

ISOLATION AND IDENTIFICATION OF BACTERIA CAPABLE OF SULFATE
REDUCTION FROM PALM OIL SLUDGE

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*To all my beloved family members;
my lovely husband, Omid
and my kind child Ali*

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ABSTRACT

Sulfur in its native is a yellow crystalline solid. In nature, it occurs as the pure element or as sulfide and sulfate minerals. Inorganic sulfur compounds can be found in the form of sulfate, sulfide, sulfite, thiosulfate, elemental sulfur and polythionates. Sulfate appears to be the most stable and abundant form of sulfur available for use by living organism in the biosphere. This present study focused on the isolation and identification of bacteria capable of sulfate reduction from palm oil sludge (POS). POS is one of the most difficult and complex industrial waste produced in Malaysia from palm oil processing plants. Three different samples of POS were collected from different pond of Palm Oil effluent in palm oil processing plant in Sedenak, Kulai, Malaysia. The concentration of sulfate content in the samples were analysed in order to determine the sample that contain high population of bacteria capable of sulfate reduction. This ensures the possibility of isolating the bacteria of interest from the selected sample. Results from SRB-Bart kit analysis showed that POS from raw pond contain high population of SRB or related bacteria and was chosen for further isolation of the bacteria. Isolation of the bacteria was conducted using selective enrichment method followed by growth on solid medium using rolling bottle technique. The isolation has successfully separated five different pure culture coded X2 , X1, E, B and C that were further identified using the analysis of amplified 16S rRNA sequences of the individual bacterium. Four of the bacteria namely E, B, X1, and X2 were found able to reductively degrade sulfate. These bacteria were able to grow and reduce limited amount of sulfate thus indicated to the assimilatory reduction of sulfate activity of these bacteria.

ABSTRAK

Sulfur asli adalah pepejal kristal kuning. Secara semula jadi ia berlaku sebagai elemen tulen atau sebagai mineral sulfida dan sulfat. Sebatian sulfur bukan organik boleh didapati dalam bentuk sulfida sulfat, sulfit, thiosulfate, sulfur unsur dan polythionates. Sulfat merupakan bentuk yang paling stabil dan banyak didapati untuk digunakan oleh organisma hidup dalam biosfera. Kajian ini memberi tumpuan kepada pengasingan dan pengenalpastian bakteria yang mampu mengurangkan sulfat dari enapcemar kelapa sawit (POS). POS adalah salah satu sisa yang paling sukar dan kompleks oleh perindustrian yang dihasilkan di Malaysia dari kilang pemprosesan minyak sawit. Tiga sampel POS yang berbeza telah dikumpulkan dari kolam minyak sawit efluen yang berbeza di loji pemprosesan minyak sawit di Sedenak, Kulai, Malaysia. Kepekatan kandungan sulfat dalam sampel dianalisis untuk menentukan sampel yang mengandungi populasi bakteria yang mampu mengurangkan sulfat tertinggi. Hal ini bagi memastikan kemungkinan bakteria berfaedah dapat diisolasi daripada sampel yang dipilih. Keputusan dari SRB Bart kit analisis menunjukkan bahawa POS dari kolam mentah mengandungi populasi bakteria SRB yang tinggi dan telah dipilih untuk diisolasi bagi proses selanjutnya. Pengisolasian bakteria telah dijalankan menggunakan kaedah pengayaan terpilih diikuti oleh pertumbuhan pada medium pepejal menggunakan teknik botol *rolling*. Pengisolasian telah berjaya mengasingkan lima kultur tulen yang dikodkan sebagai X2, X1, E, B dan C yang seterusnya dikenal pasti menggunakan analisis 16S rRNA urutan bakteria individu. Empat bakteria iaitu E, B, X1, dan X2 telah didapati mampu untuk mengurangkan kandungan sulfat. Bakteria ini mampu untuk berkembangbiak dan mengurangkan jumlah sulfat dalam kadar terhad, lantas menunjukkan pengurangan asimilasi aktiviti sulfat bakteria ini.

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LIST OF ABBREVIATIONS

H ₂ S	-	Hydrogen Sulfide
SRB	-	Sulfate Reducing Bacteria
PAPS	-	3'-Phosphadenosine 5'-Phosphosulfate
APS	-	Adenosine-5'-Phosphosulfate
FeS ₂	-	Iron Disulfide
CS ₂	-	Carbon Disulfide
ATP	-	Adenosine-3'-Phosphosulfate
PAP	-	3', 5'-Diphosphadenosine
AMP	-	Adenosine-1'-Phosphosulfate
AMD	-	Acid Mine Drainage
ARD	-	Acid Rock Drainage
SRM	-	Sulfate Reducing Microorganism
PCE	-	Perchloroethylene
FGD	-	Flue Gas Desulfurization
MIC	-	Microbiologically Influenced Corrosion
OD	-	Optical Density EDTA
EDTA	-	Ethylenediaminetetraacetic Acid
TAE Buffer	-	Tris-acetate-EDTA buffer

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Insulating Sulfur is the tasteless, odorless and plentiful. It is yellow crystalline solid in the native and in the nature; it is as the pure element, sulfide and sulfate minerals. The H₂S, which is produced from sulfur, has the odor and the smell of it is compared to the rotten eggs. Inorganic sulfur compounds can be found in different forms such as sulfide, sulfite, thiosulfate, polythionates and elemental sulfur. One of the abundant and stable forms of sulfur, which is use with the living organism, is sulfate (Komarnisky *et al.*, 2005).

Different groups of microorganism have the ability to reduce sulfate (Peck, 1961). These microorganisms can be divided in to two groups, the first group which Reduce sulfate in the small amounts such as *Pseudomonas* sp, *Bacillus* sp, *Proteus mirabilis*, *heterotrophy-Proteus vulgaris* and *Saccharomyces* were called assimilatory sulfate reducers (Berndt and Vargas, 1987). The other group is restricted to the bacteria and archaeal, they used sulfate as the terminal electron acceptor in the anaerobic condition and they were called dissimilatory sulfate reducers.

These two groups have the very important role in the sulfur cycle (Peck, 1961). The important group of dissimilatory sulfate reducer are sulfate reducing bacteria. They are nonpathogenic bacteria. They can live in the environment that is strictly anaerobic, but recently it has been found that some species of them can tolerate oxygen for the short time (Cypionka, 2000). These bacteria have the ability to tolerate the toxic environment more than the other anaerobic bacteria, this makes them special for using them in the remediation process. They can use sulfate, sulfite, thiosulfate as the electron acceptor, also they contribute in the recycling of elemental sulfur in nature (Butlin *et al.*, 1949; Zhang *et al.*, 2009).

By reduction of sulfate to the H_2S , the net alkalinity is generated. These bacteria can be found in different environments such as soil, mud and sediments of freshwaters (rivers and lakes), thermal environments, waters deposited from petroleum processing and many others (Wargin *et al.*, 2007).

Determining the kinetics of growth and sulfur reducing activity in different physicochemical conditions will be useful in determining growth and survival of particular type of bacteria, which are able to reduce sulfate in specific environment. This will give significant contribution in the planning and modulating of industrial process and installations. For example in the case of corrosion prevention, the choice of biocide can be easily determined when the structure and characteristics of these bacteria community in the system can be predicted. The bacteria which degrade sulfate are known as the source of 75% corrosion occurs in production wells and more than 50% failures of buried pipelines and cables (Anandkumar *et al.*, 2009).

1.2 Scope of the Study

In this research, three samples of Palm oil sludge from different ponds (anaerobic, aerobic and raw) were collected from Mahamurni Plantations, Sedenak, Kulai, Malaysia. The samples were used to isolate and identify culturable bacteria, which able to reduce sulfate. The selection of palm oil sludge was carried out in order to determine the density of these bacteria in the sample. SRB-BART kit was used to analyze the presence of population of these bacteria in all samples collected. Isolation were carried out employing enrichment technique, under anaerobic condition. Pure culture of the bacteria was selected for further identification based on phylogenetic characterization. Growth and sulfate reducing activities of the selected bacteria were investigated.

1.3 Problem of the Statement

The bacteria which reduce sulfate causing the very bad odour , the smell of it is like the rotten egg. This smell is due to the H_2S and this gas is very toxic. The presence of this gas in the equipments of industries specially in the petroleum tanks caused damage in the subsurface and surface of the equipments and this the main problems in the industries (ZoBell, 1958). One of the reasons of reservoir souring is due to the production of H_2S which is called biogenetic H_2S which sulfate reduces to sulfide due to anaerobic bacteria activity (Fitzgerald *et al.*, 1998).

These bacteria have the important role in the corrosion in the pipelines and other industrial installation. The oil industry estimates that SRB are the cause of large economically losses due to corrosion damages in pipelines (Anandkumar *et al.*, 2009; Gittel *et al.*, 2009; Sungur *et al.*, 2010)

1.4 Objective of the Study

This study was designed to meet several objectives as follow:

(a) To isolate the bacteria with the ability of reduced sulfate from suitable palm oil sludge using enrichment technique

(b) To identify physiological and phylogenetic characteristics of selected pure bacteria

(c) To determine the kinetics of growth and sulfur reducing activity of selected bacteria

1.5 Significance of the Study

The interest in bacteria due to the ability of removal of sulfate and heavy metals, so the culturing and identification of these bacteria from the environment is very important for understanding the mechanism of them to help in control the growth and activities of them (Luptakova, 2007).

The degradation of sulfate to produce hydrogen sulfide by these bacteria causes the significant production of alkalinity. On the other hand hydrogen sulfide capable of binding with heavy metals and caused precipitation of the metal sulfide, so it helps to the process of metal removal. Successful isolation and cultivation of SRB enable further manipulation of the bacteria to enhance environmental

bioremediation such as in the treatment of acid mine drainage (AMD) developed from accumulation of high concentration of sulfur or sulfate (Zhang *et al.*, 2009).

Some microorganisms have the ability to degrade sulfur-containing crude oil; one of the famous families that used in industries is SRB. Some species of Gamaproteobacteria have this ability by recognizing the bacteria which capable to reduce sulfate can be help to control the removal of sulfur content from crude oil and improving the quality of it (Sherry *et al.*, 2012; Suárez-Suárez *et al.*, 2011).

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