



UNIVERSITI PUTRA MALAYSIA

***ISOLATION, PARTIAL PURIFICATION AND CHARACTERIZATION OF
MOLYBDENUM-REDUCING ENZYMES FROM AN ANTARTICA
BACTERIUM (*gamma-Proteobacterium* STRAIN DR. Y1)***

SITI AQLIMA BINTI AHMAD

FBSB 2006 30



**ISOLATION, PARTIAL PURIFICATION AND CHARACTERIZATION OF
MOLYBDENUM-REDUCING ENZYMES FROM AN ANTARTICA
BACTERIUM (γ -Proteobacterium STRAIN DR.Y1)**

By

SITI AQLIMA BINTI AHMAD

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

December 2006



Dedicated to my parents, family and friends.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

**ISOLATION, PARTIAL PURIFICATION AND CHARACTERIZATION OF
MOLYBDENUM-REDUCING ENZYMES FROM AN ANTARTICA
BACTERIUM (*gamma-Proteobacterium* STRAIN DR.Y1)**

By

SITI AQLIMA BINTI AHMAD

December 2006

Chairman: Professor Nor Aripin Shamaan, PhD

Faculty : Biotechnology and Biomolecular Sciences

Bacterial Isolate no. J7A was isolated from Jubany Station, Antarctica and it has the capability to reduce the heavy metal molybdenum (molybdate) to molybdenum blue in a solid medium agar, pH 7 at 10°C, after for 4 days of incubation. Isolate J7A was identified as Gram-negative and *gamma-Proteobacterium* Strain Dr.Y1 through molecular phylogenetics analysis of the sequenced 16s rRNA gene. The optimization studies were carried out to optimize the production of molybdenum blue. The combination of 1% (w/v) glucose, 0.3% (w/v) ammonium sulphate, 0.1% (w/v) of yeast extract, 30mM molybdate, and low phosphate medium at pH 7 give the optimum production of Molybdenum blue. Partial purification and characterization were conducted on molybdenum reducing enzyme with anion exchange chromatography using Macro-Prep High-Q™ column and gel filtration chromatography using Agilent Zorbax™ (GF-250) column. Three bands were visualized on the gel filtration fraction at 39, 36 and 33 kDa using the SDS polyacrylamide-gel electrophoresis (SDS-PAGE) suggesting that purification was



not achieved. In enzyme kinetic studies, NADH serves as the substrate for electron donor and 12-MP act as the substrate for electron acceptor. The K_m and V_{max} for NADH were 0.4838 mM and 21.51 units/mg enzyme respectively. While the values for 12-MP were 5.347 mM and 64.04 units/mg enzyme respectively. The characterization of Mo-reducing enzyme studies were carried out at the optimum pH of 7.5 using 50mM Tris-HCl at 15°C. The enzyme is stable at -20°C for six days in Tris-HCL buffer at pH 7.5.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PEMENCILAN, PENULENAN SEPARA DAN PENCIRIAN ENZIM
PENURUNAN MOLYBDENUM OLEH BAKTERIA ANTARCTICA
(*gamma-Proteobacterium* STRAIN DR.Y1)**

Oleh

SITI AQLIMA BINTI AHMAD

Disember 2006

Pengerusi: Profesor Nor Aripin Shamaan, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Bakteria nombor J7A telah dipencilkan daripada Jubany Station, Antarctica dan mempunyai kebolehan untuk menurunkan logam berat molybdenum (molybdate) kepada molybdenum biru di dalam medium rendah fosfat, pH 7 dalam keadaan anaerobic pada 10°C selama empat hari. Pemencilan J7A telah diidentifikasi sebagai Gram-negatif dan strain baru untuk jujukan DNA yang dikenali sebagai *gamma-Proteobacterium* strain DR.Y1 menggunakan analisis filogenetik molekul 16S rRNA. Pengoptimuman telah dikaji untuk menentukan kadar optimum penghasilan molybdenum biru. Kombinasi 1% (w/v) kepekatan glukos sebagai sumber karbon, 0.3% (w/v) kepekatan ammonium sulfat sebagai sumber nitrogen, 0.1% (w/v) kepekatan yis, 30mM kepekatan molybdate, dan medium rendah fosfat pada pH 7 memberikan penghasilan optimum molybdenum biru. Penulenan separa dan pencirian telah dikonduksikan oleh enzim penurunan-molybdenum dengan kromatografi penukaran anion menggunakan kolum Macro-Prep High-Q™ dan kromatografi penurasan gel menggunakan kolum Agilent Zorbax™ (GF-250). Tiga ikatan telah visualisasikan pada fraksi gel filtrasi pada 39,36 dan 33 kDa



menggunakan SDS elektroforesis-gel poliakrilamid yang menunjukkan penulenan tidak tercapai.. Dalam kajian enzim, NADH bertindak sebagai substrat untuk penderma electron dan 12-MP bertindak sebagai substrat untuk penerima electron. K_m dan V_{max} untuk NADH ialah 0.4838 mM dan 21.51 unit/mg enzim. Manakala nilai untuk 12-MP ialah 5.347 mM dan 64.04 units/mg enzim. Pencirian enzim penurun-Mo telah didapati optimum pada pH 7.5 menggunakan 50mM Tris-HCl pada 15°C. Enzim ini stabil pada -20°C selama enam hari di dalam buffer Tris-HCl pada pH 7.5.

AKNOWLEDGEMENTS

In the name of Allah, the Most Beneficent, Most Gracious, Most Merciful

Writing the acknowledgements is a wonderful phase to express in so few words all the gratitude and deepest appreciation to people who made this Msc. dissertation possible.

Thank you to Prof. Dr. Nor Aripin Shamaan, my first supervisor. My sincere gratitude also goes to Dr. Mohd. Yunus Abdul Shukor and Prof. Mohd Ariff Syed, my supervisor. The quality of their supervision is the best. Thank you for guiding me the step of the way out to achieve this goal. Thank you indeed.

My appreciation also goes to Universiti Putra Malaysia, Malaysian Antarctica Research Program, and Academic Science Malaysia for the opportunity given to pursue my goals.

I wish to dedicate my dissertation to all parents. Most dedicated is to my parents Ahmad Hassan and Che Ramlah Dollah who always proud of me, believed in me and knew that I would do well. I feel very privileged to have been brought up in this family and believe that my achievements are a reflection of the love, effort and pray that I have received from them. My success belongs to them.

My most profound gratitude goes to family, Dr. Affizal Ahmad, Mohd Fizzik Ahmad, Affiaine Ahmad, Rasidi Naim, Daniel Iskandar Rasidi, Ameera Suhaila



Rasidi, Amni Batrisya Rasidi and Jasmine Safura Rasidi, who supported me throughout my Msc. Their encouragement during the hard and stressful times was very crucial. In their undoubting belief and esteem in me, they have provided me with the confidence that was necessary for the completion of this intense project. They were really giving inspiration for me to achieve my Master dreams into reality.

My special thanks go to my molybdenum and Antarctica partner; Fadhil Rahman and Alia Ali Hassan, for the outstanding support and service throughout my master programme. My deepest thanks to my lab partners, lab 204 and 115; Nina Azmi, Farah Dahalan, Nata Rajan, Neni Gusmanizar, Wan Surini, Arif Khalid and Sim Han Koh for their kindness and happiness they have shared with me. Gratitude is also extended to my friends especially to Baizura Desa, Nadia Yaacob, Hanisah Shafie, Shafina Habib, Shikin Rashid, Maizirah Ibrahim and Noraida Yunus for their unselfishness in sharing their knowledge and helping hands. They give me the emotional and spiritual strength to success in this Msc. research.

I always believe that Allah always with me, no matter how hard this journey is. And, I keep on belief that “In every difficulty, lies opportunity”. Thank you God. Thanks indeed.

Siti Aqlima Ahmad, 2006.



I certify that an Examination Committee met on 20th December 2006 to conduct the final examination of Siti Aqlima binti Ahmad on her Master of Science thesis entitled “Isolation, Partial Purification and Characterization of Molybdenum-reducing Enzyme from An Antarctica Bacterium (The gamma-Proteobacterium strain DR.Y1)” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Dr. Muhajir Hamid, PhD

Lecturer

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Chairman)

Assoc. Prof. Dr. Shuhaimi Mustafa, PhD

Lecturer

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Internal examiner)

Dr. Muskhazli Mustafa, PhD

Lecturer

Faculty of Sciences

Universiti Putra Malaysia

(Internal examiner)

Assoc. Prof. Dr. Mushrifah Idris, PhD

Lecturer

Faculty of Biotechnology and Sciences

Universiti Kebangsaan Malaysia

(External Examiner)

HASANAH MOHD GHAZALI, PhD

Professor/Deputy Dean

School of Graduate Studies

Universiti Putra Malaysia

Date :



This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements for the degree of Master of Science. The members of the Supervisory Committee are as follows:

Nor Aripin Shamaan, PhD

Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Chairman)

Mohd Arif Syed, PhD

Professor

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Member)

Mohd Yunus Shukor, PhD

Lecturer

Faculty of Biotechnology and Biomolecular Sciences

Universiti Putra Malaysia

(Member)

AINI IDERIS, PhD

Professor/Dean

School of Graduate Studies

Universiti Putra Malaysia

Date :



DECLARATION

I hereby declare that the thesis is based on my original work except for quotation and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

SITI AQLIMA BINTI AHMAD

Date:

TABLE OF CONTENTS

	Page
DEDICATION	ii
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGEMENTS	vii
APPROVAL	ix
DECLARATION	xi
LIST OF TABLES	xv
LIST OF FIGURES	xvi
LIST OF ABBREVIATIONS	xviii
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	3
2.1 Molybdenum.	3
2.1.1 History on Molybdenum	3
2.1.2 Molybdenum in Industry	4
2.1.2.1 Molybdenum Sources	4
2.1.2.2 Molybdenum Applications	6
2.1.3 The Chemistry of Molybdenum	7
2.1.3.1 The Molybdate Ion	8
2.1.4 Molybdenum in Biochemistry	10
2.1.4.1 Molybdenum in Plants and Soil	10
2.1.4.2 Molybdenum in Humans and Animals	12
2.1.5 Molybdenum Toxicity	13
2.1.5.1 Toxicity in Animals	13
2.1.5.2 Toxicity in Human	18
2.1.6 Molybdenum Pollutions	20
2.1.7 Enzymatic and Microbial Action on Molybdenum	22
2.1.7.1 Mo-blue	22
2.1.7.2 Mo-reducing Enzyme	24
2.1.7.3 Mo-reducing Enzyme Purification	29
2.2 Bioremediation	31
2.2.1 Advantages of Bioremediation	33
2.2.2 Bioremediation of Heavy Metals	34
3 MATERIALS AND METHODS	37
3.1 Equipments, Chemicals, Buffer and Chemical Solutions	37
3.2 Isolation and Screening of Mo-reducing Bacteria	37
3.2.1 Bacterial Sampling	37
3.2.2 Isolation of Bacteria	39
3.3 Maintenance and Growth of Bacterial Isolates	40



3.4	Screening of Mo-reducing Bacteria	41
3.5	Identification of Mo-reducing Bacteria	41
3.5.1	Gram Staining	42
3.5.2	16S rRNA Analysis	43
3.5.2.1	Genomic Extraction	43
3.5.2.2	Quantification of DNA Concentration	44
3.5.2.3	Polymerase Chain Reaction (PCR)	44
3.5.2.4	Purification of Amplified PCR Products	45
3.5.2.5	Sequence Analysis	45
3.5.2.6	Phylogenetic Analysis.	46
3.6	Growth Optimization of Uncultured Bacteria Strain DR.Y1	48
3.6.1	Optimization of Growth Over Time	48
3.6.2	Optimization of Temperature	49
3.6.3	Optimization of Carbon Sources	50
3.6.4	Optimization of Glucose Concentration	50
3.6.5	Optimization of Nitrogen Sources	51
3.6.6	Optimization of Ammonium Sulphate Concentration	52
3.6.7	Optimization of Yeast Concentration	53
3.6.8	Optimization of MoO_4^{2-} and Phosphate	54
3.6.9	Optimization of pH	55
3.7	Partial Purification of Mo-reducing Enzyme	56
3.7.1	Scale-up Culture of Isolate J7A	56
3.7.2	Preparation of Enzyme Extracts	58
3.7.3	Determination of Protein Concentration	60
3.7.4	Partial Purification Using Macro-Prep High-Q™ Anion Exchanger	61
3.7.5	Partial Purification Using Agilent Zorbax™ (GF-250) Gel Filtration	62
3.7.6	SDS Polyacrylamide Gel Electrophoresis	63
3.7.6.1	Preparation of Gel	63
3.7.6.2	Sample preparation and Loading The Gel	64
3.7.6.3	Staining and Distaining	65
3.8	Enzymatic Studies on Reduction of Molybdenum.	66
3.8.1	Mo-reducing Enzyme Assay	66
3.8.2	Quantification of molybdenum blue	67
3.8.3	Determination of Mo-reducing Enzyme K_m and V_{max}	68
3.8.3.1	The K_m and V_{max} NADH as The Substrate Electron Donor.	68
3.8.3.2	The K_m and V_{max} of 12-MP as The Substrate Electron Acceptor	69
3.8.4	Effect of Different Temperatures on Mo-reducing Enzyme Activity	69
3.8.5	Effect of Different pH on Mo-reducing Enzyme Activity	70
3.9	Determination of Mo-reducing Enzyme Temperature Stability	70

4	RESULTS AND DISCUSSIONS	72
4.1.	Isolation and Screening of Mo-reducing Bacteria	72
4.1.1	Isolation of Mo-reducing Bacteria	72
4.1.2	Screening of Mo-reducing Bacteria	75
4.2	Identification of Mo-reducing Bacteria	77
4.2.1	Gram Staining	77
4.2.2	16S rRNA Analysis	80
4.2.2.1	Genomic Extraction	80
4.2.2.2	Polymerase Chain Reaction (PCR)	80
4.2.2.3	16S rRNA Gene Sequencing	82
4.2.2.4	Phylogenetic Analysis	84
4.3	Molybdate reduction Optimization of gamma-Proteobacterium Strain DR.Y1	87
4.3.1	Optimization of gamma-Proteobacterium Strain DR.Y1 Molybdate reduction Over Time	87
4.3.2	Optimization of Temperature	90
4.3.3	Optimization of Carbon Sources	92
4.3.4	Optimization of Glucose Concentrations	94
4.3.5	Optimization of Nitrogen Sources	96
4.3.6	Optimization of Ammonium Sulphate Concentrations	99
4.3.7	Optimization of Yeast extract Concentrations	101
4.3.8	Optimization of MoO_4^{2-} and Phosphate	103
4.3.9	Optimization of pH	105
4.3.10	Molybdenum blue production and pH profile during molybdate reduction	107
4.4	Partial Purification of Mo-reducing Enzyme	110
4.5	SDS Polyacrylamide Gel Electrophoresis	115
4.6	Enzymatic Studies on Reduction of Molybdenum	117
4.6.1	Kinetic Studies by Mo-reducing Enzyme	117
4.6.1.1	Kinetic Studies Using NADH as The Substrate Electron Donor	117
4.6.1.2	Kinetic Studies Using 12-MP as The Substrate Electron Acceptor	120
4.6.2	Effect of Different Temperatures on Mo-reducing Enzyme Activity	122
4.6.3	Effect of pH on Mo-reducing Enzyme Activity	124
4.7	Determination of Mo-reducing Enzyme Temperature Stability	126
5	CONCLUSIONS	130
	REFERENCES	132
	APENDICES	145
	BIODATA OF THE AUTHOR	156

LIST OF TABLES

Tables		Page
1	The toxicity of molybdenum to fresh water based on their species	17
2	Techniques of <i>in situ</i> bioremediation	31
3	The effects of glucose concentration growth	51
4	The effects of (NH ₄) ₂ SO ₄ concentration on growth	53
5	The effects of yeast concentration of on growth	54
6	The effect of molybdate concentration on growth	55
7	Composition of resolving and stacking SDS-PAGE gels	64
8	Composition of staining, destaining and storage solution	65
9	List of Isolated and Screening Mo-reducing Bacteria	73
10	Microscopic and macroscopic observations of Isolate J7A	79
11	Mo-reducing Enzyme Purification Table	114
12	Overall results of Mo-reducing bacteria (Isolate J7A @ gamma-Proteobacterium Strain DR.Y1).	129

LIST OF FIGURES

Figure		Page
1	World molybdenum reserves (19,000,000 metric tonnes) as reported by U.S geological survey, mineral commodity summaries, January 2005.	5
2	Structure of molybdenum-blue (Mo-blue). It is formed by 12 tetrahedral MoO_4^{2-} and one phosphate (PO_4^{3-}) ion.	23
3	A schematic presentation of the mechanism of molybdenum reduction to Mo-blue by EC 48 (Ghani <i>et al.</i> , 1993).	26
4	Newly suggested schematic presentation of the mechanism of molybdate reduction to Mo-blue by EC 48 (modified from Shukor <i>et al.</i> , 2000).	28
5	Antarctica map; research stations and territorial claims.	38
6	Apparatus for scale-up of Isolate J7A growth.	58
7	Bacterial molybdate reduction in low phosphate agar (pH 7.0).	74
8	Molybdate reduction by 12 bacterial isolates.	76
9	Photomicrograph of Isolate J7A.	78
10	Agarose gel electrophoresis of genomic DNA extraction of Isolate J7A.	81
11	The region of homology between the forward and reverse complement of Isolate J7A.	83
12	Neighbour-joining method cladogram showing phylogenetic relationship between Strain Dr.Y1 and other related reference microorganisms based on the 16S rRNA gene sequence analysis.	85
13	The accession number of 16S rRNA sequence of Isolate J7A as deposited in GenBank.	86
14	Molybdate reduction curve of gamma-Proteobacterium Strain DR.Y1.	89
15	The effect of temperature on the molybdate reduction by gamma-Proteobacterium Strain DR.Y1.	91
16	The effect of carbon sources on the molybdate reduction by gamma-Proteobacterium Strain DR.Y1.	93

17	The effect of glucose concentration on the molybdate reduction by gamma-Proteobacterium Strain DR.Y1.	95
18	The effect of nitrogen source on the molybdate reduction by gamma-Proteobacterium Strain DR.Y1.	98
19	The effect of ammonium sulphate concentration on the molybdate reduction by gamma-Proteobacterium Strain DR.Y1.	100
20	The effect of yeast extract concentrations on the molybdate reduction by gamma-Proteobacterium Strain DR.Y1.	102
21	The effect of molybdate concentration on the molybdate reduction by gamma-Proteobacterium Strain DR.Y1.	104
22	Effect of pH on the molybdate reduction by gamma-Proteobacterium Strain DR.Y1.	106
23	Scanning spectra of molybdenum blue from bacterium Strain Dr.Y1.	108
24	Changes in pH (○) of the media during the course of molybdate reduction (●) by Strain Dr.Y1.	109
25	Elution profile of Mo-reducing enzyme using Macro-prep High-Q TM anion-exchanger.	111
26	Elution profile of Mo-reducing enzyme using Zorbax TM (GF250) gel filtration column.	113
27	SDS polyacrylamide gel analysis of partially purified Mo-reducing enzyme	116
28	Michaelis-Menten and Lineweaver-Burk plot with NADH as electron donor substrate.	119
29	Michaelis-Menten and Lineweaver-Burk plot with 12-PM as electron acceptor.	121
30	The Effect of Temperature on Mo-reducing Enzyme Activity.	123
31	Effects of different pHs and buffers on Mo-reducing enzyme activity.	125
32	Effect of prolonged pre-incubation temperatures on Mo-reducing enzyme.	128

LIST OF ABBREVIATIONS

%	Percent
(NH ₄) ₂ SO ₄	Ammonium sulphate
<	Less than
>	More than
°C	Degree celsius
μl	Microlitre
μM	Micromolar
12-MP	Twelve-molybdophosphate
Ag	Argentum
As	Asenic
ATP	Adenosine triphosphate
Cd	Cadmium
cm	Centimeter
Co	Cuprum
Cr	Chromium
Cu	Copper
DEAE	Diethylaminoethylamine
dH ₂ O	Distilled water
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
EDTA	Ethylene diamine tetraacetic acid
<i>et al</i>	And friends



Fe	Ferum
Glc	Glucose
g	Gravity (relative centrifugal force)
HCl	Hydrogen chloride
Hg	Mercury
HPLC	High performance liquid chromatography
HPM	High phosphate medium
hr	Hour
K	Kelvin
kb	Kilobase
kDa	Kilodalton
Kg	Kilogram
K_m	Michaelis-Menten constant
L	Litre
LPM	Low phosphate medium
m	Meter
M	Molar
mA	Milliampere
mAu	Mili absorbance unit
mg	Miligram
MgSO ₄	Magnesium sulphate
min	Minutes
mM	Milimolar
Mo	Molybdenum
Mo-blue	Molybdenum blue

Mo-reducing bacteria	Molybdenum reducing bacteria
Mo-reducing enzyme	Molybdenum reducing enzyme
MT	Milestones
MW	Molecular weight
$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$	DiSodium-hidrophosphate
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	DiSodium molybdate
NaCl	Sodium chloride
NAD^+	Nicotinamide adenine dinucleotide oxidized form
NADH	Nicotinamide adenine dinucleotide reduced form
Ni	Nikel
nm	Nanometer
OD	Optical density
PAGE	Polyacrylamide gel electrophoresis
Pb	Plumbum
PCR	Polymerase chain reaction
pH	-Log concentration of H^+ ion (<i>Puissance hydrogene</i>)
PMSF	Phenylmethylsulfonylfluoride
PO_4^{3-}	Phosphate
RNA	Ribonucleic acid
rpm	Revolution per minute
SDS	Sodium dodecyl sulphate
Sn	Stanium
T50-7.5-buffer	50 mM Tris-HCl at pH 7.5
TEMED	N,N,N',N'-tetramethyl-ethylenediamine
UV	Ultraviolet



v/v	Volume/ volume
V_{max}	Maximum velocity
w/v	Weight/ volume
XOD	Xanthine oxidase
Zn	Zink

CHAPTER 1

INTRODUCTION

Water pollution due to heavy metals is a very important issue as it reduces the viable water resource by creating a negative feedback loop involving increasing economic pressure and decreasing quality of supply. Water covers nearly 70% of our planet, yet the majority of this water is salt water. We have very little reserves of fresh water for use. However, we have polluted or contaminated a great majority of our water sources with little thought about our future needs.

Heavy metals in water, air and soil are global problems that have become a growing threat to the environment and humanity. Heavy metal such as mercury, lead and arsenic are widely recognized as highly toxic and dangerous to organism (He *et al.*, 2005; Patra *et al.*, 2004). As a result of widespread application in numerous industrial processes, heavy metal has become a contaminant of many environmental systems (Bird *et al.*, 2005; Hasselriis and Licata, 1996). Major sources of heavy metal pollution today come from the combustion of leaded gasoline, mining and processing, steel, iron, cement and fertilizers production, nuclear and other industrial effluents and sludges, dumping and land filling of industry wastes, biocides and preservatives including organometallic compounds.

From industrial applications, molybdenum has been found in discharged effluents, which results in the widespread contamination of molybdenum to the environment (Davis, 1991). There were many reports on molybdenum pollution due to



molybdenum mining activity such as at Tokyo Bay and Black Sea in 1991, Red Sea in 1996 and Tyrol in 2000 (Davis, 1991; Slifer, 1996; Neuhauserer *et al.*, 2000). Exposure to high concentration of molybdenum affected the reproduction and caused mortality in humans and animals.

Heavy metal is different from to organic pollutants because it cannot be detoxified by degradation and remains in the ecosystem (Shukor *et al.*, 2000). So, the best strategy is to remove the heavy metals by bioremediation. Bioremediation is a process which involves the transformation/detoxification of pollutants using microorganisms and plants. Bioremediation cleans up the environment effectively and is cheaper than any other methods (King *et al.*, 1992; Vidali, 2001). This research emphasizes the biotransformation of molybdenum using bacterium isolated from Antarctica. The first psychrophiles Mo-reducing bacterium and Mo-reducing enzyme that have high potential for bioremediation will be studies.

The objectives of this study are:

- to isolate and screen psychrophilic Mo-reducing bacteria.
- to determine the optimum environmental and nutrient conditions of a screened bacterium.
- to identify the selected Mo-reducing bacterium to species level.
- to partially purify and characterize the Mo-reducing enzyme.

CHAPTER 2

LITERATURE REVIEW

2.1 Molybdenum

2.1.1 History on Molybdenum

A 14th century Japanese sword has been found to contain molybdenum. However, molybdenum was only discovered during the latter part of the 18th century and did not occur in metallic form in nature. Molybdenum has been discovered by the Swedish scientist, Carl Wilhelm Scheele, in 1778. He was able to positively identify molybdenum. He decomposed molybdenite, which is molybdenum predominant metal, by heating it in air to obtain a white oxide powder. Four years later, in 1782, Peter Jacob Hjelm reduced the oxide with carbon to obtain a dark metallic powder, which he named “Molybdenum”. Molybdenum came from the Greek word “molybdos”, which means lead-like.

Molybdenum was first used in 1891 by the French company, Schneider & Co. as an alloying element in the production of armour plates. Molybdenum was found to be an effective replacement for tungsten in numerous steel alloying applications. In World War 1, molybdenum has been extensively used as a substitute for tungsten in many hard and impact-resistant steels, and the increased demand has initiated an intensive search for new sources of molybdenum supply. There were marked developments of the massive Climax deposit in Colorado, USA in 1918. After 12 years, in 1930, the proper temperature range for the forging and heat treatment of