



UNIVERSITI PUTRA MALAYSIA

***TREATMENT OF OILFIELD WASTEWATER USING MICROORGANISMS
ISOLATED FROM BUDU BASED PRODUCT***

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ITMA 2014 6



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ISOLATED FROM *BUDU* BASED PRODUCT**

By

NUR 'IZZAH EZHAR

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in
Fulfilment of the Requirements for the Degree of Masters of Science**

September 2014

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

**TREATING OILFIELD WASTEWATER USING MICROORGANISMS
ISOLATED FROM *BUDU*-BASED PRODUCT (*BUDU*)**

By

NUR 'IZZAH EZHAR

September 2014

Chairman : Professor Fakhru'l-Razi B Ahmadun, PhD
Institute : Institute of Advanced Technology

This study aimed to use crude oil degrading bacteria isolated from Malaysian traditional salt food product (*Budu*) for biological treatment of oilfield wastewater. Out of five species observed, two species showed dominant growth and have been successfully isolated. Both species are proven to be able to utilize crude oil as sole carbon source and shown to have salt tolerance up to 7.5% NaCl. The species identified were *Staphylococcus Hominis sp.* and *Corynebacterium Auris sp.*

Preliminary study of oilfield wastewater treatment by mixed culture of *Staphylococcus Hominis sp.* and *Corynebacterium Auris sp.* has been evaluated by acclimatizing it with real oilfield wastewater. It takes about 21 days for the mixed culture to acclimatize to the new environment. This study also evaluates the kinetic growth of mixed bacterial culture during the acclimatization process. Based on the graph of specific growth rate (μ) versus substrate plotted, the curve obtained agreed with the Monod equation evaluation. The maximum specific growth rate (μ_{\max}) and half saturation constant (K_s) value obtained were 0.328 d⁻¹ and 0.171 mg/L, respectively.

A lab scale sequencing batch reactor (SBR) with a volume of 2L was applied in this study to investigate the removal efficiency of chemical oxygen demand (COD) and oil and gas (O&G) based on four different hydraulic retention times (HRT) of 24 h, 36 h, 48 h and 60 h. Influent COD was recorded in the range of 2300 – 2500 mg/L while influent O&G was in the range of 12.4 – 13.2 mg/L. During the SBR treatment, the pH value recorded was in the range of 8.0 – 9.0. Out of four HRTs operated, the longest HRT (60 h) showed the highest removal efficiency of COD and O&G (76.8% and 75.1% respectively). Initial MLVSS operated with 60 h HRT was 1060 mg/L and then increased to 3100 mg/L, approximately 3 times its initial value.

An investigation on the hydrocarbon degradation of oilfield wastewater was also carried out. Gas chromatography mass spectrometer was used to analyze the percentage of hydrocarbon degradation. The mixed culture of *Staphylococcus Hominis sp.* and *Corynebacterium Auris sp.* shown to be able to degrade C₁₀ – C₁₉ n-alkanes. The

percentage of hydrocarbon degradation decreased as the molecular weight of *n*-alkanes increased. At 60 h HRT, more than 90% hydrocarbon degradation was achieved for C₁₀-C₁₄. As the hydrocarbon chain increase from C₁₅-C₁₉, the percentage of hydrocarbon degradation decreased to 70%. Besides, *Staphylococcus Hominis* sp. and *Corynebacterium Auris* sp. were also found to be able to degrade low molecular weight aromatic compound such as ethylbenzene, 2 isomers of xylene (*m*-xylene and *p*-xylene), methyl phenol, naphthalene, and methyl naphthalene effectively at 60 h HRT. The degradation percentage recorded at 60 h HRT for ethylbenzene, *m*-xylene and *p*-xylene was 85.2%, 88.6%, 85%, 95.4% and 90.8% respectively.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana

**MERAWAT AIR HASILAN DARI PERLOMBONGAN MINYAK DENGAN
KAEDAH BIOLOGI MENGGUNAKAN MIKROORGANISMA DIASING DARI
PRODUK BERASASKAN *BUDU***

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Kajian ini bertujuan untuk menggunakan bakteria yang diasingkan dari produk makanan tradisional Malaysia berasaskan garam (*Budu*) untuk rawatan air buangan industri minyak dengan kaedah biologi. Daripada lima spesies yang diperhatikan, dua spesies telah menunjukkan pertumbuhan yang dominan dan telah berjaya diasingkan. Kedua-dua spesies terbukti dapat menggunakan minyak mentah sebagai sumber karbon dan terbukti mempunyai toleransi garam sehingga 7.5% NaCl. Spesies ini telah dikenal pasti sebagai *Staphylococcus Hominis* sp. dan *Corynebacterium Auris* sp.

Kajian awal rawatan air buangan industri minyak oleh kultur campuran antara *Staphylococcus Hominis* sp. dan *Corynebacterium Auris* sp. telah dinilai dengan menyesuaikan campuran bakteria tersebut ke dalam air buangan industri minyak yang asli. Ia mengambil masa selama 21 hari untuk kultur campuran itu menyesuaikan diri dengan persekitaran baru. Kajian ini juga menilai pertumbuhan kinetik budaya bakteria campuran semasa proses penyesuaian ini. Berdasarkan graf kadar pertumbuhan spesifik (μ) melawan substrat yang telah diplot, lengkung yang diperolehi tidak bercanggah dengan penilaian persamaan Monod. Kadar maksimum tertentu pertumbuhan (μ_{max}) dan nilai setengah tepu malar (K_s) yang diperolehi masing-masing ialah 0.328 d⁻¹ dan 0.171 mg / L.

Skala makmal reactor kumpulan turutan (RKT) dengan jumlah muatan 2L telah diaplikasikan dalam kajian ini untuk menyiasat kecekapan penyingkiran keperluan oksigen kimia (KOK) dan minyak dan gris (M&G) berpandukan masa tahanan hidraulik (TH) yang berbeza iaitu 24 j, 36 j, 48 j dan 60 j. Pengaliran masuk KOK tercatat dalam julat 2300 - 2500 mg/L manakala pengaliran masuk M&G adalah dalam julat 12.4 - 13.2 mg/L. Semasa proses rawatan RKT, nilai pH yang dicatat ialah dalam julat 8.0 - 9.0. Daripada empat RKT yang dikendalikan, RKT yang paling lama iaitu 60 j menunjukkan kecekapan penyingkiran KOK dan M&G tertinggi dengan catatan masing-masing 76.8 % dan 75.1%. MLVSS awal dikendalikan dengan 60 j HRT direkodkan sebanyak 1060

mg/L dan kemudian ia meningkat kepada 3100 mg/L, lebih kurang tiga kali ganda nilai asal.

Siasatan berhubung kadar degradasi hidrokarbon dalam air buangan industri minyak juga telah dijalankan. Kromatografi gas spektrometer telah digunakan untuk menganalisis peratusan penguraian hidrokarbon. Campuran bakteria *Staphylococcus Hominis sp.* dan *Corynebacterium Auris sp.* telah terbukti dapat mengurai *n*-alkane C₁₀-C₁₉. Peratus degradasi hidrokarbon didapati berkurangan apabila berat molekul *n*-alkana bertambah. Pada TH 60 j, lebih daripada 90% penguraian hidrokarbon C₁₀- C₁₄ tercapai. Peningkatan rantaian hidrokarbon dari C₁₅ – C₁₉ telah mengakibatkan penurunan pada peratus penguraian hidrokarbon sehingga julat 70%. Selain itu, *Staphylococcus Hominis sp.* dan *Corynebacterium Auris sp.* juga mampu menguraikan karbon aromatic dalam kumpulan berat molekul yang rendah seperti ethylbenzene , 2 isomer xilena (m- xilena dan p-xilena), metil fenol, naftalena dan metilnaftalena. Peratusan kemerosotan etilbenzena, m-xilena dan p-xilena yang dicatatkan pada 60 h TH adalah masing-masing 85.2%, 88.6%, 85%, 95.4% dan 90.8%.

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I certify that a Thesis Examination Committee has met on 4th September 2014 to conduct the final examination of Nur Izzah Ezhar on her thesis entitled “Treatment of Oilfield Wastewater Using Microorganisms isolated from *Budu*-Based Product” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

ASBR	Anaerobic Sequencing Batch Reactor
BTEX	Benzene, Toluene, Ethylbenzene and Xylene
CFU	Colony Forming Unit
COD	Chemical Oxygen Demand
GCMS	Gas Chromatograph Mass Spectrometer
HRT	Hydraulic Retention Time
MLVSS	Mixed Liquor Volatile Suspended Solids
MSM	Mineral Salt Medium
NORM	Naturally Occurring Radioactive Material
O&G	Oil and Grease
PAH	Polycyclic Aromatic Hydrocarbon
RHO	Ring Hydroxylating Oxygenases
SBBR	Sequencing Batch Biofilm Reactor
SBR	Sequencing Batch Reactor
SS	Suspended Solids
TOC	Total Organic Carbon
TSS	Total Suspended Solids
XRD	X-Ray Diffraction

CHAPTER 1 INTRODUCTION

1.1 Background

Produced water is generally known to be the largest volume waste stream in oil and gas exploration activities. It is a by-product inevitably produced during the exploitation process of oil and gas (Scurtu, 2009). In the early stage of production, relatively little amount of wastewater is produced. However, as the oil and gas reservoirs become older and moving towards their completion stages, the proportion of waste water may rise to as high as 80% (Lu et al., 2006).

Formation water, injection water, and process water that have been extracted during petroleum production are included as produced water. Also, chemicals added during processing may attach to the produced water. Normally, produced water is used for re-injection into the well for oil enhancing recovery but in some cases re-injection is not practical due to geographic and cost consideration. Hence, disposal or reuse will be another option (Veil et al., 2004). However, both options are still required to undergo treatment processes due to the composition of produced water that may lead to serious environmental impacts (Arthur et al., 2005).

Oilfield wastewater is usually brackish or salty in quality. The constituents of wastewater that are greatly considered as the major threat to the environments are dispersed oil, partially soluble hydrocarbons, phenols, and some hazardous chemicals (Faksness et al., 2004). However, the composition of wastewater from oil production is different compared to the wastewater generated from gas production. Wastewater generated from gas production contains condensed water which is originally in vapour state while in the reservoir but later condensed to be in the liquid state in the production separation system (Clark and Veil, 2009).

Disposal of oilfield wastewater is prohibited in many oil producing countries unless certain regulatory requirements is achieved. Physical treatment, chemical treatment, and biological treatment are the most common treatment applied in treating wastewater. In recent years, many technologies have been developed in order to remove toxic and hazardous pollutants.

Biological treatments systems are widely used in the wastewater treatment and have been demonstrated to be environmentally friendly and cost-effective technologies (Vieira et al., 2005). However, treating oilfield wastewater can be challenging as this type of wastewater is categorized as hypersaline waste. Conventional microorganisms demonstrate poor degradation in hypersaline environments. Therefore, extremophiles such as halophilic microorganisms can be another alternative in treating hypersaline wastewater (Song Yan et al., 2004).

Sequencing batch reactor (SBR) system is a promising biological system in treating wastewaters. It is a modification of activated sludge system which has been successfully used in treating various wastewaters. Besides, SBR is able to result a high percentage removal of biological oxygen demand and chemical oxygen demand (Mahvi, 2008).

1.2 Problem Statement

A number of studies on biological treatment of hypersaline wastewater have proven that microorganisms specifically bacteria, yeast, and fungi can act as petroleum-hydrocarbon degraders (Li et al., 2005). However, because of the characteristics of produced water where the salinity concentration is rather high, the activity rate of microorganisms fell significantly (Pendashteh et al., 2010).

In a hypersaline environment such as oilfield wastewater, extremophiles such as halo tolerant and halophilic microorganisms have the ability to survive. In fact, certain halophilic species need high salinity environment to live. Non-halophilic microorganisms cannot survive such hypersaline environments and could not function properly as the environmental conditions tend to induce plasmolysis (Peyton et al., 2002).

Halophilic extremophiles are easily found in salt lake. As in Malaysia, there is no salt lake and the salinity of estuaries in Malaysia is approximately the same as seawater. Another source that halo tolerant and halophilic microorganisms are likely to be found is from salt food product.

As salt concentration increases, the biological diversity will decrease and it is said when the concentrations are approximately 150 g/L to 200 g/L, microorganisms will be less active and eventually did not manage to survive. However, recent discovery shows that these highly salt-tolerant and highly salt-requiring microorganisms can be found in high salt concentrations more than 300 g/L. Such environments can be found at a place such as Dead Sea, Great Salt Lake, in salt crystallizer ponds, estuaries, and even salt-food products (C. Gerday, 2007).

1.3 Objectives of Research

The main goal of this research is to investigate the ability of microorganisms from salt food product in treating oilfield wastewater. Specific goals and objectives of this research include:

1. To identify bacterial species from salt food product that can be utilised for oilfield wastewater treatment and investigate the removal efficiency of COD and O&G in effluent wastewater for four different hydraulic retention time (HRT).
2. To analyse the effectiveness of hydrocarbon biodegradation in oilfield wastewater by the mixed bacterial consortium isolated from salt food product.

1.4 Scope of Research

Produced water is a general term of wastewater generated during the production of oil and gas. This research focuses mainly on biological treatment of wastewater generated from oil production, which is also known as oilfield wastewater.

Biological treatment gives many benefits to operators including cost effective and more environmental friendly compared to physical and chemical treatment. A lab-scale sequencing batch reactor (SBR) is selected to be applied in this study. A lot of study has proved that SBR method has been successfully applied in wastewater treatment. Besides, this process offers advantages of better stability and better operational flexibility (Al-Harazin, 1992).

The source of microorganisms used to degrade petroleum-hydrocarbon is isolated from salt food product. Since salt food product is originally in high salinity environments, the chances to discover halophilic oil-degrading microorganisms are high. This research may then prove that the source of petroleum-hydrocarbon degrading microorganisms is not limited to polluted soil, unpolluted soil, reservoir, or sediments.

The characteristic of oilfield wastewater is analysed in this research. The purpose is to determine the quality of wastewater before and after biological treatment process whether it will meet the regulatory compliance or need further treatment. Besides, the removal efficiency of certain parameters such as chemical oxygen demand (COD), and Oil and Grease (O&G) are studied. Hydrocarbon degradation is also investigated in this study by observing the percentage degradation of n-alkanes and aromatic compound.

1.5 Novelty of Research

Salt food product can pose as another source of petroleum hydrocarbon degrading bacteria.

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