

**ALUMINIUM (III) BIOSORPTION MAGNETOTACTIC
ALCALIGENES SP. SUM 123 ISOLATED FROM
SKUDAI RIVER JOHOR**

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ALUMINIUM (III) BIOSORPTION MAGNETOTACTIC
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SKUDAI RIVER JOHOR

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This thesis work is dedicated to my husband, Abdelhafid, who has been a constant source of support and encouragement during the challenges of graduate school and life. I am truly thankful for having you in my life. This work is also dedicated to my parents, Muftah and Fatima, who have always loved me unconditionally and whose good examples have taught me to work hard for the things that I aspire to achieve.

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ABSTRACT

The increasing application of aluminium metals in various industrial processes have raised significant concerns and health risks for humans and its environments. In its ionic form, aluminium poses higher threats to human health due to its ability to cause cellular impairment. As a remediation tool, biosorption by magnetotactic bacteria (MTB) is considered in this study for the removal of this metallic pollutant due to its ability to adsorb heavy metals. The isolation of iron oxide-producing *Alcaligenes* sp. strain SUM 123 for aluminium(III) biosorption was conducted from samples collected from Skudai River. Biochemical tests and 16S rRNA characterization was employed for the identification of the isolated magnetotactic bacterium (MTB). The characterization of this MTB was determined using a High Resolution Transmission Electron Microscope (HRTEM), X-Ray Diffraction (XRD), Scanning Transmission Electron Microscope and Energy Dispersive x-ray Spectroscopy (STEM-EDX) and the Fourier Transform Infrared Spectroscopy (FTIR). Observation by HRTEM shows the lattice spacing of iron oxide at 0.24 nm and 0.31 nm while the XRD analysis depicts the presence of crystalline planes of iron oxide at (311) and (220). The magnetosomes observed via STEM-EDX analysis confirms the presence of iron oxide and the composition of P-granules containing different heavy metals in the isolated *Alcaligenes* sp. strain SUM 123. The appearance of Fe-O groups of magnetosomes were observed via FTIR analysis. In addition, the decomposition of these iron oxide components was at 270-500 °C according to thermogravimetric analysis (TGA). The MTB growth and magnetosomes formation were studied at different pH (5-8), temperature (20-40 °C), and ferric quinate concentration (20-120 µM). It was observed that magnetosomes formation is significantly influenced by pH change and relatively unaffected by variations in temperature and ferric quinate concentrations. Aluminium(III) adsorption by the isolated *Alcaligenes* sp. strain SUM 123 was examined at pH 2-9, temperature 10-40 °C, initial Al(III) concentration 80-500 mg.L⁻¹, contact time 10-60 mins and adsorbent dosage 2-12 g.L⁻¹. The optimal adsorption of Al(III) by SUM 123 was observed at pH 5, temperature 25 °C, 80 mg.L⁻¹ initial Al(III) concentration, 60 mins contact time and an adsorbent dosage of 10 g.L⁻¹. The biosorption process of Al(III) by SUM 123 was best fitted to the Langmuir isotherm model, while the pseudo-second order was found to be the best describe the experimental data. According to FTIR analysis, it was found that the hydroxyl, amide, and amine groups of the magnetosomes were involved in the biosorption process. It is therefore established from this study that the iron oxide-producing *Alcaligenes* sp. strain SUM 123 is a potentially effective and economical remediation tool for aluminium(III) removal in industrial applications.

ABSTRAK

Penggunaan logam aluminium dalam pelbagai industri telah menimbulkan kebimbangan dan risiko kesihatan yang tinggi terhadap manusia dan persekitarannya. Logam aluminium dalam bentuk ion membawa ancaman yang lebih besar kepada kesihatan manusia kerana keupayaannya untuk menyebabkan kemerosotan sel. Sebagai alat rawatan, bioerapan menggunakan bakteria magnetotaktik (MTB) digunakan dalam kajian ini untuk penghapusan pencemar metalik disebabkan oleh kebolehan penjerapan logam beratnya. Pengasingan ferum oksida yang menghasilkan *Alcaligenes* sp. strain SUM 123 bagi bioerapan aluminium(III) dijalankan melalui sampel yang dikutip daripada Sungai Skudai. Ujian biokimia dan protokol analisis gen 16S rRNA digunakan untuk mengenal pasti bakteria magnetotaktik terpencil (MTB). Pencirian MTB ditentukan dengan menggunakan Mikroskop Pancaran Elektron Beresolusi Tinggi (HRTEM), Pembelauan Sinar-X (XRD), Mikroskop Pengimbas Transmisi Elektron dan Spektroskopi Sinar-X Tenaga Serakan (STEM-EDX) dan Spektroskopi Inframerah Transformasi Fourier (FTIR). Kajian HRTEM menunjukkan ruang kekisi ferum oksida pada 0.24 nm dan 0.31 nm manakala analisis XRD menggambarkan kehadiran satah berhablur ferum oksidapada (311) dan (220). Tinjauan ke atas magnetosom melalui analisis STEM-EDX mengesahkan kehadiran ferum oksida dan komposisi granul-P yang mengandungi pelbagai jenis logam berat dalam *Alcaligenes* sp strain SUM 123. Kemunculan kumpulan magnetosom Fe-O dicerap melalui analisis FTIR. Berdasarkan Analisis Termogravimetrik (TGA) penguraian ferum oksida adalah pada 270-500 °C. Pertumbuhan MTB dan pembentukan magnetosom telah dikaji pada pelbagai julat pH (5-8), suhu (20-40 °C), dan kepekatan ferik quinate (20-120 µM). Hasil tinjauan menunjukkan bahawa pembentukan magnetosom dipengaruhi oleh perubahan pH dan secara relatifnya tidak terjejas oleh perubahan suhu dan kepekatan ferik quinate. Penjerapan aluminium(III) oleh *Alcaligenes* sp. strain SUM 123 dikaji pada pH 2-9, suhu 10-40 °C, kepekatan awal Al(III) 80-500 mg.L⁻¹, masa sentuhan 10-60 minit dan dos penjerap 2-12 g.L⁻¹. Penjerapan optimum Al(III) oleh SUM 123 yang ditinjau adalah pada pH 5, suhu 25 °C, kepekatan awal Al(III) 80 mg.L⁻¹, masa sentuhan 60 minit dan dos penjerap 10 g.L⁻¹. Proses bioerapan Al(III) dengan SUM 123 paling menepati model isoterma Langmuir, manakala model aturan pseudo-kedua didapati paling baik menggambarkan data eksperimen. Analisis FTIR menunjukkan penglibatan kumpulan hidroksil, amida dan amina daripada magnetosom dalam proses bioerapan. Hasil daripada kajian ini menunjukkan *Alcaligenes* sp. strain SUM 123 yang menghasilkan ferum oksida merupakan alat rawatan yang berkesan dan ekonomikal untuk penyingkiran aluminium(III) dalam aplikasi industri.

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LIST OF ABBREVIATIONS

μM	-	Micromolar
μm	-	Micrometer
AAS	-	Atomic Absorption Spectroscopy
BCM	-	Biological-Controlled Mineralization
CRM	-	Capillary method
CSD	-	Crystal Size Distribution
DDW	-	Distilled deionized water
DIC	-	Differential Interference Contrast
DNA	-	Deoxyribonucleic acid
DOE	-	Department of Environment
<i>E. coli</i>	-	<i>Escherichia coli</i>
EDX	-	Energy X-ray diffraction
EMA	-	European Medicines Agency
FDA	-	Food and Drug and Administration
FESEM-EDX	-	Field Emission Scanning Electron Microscopy-Energy X-ray Diffraction
FTIR	-	Fourier Transform Infrared Spectroscopy
GM	-	Growth medium
HRTEM	-	High Resolution Transmission Electron Microscope
IM	-	Isolation medium
LM	-	Light microscopy
M	-	Molar
mbar	-	millibar
MMP	-	Multicelled magnetic prokaryote
MSs	-	Magnetosomes
MTB	-	Magnetotactic bacteria

NCBI	-	National Center of Biotechnology Information
nm	-	nanometer
nmol	-	Nanomole
NS	-	North Seeking
OD	-	Optical Density
OTAZ	-	Oxic-anoxic transition zones
ppm	-	part per million
RLS	-	rate limiting step
rpm	-	Revolutions per minute
SD	-	Single-magnetic Domain
SP	-	Superparamagnetic
SS	-	South Seeking
STEM	-	Scanning Transmission Electron Microscope
TEM	-	Transmission electron microscopy
TGA	-	Thermogravimetric Analysis
USA	-	United States of America
XRD	-	X-ray Diffraction

LIST OF SYMBOLS

$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	-	Aluminium chloride
C_e	-	Equilibrium aluminium concentration (ppm)
C_o	-	Initial aluminium concentration (ppm)
Fe_3O_4	-	magnetite
Fe_3S_4	-	greigite
FeS	-	Mackinawite
K_1	-	Equilibrium rate constant of pseudo-first order kinetic model (1/min)
K_2	-	Equilibrium rate constant of pseudo-second order kinetic model (g/mg.min)
K_D	-	Dissociation constant
K_F	-	Freundlich constant (dm ³ /mg)
n	-	Intensity of adsorption
q_e	-	Amount adsorbed at equilibrium condition (mg/g)
q_t	-	Adsorption capacity at time t (mg/g)
R^2	-	Correlation coefficient
RL	-	Langmuir parameter

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CHAPTER 1

INTRODUCTION

1.1 Introduction

There are many occasions when there is a direct overlap between geology and biology. Perhaps one of the best examples of this is magnetotactic bacteria (MTBs). They appear to be a distinctive Gram-negative prokaryote that have the ability to synthesize small crystals of magnetite inside their cells. They do this by transporting iron from the surrounding environment into their body. They use a number of proteins to biomineralize the iron into nano-sized magnets of magnetite (Fe_3O_4), greigite (Fe_3S_4) or combination of both (Bazylinski *et al.*, 1993a, 1995; Lower and Bazylinski, 2013). These aquatic microorganisms use the magnets to allow them to orient themselves in the Earth's magnetic field. They use flagella as a source of motility and they are sensitive to the changes in oxygen concentration (Bazylinski and Frankel, 2004).

MTB vary in many aspects but they are united by the presence of magnetosomes. Most discovered MTB are affiliated with *alphaproteobacteria*, but MTB belonging to the *Gammaproteobacteria*, the *Deltaproteobacteria*, and *Nitrospirae* have also been described (Amann *et al.*, 2007; Lefèvre and Bazylinski, 2013). The variations in cell morphology are represented by coccoid, rod-shaped,

spirilla, vibrio and multicellular microorganisms (Keim *et al.*, 2004; Schuler, 2002).

MTBs can be found worldwide in the sediment from various aquatic environments including brackish and fresh water, rivers, lakes, and hot springs (Blackmore, 1975; Moench and Konetzka, 1978; Spring *et al.*, 1994; Rulík and Chaudhary, 2014; Ghazvini *et al.*, 2014; Lin *et al.*, 2009; Oestreicher *et al.*, 2012; Lefèvre *et al.*, 2010b). Bacterial magnetite contributes to the magnetic signal of the sediments and is widely distributed mainly in natural habitats (Oestreicher *et al.*, 2012) such as marine region (Zhu *et al.*, 2010), pond ecosystem (Simmons and Edwards, 2007; Lin *et al.*, 2013), iron ore soil (Liu *et al.*, 2006) and estuarine region (Hergt *et al.*, 2005).

The magnetosome is the defining feature of magnetotactic bacteria. It is denoted as an intracellular, lipid membrane-bound, magnetic iron-containing inorganic crystal (Frankel and Bazylinski, 2006; Lefèvre and Bazylinski, 2013). Two types of iron-containing crystals are known to be produced by magnetotactic bacteria: an iron oxide, magnetite (Fe_3O_4), and an iron sulfide, greigite (Fe_3S_4). Only a unique collection of marine magnetotactic multicellular prokaryotes (MMP), of the *Deltaproteobacteria* class, are reported to biomineralize both types of minerals (Lefèvre *et al.*, 2011). The crystal morphologies of magnetosomes in MTB include cuboidal (cubo-octahedral), elongated prismatic (quasi-rectangular), arrowhead-shaped (bullet-shaped) and unusually large elongated prismatic crystals (Bazylinski and Frankel, 2003; Lins *et al.*, 2005; Yan *et al.*, 2012). It is shown that each MTB species have their own crystal habits that are different from a biotically produced magnetite particles. The morphology of biologically synthesized magnetic particles is strain specific. The size is almost equal within one strain; however, sizes can range from 35 to 120 nm for different MTB (Bazylinski and Frankel, 2004).

Magnetosomes (MSs) of MTB composed of various sizes and types of amorphous minerals granules such as calcium, oxygen, and phosphorus and to a lesser extent aluminium, iron, and zinc (Balkwill *et al.*, 1980; Ulysses and Marcos,

1999). Some granules containing polyphosphates represent a new category of MSs (Ulysses and Marcos, 1999). Because of their involvement with various metals ions, MTB probably play a significant role in geochemical cycling (Simmons *et al.*, 2007). It is common for MTB to contain internal granules, especially phosphorus (Lins and Farina, 1999), and sulphur (Keim and Farina, 2005).

Researchers revealed that MTB can be used to recover precious metals, which is apparently a major solution for industrial processing concerns (Gao *et al.*, 2007). MTB can adsorb heavy metal ions more than other bacteria and it can easily be extracted from an aqueous medium after biosorption (Qu *et al.*, 2014). The presence of magnetosomes helps to separate MTB from their environment using permanent magnet or electromagnetic field.

Globally, toxic metal ions mediated pollution is continuously growing. Industries such as mining, smelting, and metal plating cause heavy metals contamination in water (Vijayaraghavan and Yun, 2008). Heavy metals accumulation in the food chain cause tremendous ecological imbalances and is detrimental unless inhibited. Thus, there is a need to explore the cheap and environmental friendly process which can act as a shield to these threats to increase the standard of living and to make world a better place to live (Magdalena and Małgorzata, 2014). Countries having strong environmental laws to limit the use of contaminant being wasted in the environment (without being treated under consideration) are urged to developed on site or in plant facilities to treat the effluents to make the pollutants under the acceptable concentration (Banat *et al.*, 1996; Vijayaraghavan and Yun, 2008).

Aluminium (Al) is a type of toxic heavy metal. Its exposure to human results in the increase rates of neurological disorder including Alzheimer, Parkinson, and Skeletal diseases (osteomalacia) (Akesson *et al.*, 2014; Stephen, 2010). Often, several industries such as food (canning, and packing), kitchen utensils (Tuzen and Soylak, 2008; Yokel, 2016), transportation (Tuzen and Soylak, 2008) and chemicals

(catalyst, pigment, tanning agent and as a mordant) predominantly use this heavy metal. It is also used to make abrasives, cement, explosives, and ink (Tuzen and Soyak, 2008). Despite its toxicity, it is used in pharmaceutical industry to create antacids and anti-diarrhoea medicines. This predominant usage of Aluminium in industries makes it abundant in the natural environment. Therefore, Scientists and engineers are using several methods to reduce the concentration of metals in the industrial wastewater; it includes agglomeration, neutralization, complexation, ion-exchange resin, separation and elution (Meshram *et al.*, 2014).

Therefore, the development in reducing the metals in environment finds a new method, which is called biosorption. This method has received great attention in the recent years due to its low cost and high capacities. The mechanism of adsorption by biomass can be described as a passive immobilization of metal ions. It is essentially based on physicochemical interaction between functional groups of the cell wall and metal. Likewise, the cell wall of bacteria generally consists of proteins, lipids and polysaccharides which contain functional groups, such as amino groups, phosphate, hydroxyl and carboxylate so these functional groups offer binding sites for metals (Won *et al.*, 2013).

In this study, a new variant of MTB (*Alcaligenes sp* SUM 123) was isolated from Skudai River water as potential resource to remove Al (III) from aqueous solution. The influence of several external factors such as temperature, pH, Al (III) concentration, and biomass dosage on Aluminium ion biosorption efficacy of such MTB was scrutinized. Earlier studies are mainly focused on the isolation as well as cultivation of MTB and MSs (Schüler and Heyen, 2003; Ghazvini *et al.*, 2014). However their biosorption effectiveness towards heavy metals ions is seldom reported (Wu *et al.*, 2008).

1.2 Problem Statement

Currently, the chemical explosion from rapid industrialization and expansion of modern agriculture area is the main concern of the environment and human health. Toxic Aluminium metal accumulated and degenerations the environment (Denizli *et al.*, 2003). Methods such as chemical precipitation, chemical oxidation or reduction, membrane and evaporation technology, electrochemical treatment, and filtration which are introduced to remove the accumulation of heavy metals are expensive, inaccurate and inefficient (Gunatilake, 2015). To overcome such limitations, an appropriate biological method need to be developed.

Magnetotactic bacteria (MTBs) are ubiquitous in diverse terrestrial and aquatic ecosystem. Malaysia is a tropical country surrounded by oceans, rivers, and lakes and can be a great source of MTB. Thus, it is worth to explore the sediment of Skudai River in Johor, Malaysia as potential resource for MTB isolation. The isolated MTB can be a usefull tools in different area of study such as heavy metals biosorption.

Although much attention has been focused on the mechanisms of biomineralization in MTB (Bazylinski *et al.*, 1995; Taylor and Barry, 2004; Rahn-Lee and Komeili, 2013), lack information available on MTB growth and magnetosomes formation under different environmental conditions such as pH, temperature, and iron concentration, since these conditions will affect the magnetosome formation and the performance of the MTB in any area of study. Thus, determining the survival and growth conditions of these MTB together with the formation of MSs in a broad range of environmental parameters is a challenging task.

Pervious studies used MTB to remove different types of metals such as Cr (V) ion from wastewater (Qu *et al.*, 2014). In addition, Au (III), Cu (III) ions adsorbed from aqueous solution using MTB (Song *et al.*, 2007). Therefore, the

present thesis exploits a new variant of MTB (*Alcaligenes sp* SUM 123) isolated from Skudai River as potential resource to remove Al (III) ions metal from aqueous solution with various range of factors such as temperature, pH, Al (III) concentration, and biomass dosages. The magnetotactic bacteria have the ability to adsorb high concentration of heavy metals from aqueous solution in a short time compared to other microorganisms. Besides, The presences of magnetosomes in MTBs will help in extracting them from the treatment system just by using a magnet after the biosorption processes.

1.3 Research Objectives

Based on the problem statement the following objectives are set:

- i. To isolate MTB from Skudai River sediment (Johor Bahru, Malaysia) and identify them using 16S rRNA sequencing analysis and biochemical method.
- ii. To characterize the structure and properties of MTB and magnetosomes.
- iii. To determine the influence of temperature, pH, ferric quinate concentration as iron source on the growth of MTB and magnetosomes formation.
- iv. To evaluate the Al (III) ions biosorption efficiency of newly isolated MTB from aqueous solution under varying temperature, pH, biomass concentration and Al concentration.
- v. To assess biosorption potency via isotherm (Langmuir and Freundlich model) and kinetics study.

1.4 Scope of the Study

Based on the objectives the research scopes are limited to the following aspects:

- i. The MTB was isolated from Skudai River water sediment and was identified using 16S rRNA gene analysis and biochemical methods such as catalase, nitrate, indole, motility, MacConkey, starch, lipase, ureas, and citrate tests.
- ii. Characterization of the isolated bacterium as magnetotactic bacteria was conducted using Scanning Transmission Electron Microscope-Energy x-ray diffraction (STEM-EDX) that showed the appearance of magnetosomes inside bacterium and P-granules which is a common trait of MTB. While, X-Ray Diffraction (XRD) detected the crystalline structure of magnetite (Fe_3O_4) in MTB, and High Resolution Transmission Electron Microscope (HRTEM) also confirmed the lattice space of magnetite. Infrared Fourier Transform Spectroscopy (FTIR) revealed the band of Fe-O at 585 cm^{-1} wavenumber. In addition, the domain structure by magnetotactic bacterium detected the magnetism of magnetite as trait for magnetotactic bacterium.
- iii. The effects of pH at (5, 6, 7, 8), temperature at (25, 30, 35, 40 °C) , and ferric quinate as iron source (20- 120 μM) on the MTB growth and magnetosomes formation was determined.
- iv. The biosorption of Al (III) from aqueous solution by isolated MTB was studied under varying parameters such as pH (2-9), contact time (5-70 minutes), temperature (10- 40 °C), biosorbent dosage (2- 12 g/L), and initial Al concentration (100-500 mg/L).
- v. The behavior and mechanism of biosorption study conducted via isotherm (Langmuir and Freundlich model), kinetics models (Pseudo-first order and Pseudo-second order), and thermodynamic equations.

1.5 Significance of the Study

The isolation of these MTB is expected to bring new knowledge in terms of their growth, morphology, formation of MSs under various external conditions. Upon understanding their structures, growth mechanisms, and magnetic properties it would be possible to implement them for various potential applications in medicine, biotechnology, and bioremediation. For the first time, this work would attempt to isolate new MTB species from Skudai River mud (Malaysia) as new biological source useful for Al (III) biosorption. Thus, major environmental concern regarding the pollution of water by toxic heavy metals and its consequences in ecological imbalances would be mitigated. The isolation of MTB from polluted soil will aid in the use such MTB for removing heavy metals from wastewater and soil. The use of biomass has received a lot attention nowadays due to it has low cost and high adsorption capacity. Furthermore, the MTB used in this study has been screened for its ability to resist high concentration of Al (III). Thus, it can be an economical adsorbent after we got the motivation to remove high concentration of Aluminum. The outcome of the research can be used to prove the existence of specific microorganism can survives in a polluted environment.

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