petroleum Science and Engineering*. 18, 147–160.
- Young, K. D. (2006) The selective value of bacterial shape. *Microbiology and Molecular Biology Reviews*. 70(3), 660-703.
- Youssef, N., Simpson, D. R., Duncan, K. E., McInerney, M. J., Folmsbee, M, Fincher, T. and Knapp, R. M. (2007) *In-situ* biosurfactant production by *Bacillus* strains injected into a limestone petroleum reservoir. *Applied and Environmental Microbiology*. 73(4), 1239–1247.
- Youssef, N., Elshahed, M. S. and McInerney, M. J. (2009) *Microbial Processes in Oil Fields: Culprits, Problems, and Opportunities*, in Laskin, A. I., Sariaslani, S. and Gadd, G. M. (eds.) *Advances in Applied Microbiology*. Burlington: Academic Press. 66, pp. 141-251.
- Yue, M., Zhua, W., Song, Z., Long, Y. and Song, H (2017) Study on distribution of reservoir endogenous microbe and oil displacement mechanism. *Saudi Journal of Biological Sciences*. 24, 263-267.
- Zhang, J., Xue, Q., Gao, H., Lai, H. and Wang, P. (2016) Production of lipopeptide biosurfactants by *Bacillus atrophaeus* 5-2a and their potential use in microbial enhanced oil recover. *Microbial Cell Factories*. 15, 168-179.
- Zhao, L., Ma, T., Gao, M., Gao, P., Cao, M., Zhu, X. and Li, G. (2012) Characterization of microbial diversity and community in water flooding oil reservoirs in water flooding reservoirs in China. *World Journal of Microbiology and Biotechnology*, 28, 3039–3052.

- Zheng, C., Li, Y., Huang, L., Xiu, J. and Huang, Z. (2012) Investigation of a hydrocarbon-degrading strain, *Rhodococcus ruber* Z25, for the potential of microbial enhanced oil recovery. *Journal of Petroleum Science and Engineering*. 81, 49–56.
- Zita, A and Hermansson, M. (1997) Effects of bacterial cell surface structures and hydrophobicity on attachment to activated sludge flocs. *Applied and Environmental Microbiology*. 63(3), 1168–1170.
- Zulkefli bin Daud (2014) Biological treatment of actual textile wastewater using a sequential facultative anaerobic-aerobic bioreactor. PhD Thesis. Universiti Teknologi Malaysia, Skudai.