
**UNIVERSITI PUTRA MALAYSIA**

***MICROBIAL MOLYBDATE REDUCTION TO MO-BLUE BY A CYANIDE-DEGRADING BACTERIUM***

**HAFEEZ MUHAMMAD YAKASAI**

**FBSB 2018 2**

# MICROBIAL MOLYBDATE REDUCTION TO MO-BLUE BY A CYANIDE-DEGRADING BACTERIUM

By

**HAFEEZ MUHAMMAD YAKASAI**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy**

**December 2017**

**COPYRIGHT**

**DEDICATION**

This thesis is dedicated to my beloved family whose moral support, prayers and encouragement are undoubtedly the driving force to my achievements.

Abstract of thesis presented to Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

**MICROBIAL MOLYBDATE REDUCTION TO MO-BLUE BY A CYANIDE-DEGRADING BACTERIUM**

By

**HAFEEZ MUHAMMAD YAKASAI**

**December 2017**

**Chairman : Associate Professor Mohd Yunus Shukor, PhD**
**Faculty : Biotechnology and Biomolecular Sciences**

Molybdenum, an emerging pollutant, has recently being demonstrated to be toxic to spermatogenesis in several animal models. It is also very toxic to ruminants causing death at very low level (parts per million). Molybdenum is mined as a small by-product of copper and gold mining in Malaysia. Molybdenum pollution is also found at dumping sites of waste oil lubricant containing molybdenum disulfide, with levels of up to hundreds of parts per million found. Molybdenum, in the soluble form, molybdate can be reduced to molybdenum blue, a colloidal and relatively insoluble product and this phenomenon forms the basis for bioremediation of molybdenum. This research is therefore, aimed at screening, phylogenetic identification, characterization and optimization of molybdenum-reducing activity of the best isolate via one-factor-at-a-time (OFAT) and response surface method (RSM); modelling the kinetics of molybdenum reduction through primary and secondary models; finally purify and characterize the molybdenum-reducing enzyme activity. One of the ten previously isolated cyanide-degrading bacteria from gold mine soils in Malaysia exhibited a novel molybdenum reduction to molybdenum-blue, with this best molybdate-reducer further studied on a molybdate low phosphate minimal salts media supplemented with glucose and ammonium sulfate as the carbon and nitrogen source, respectively. Strain HMY3 via phylogenetic analyses reveals that the isolate belongs to *Serratia* genus. Sucrose was the best carbon source supporting molybdate reduction in this strain and was optimum at 20 g/L. Ammonium sulfate was the best source of nitrogen for strain HMY3 and was optimal at 10 g/L. Strain HMY3 grew best at 35 $^{o}$C and at pH 6.5. Response Surface Method shows the best conditions for molybdenum reduction were molybdate concentration between 55 and 57.5 mM, phosphate concentration of 3.95 mM, pH 7, sucrose concentration between 15 and 17.5 g/L and incubation time between 48 and 60 h. Molybdenum reduction was inhibited by the heavy metals such as copper, mercury, chromium and arsenic at concentrations higher than 1 ppm. However, prolonged incubation succeeded in overcoming this inhibition. Analysis of the reduction kinetics showed that molybdenum reduction over time can best be

modelled using the modified Gompertz model while the reduction kinetics was best modelled using the Luong model. Statistical analysis of these models has been carried out and they exhibit low values for RMSE and AICc, highest adjusted $R^2$ values, F-test and with Bias Factor and Accuracy Factor nearest to unity (1.0) over other models. The calculated value for the Luong's constants which are maximal reduction rate, half saturation constant for maximal reduction, maximal concentration of substrate tolerated and curve parameter that defines the steepness of the growth rate decline from the maximum rate symbolized by $q_{max}$, $K_s$, $S_m$, and $n$ were 0.06±0.1 $hr^{-1}$, 47.95±10.12 mM, 69.63±0.8 mM and 0.69±0.11, respectively. The Luong model clearly shows strong substrate inhibition to rate of reduction at high substrate (Mo) concentration. The Mo-reducing enzyme has been purified using an ammonium sulfate precipitation followed by gel-filtration chromatography. The results showed that the best ammonium sulfate fraction giving the highest enzyme activity was between 50 and 60% ammonium sulfate concentration. The molybdenum-reducing enzyme was monomeric with an estimated mwt of 105 kDa. The enzyme was further characterized, and the results show that the enzyme is most stable in Tris-buffer pH 7 containing 0.1 mM DTT, temperature 4 $^oC$ and not affected by metabolic inhibitors and heavy metals (1 ppm). The enzyme attains optimum catalysis at pH 5.5, temperature between 25 and 35 $^oC$, substrate concentration (LPPM) 12 mM and electron donor (NADH) concentration 5 mM. In conclusion, a novel cyanide-degrading bacterium with a molybdenum reduction capacity has been isolated, and statistical method using RSM has succeeded in optimizing reduction. Also, the enzyme has been purified to homogeneity. The characteristic of this bacterium makes it suitable for future bioremediation works in polluted soil containing cyanide and molybdenum metal.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENURUNAN MOLIBDAT BERASASKAN MIKROB KEPADA MOLIBDENUM BIRU OLEH BAKTERIA-PENGURAI SIANIDA**

Oleh

**HAFEEZ MUHAMMAD YAKASAI**

**Disember 2017**

**Pengerusi : Profesor Madya Mohd Yunus Abd. Shukor, PhD**
**Fakulti : Bioteknologi dan Sains Biomolekul**

Molibdenum, bahan pencemar yang baru muncul pada waktu kebelakangan ini adalah toksik kepada proses spermatogenesis dalam beberapa model haiwan. Ia juga sangat toksik kepada ruminan menyebabkan kematian pada kepekatan yang sangat rendah (bahagian per juta). Molibdenum dilombong sebagai bahan produk sampingan kecil dari perlombongan kuprum dan emas di Malaysia. Pencemaran molibdenum juga dijumpai di tapak pelupusan pelincir minyak sisa yang mengandungi molibdenum disulfida di beberapa tempat di Malaysia pada kepekatan sehingga beratus-ratus bahagian per juta. Molibdenum, dalam bentuk terlarutnya adalah molibdat, dan boleh diturunkan kepada molibdenum biru, satu produk koloidal dan tidak larut, dan fenomena ini menjadi asas bagi bioremediasi molibdenum. Oleh itu, objektif penyelidikan ini adalah bertujuan untuk menyaring, mengenalpasti secara filogenetik, membuat pencirian dan memoptimumkan aktiviti penurunan molibdenum melalui kaedah satu-faktor-pada-satu-masa (OFAT) dan kaedah permukaan tindak balas (RSM); memodelkan kinetik penurunann molibdenum melalui model-model primer dan sekunder; dan terakhir sekali menulenkan dan mencirikan aktiviti enzim penurunan molibdenum. Salah satu daripada sepuluh bakteria pengurai sianida yang sebelumnya dipencilkan dari tanah perlombongan emas di Malaysia mempamerkan keupayaan novel penurunan molibdenum kepada molibdenum-biru, dengan bakterium penurunan molibdat ini dikaji selanjutnya menggunakan media garam minimal molibdat dengan fosfat rendah ditambah dengan glukosa dan ammonium sulfat sebagai sumber karbon dan nitrogen, masing-masing. Melalui analisa filogenetik strain HMY3 ini didapati tergolong dalam genus *Serrati*a. Sukrosa adalah sumber karbon terbaik yang menyokong penurunan molibdat dalam strain ini dan adalah optimum pada 20 g/L. Ammonium sulfate adalah sumber terbaik nitrogen untuk strain HMY3 dan adalah optimum pada 10 g/L. Strain HMY3 tumbuh paling baik pada suhu 35 °C dan pada pH 6.5. Melalui kaedah permukaan tindak balas, keadaan terbaik untuk penurunan molibdenum adalah pada kepekatan molibdat di antara 55 dan 57.5 mM,

kepekatan fosfat pada 3.95 mM, pH 7, kepekatan sukrosa di antara 15 dan 17.5 g/L dan masa inkubasi di antara 48 dan 60 jam. Penurunan molibdenum direncat oleh logam berat seperti kuprum, merkuri, kromium dan arsenik pada kepekatan yang lebih tinggi daripada 1 bahagian per juta. Walau bagaimanapun, waktu pengeraman yang berpanjangan telah berjaya mengatasi perencatan ini. Analisis kinetik menunjukkan bahawa penurunan molibdenum melawan masa dapat dimodelkan dengan baik sekali menggunakan model Gompertz terubahsuai manakala kinetik penurunan adalah dapat dimodelkan dengan baik sekali menggunakan model Luong. Analisis statistik model-model ini mempamerkan nilai-nilai yang rendah untuk RMSE dan AICc, nilai tertinggi untuk $R^2$ yang diselaraskan, ujian F dan Faktor Bias dan Faktor Ketepatan yang paling dekat dengan satu (1.0) berbanding model-model lain. Nilai-nilai yang dikira untuk pemalar Luong seperti kadar penurunan maksima, pemalar tepu separuh untuk penurunan maksima, kepekatan maksima substrat yang diguna dan parameter lengkung yang menentukan kecuraman penurunan kadar pertumbuhan dari kadar maksimum yang diwakilkan oleh $q_{max}$, $K_s$, $S_m$, dan n adalah 0.06 ± 0.1 $jam^{-1}$, 47.95 ± 10.12 mM, 69.63 ± 0.8 mM dan 0.69 ± 0.11. Model Luong jelas menunjukkan kesan perencatan substrat yang kuat pada kadar penurunan pada kepekatan substrat (Mo) yang tinggi. Enzim penurunan Mo telah ditulenkan menggunakan pemendakan ammonium sulfat diikuti oleh kromatografi gel-penapisan. Hasilnya menunjukkan bahawa peratusan ammonium sulfat yang memberikan aktiviti enzim tertinggi adalah di antara 50 hingga 60%. Enzim penurunan molibdenum adalah monomerik dengan anggaran berat molekul 105 kDa. Pencirian enzim ini menunjukkan enzim adalah paling stabil pada pH 7 menggunakan penimbal Tris yang mengandungi 0.1 mM DTT, pada suhu 4 °C dan tidak terjejas oleh perencat metabolik dan logam berat pada 1 bahagian per juta. Enzim ini mencapai pemangkinan optimum pada pH 5.5, suhu di antara 25 dan 35 °C, kepekatan substrat substrat (LPPM) pada 12 mM dan kepekatan penderma elektron (NADH) pada 5 mM. Sebagai kesimpulannya, satu bakteria pengurai sianida novel dengan kapasiti penurun molibdenum telah diasingkan, dan kaedah statistik menggunakan RSM telah berjaya mengoptimumkan penurunan. Juga, enzim telah dapat ditulenkan sehingga homogen. Ciri-ciri bakteria ini menjadikannya sesuai untuk kerja bioremediasi pada masa hadapan di tanah tercemar yang mengandungi logam sianida dan molibdenum.

## ACKNOWLEDGEMENTS

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

My profound gratitude goes to my able supervisor Assoc. Prof. Dr. Mohd Yunus Shukor and his team Dr. Siti Aqlima Ahmad, Dr. Nur Adeela Yasid and Dr. Mohd Izuan Effendi Halmi for their tireless effort, constructive, logical and unreserved suggestions.

Sincere appreciation to my beloved parents Dr. Muhammad Dauda Yakasai and Hajiya Amina Datti Yola; to my loving wife Yusra and daughter Mariya (Mufeeda) and to my brothers; Zahraddeen, Abdulwahab and sisters Aisha, Hafsat, Asma'u and Zahra'u for your support, prayers and encouragement.

To my Teachers, families, friends and colleagues particularly my lab mates in Bioremediation and Biomonitoring laboratory for being of help at all time through this great journey in UPM.

To my employer, Bayero University Kano and sponsors, TETFund for awarding me with Ph.D. scholarship; not forgetting the mentorship of his Excellency Professor Hafiz Abubakar. Together you made my dream a reality.

**Alhamdulillah**.

Hafeez Muhammad Yakasai, 2017.

I certify that a Thesis Examination Committee has met on 13 December 2017 to conduct the final examination of Hafeez Muhammad Yakasai on his thesis entitled "Microbial Molybdate Reduction to Mo-Blue by a Cyanide-Degrading Bacterium" 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 Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

**Mohd. Puad bin Abdullah, PhD**
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

**Yaya Rukayadi, PhD**
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Internal Examiner)

**Phang Lai Yee, PhD**
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

**Subodh Kumar Maiti, PhD**
Professor
Indian Institute of Technology
India
(External Examiner)

**NOR AINI AB. SHUKOR, PhD**
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 27 February 2018

This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy.The members of the Supervisory Committee were as follows:

**Mohd Yunus Shukor, PhD**
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

**Siti Aqlima Ahmad, PhD**
Senior Lecturer
Faculty Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

**Nur Adeela Yasid, PhD**
Senior Lecturer
Faculty of Biotechnology
Universiti Putra Malaysia
(Member)

**Mohd Izuan Effendi Halmi, PhD**
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

**ROBIAH BINTI YUNUS, PhD**
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

**Declaration by graduate student**

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software

Signature: _____ Date: _____

Name and Matric No: Hafeez Muhammad Yakasai, GS43294

**Declaration by Members of Supervisory Committee**

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Associate Professor Dr. Mohd Yunus Shukor

Signature: _____
Name of Member of Supervisory Committee: Dr. Siti Aqlima Ahmad

Signature: _____
Name of Member of Supervisory Committee: Dr. Nur Adeela Yasid

Signature: _____
Name of Member of Supervisory Committee: Dr. Mohd Izuan Effendi Halmi

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| % | Percent |
| < | Less than |
| > | Greater than |
| ɤ | Gamma |
| β | Beta |
| α | Alpha |
| Abs | Absorbance |
| CCD | Central composite design |
| cm | Centimetre |
| Mo | Molybdenum |
| As | Arsenic |
| Ag | Silver |
| Cd | Cadmium |
| Ni | Nickel |
| Co | Cobalt |
| Cr | Chromium |
| Cu | Copper |
| cm | Centimetre |
| Da | Dalton |
| $dH_2O$ | Distilled water |
| EDTA | Ethylene diamine tetra acetic acid |
| *et al* | and friends |
| Fe | Iron |
| FFD | Fractional factorial design |
| G | Gram |
| h | Hour |

| | |
|---|---|
| Hg | Mercury |
| *Km* | Michaelis-Menten constant |
| $V_{max}$ | Maximum velocity |
| kb | Kilo base |
| kDa | Kilo dalton |
| kg | Kilogram |
| KCN | Potassium Cyanide |
| L | Liter |
| M | Molar |
| mAu | Milli absorbance unit |
| min | Minute |
| mL | Mililiter |
| $(NH4)_2SO_4$ | Ammonium sulfate |
| $MgSO_4$ | Magnesium sulfate |
| NaCl | Sodium chloride |
| MSM | Minimal salt medium |
| MW | Molecular weight |
| NA | Nutrient agar |
| $^oC$ | Degree celcius |
| w/v | Weight/volume |
| OD | Optical density |
| RPM | Rotation per minute |
| RSM | Response surface method |
| SDS | Sodium dodecyl sulfate |
| pH | -log concentration of $H^+$ ion (*Puissance hydrogen*) |
| $PO_4^{3-}$ | Phosphate |
| RNA | Ribonucleic Acid |
| rRNA | Ribosomal ribonucleic acid |

| | |
|---|---|
| µL | Microlitre |
| nm | Nanometer |
| nmol | Nanomole |
| µm | Micrometer |
| µM | Micromolar |
| Mo-blue | Molybdenum-blue |
| NADH | Nicotinamide adenine-dinucleotide reduced |
| DTT | Dithiothretol |
| PMSF | phenylmethylsulfonylflouride |
| DEAE | Diethylaminoethylamine |
| TEMED | N,N,N',N'-tetramethyl-ethylenediamine |
| PAGE | Polyacrylamide gel electrophoresis |
| HCl | Hydrochloric acid |
| HPLC | High Performance Liquid Chromatography |

# CHAPTER 1

# GENERAL INTRODUCTION

## 1.1 Research background

Today, our environment is under constant threat from our own activities. Humans expanding population, industrialization, urbanization and intensive agriculture have caused tremendous damage to our environment. Man's ignorance of the laws of nature and over exploitation of natural resources has further aggravated the problem (Bhatia, 2006; Hamid *et al*., 2010).

Metals and their compounds have been long recognized as toxic agents, causing a range of acute to chronic toxicity cases in occupational and environmental high-exposure settings. Heavy metals are elements that occur naturally in the environment. However, their level has increased tremendously since the pre-industrial times due to anthropogenic activities (Kaplan 2013; Meyer *et al.*, 2014; Ilyin *et al.*, 2015). Presently, increasing population and industrialization have led to a considerable and indiscriminate release of pollutants into the environment (Soares and Soares, 2012; Dixit *et al.*, 2015). When heavy metals levels exceeded the so-called critical loads, they exert harmful effects to human health and biota (Alloway, 1995; Meyer *et al*., 2014; Ilyin *et al*., 2015; Gupta *et al*., 2016). Metals such as arsenic, cadmium, chromium, cobalt, copper, lead, mercury, molybdenum, nickel, silver and zinc are known to be toxic in their elemental forms and different chemical combinations (Rosca *et al*., 2015). They are non-degradable (Sarubbo *et al*., 2015), hence, accumulate in the food chain posing a serious threat to the ecosystem (Li and Tao, 2013) due to their carcinogenic and mutagenic property (Singh and Prasad, 2011). Today, pollution by heavy metals has become a global public health concern. Thus, their removal from the environment is of great importance.

Molybdenum is an essential trace element and a micronutrient required as a cofactor by more than 50 enzymes (Zhai *et al*., 2013; Wu *et al*., 2014). It promotes cellular function by catalyzing a variety of hydroxylation and redox transfer reactions, thus play an important role in animal and plant physiology (Pandey and Singh, 2002). Earlier studies have shown that molybdenum is an endocrine disruptor and affect spermatogenesis in several animal models, nevertheless, it is ubiquitously found in a number food and water sources (Kargar *et al*., 2011; Zhai *et al*., 2013). The wide distribution of molybdenum in the industrial manufacture of ceramics, glass and contact lens solution, metallurgical processes, lubricants, pigment, catalyst, electronic products and as color additives in cosmetics has increased the risk of humans exposer to its toxicity (Pandey and Singh, 2002; Zhai *et al*., 2013). For instance, an elevated level of molybdenum in ground water around the mining areas up to 0.2 mg/L was previously reported, which is above WHO recommended limit of 0.07 mg/L in drinking water. Thus, animals in contact with molybdenum in drinking water or while foraging for plants are likely to reflect symptoms of hypocuprosis or suffer from

molybdenosis over a long exposure period (Zhai *et al*., 2013).

Microbial molybdate reduction to Mo-blue is a phenomenon that has puzzled scientists for over a century. It was until recently that the phenomenon was proven to be enzymatic rather than abiotic. Before the work of Halmi *et al.* (2013), previous works on bacterial molybdate reduction are centered towards isolating molybdenum-reducer with a higher Mo-blue production capacity as a tool in bioremediation. However, since most polluted sites contain mixed contaminants of both organics and inorganics origin, makes effective remediation a complex one. During the last five years, attention has been shifted towards isolating microorganisms with multi-reduction and/or degrading potentials that could be used to remediate co-contaminated areas. To date, about eight molybdenum-reducing bacteria with the potential to degrade other organic contaminants were isolated. Therefore, the detailed understanding of the reduction mechanism and kinetics of Mo-reducing enzyme through various optimization processes, will assist in unravelling the mystery that lingers around the phenomenon of molybdate reduction to Mo-blue, thus an important step towards effective translation of laboratory findings to the field practice.

## 1.2 Problem statements

Mining sites in Malaysia, especially gold mining often contain multi-pollutant including cyanide and heavy metals like molybdenum. These sites can be potential molybdenum toxicity source especially if the areas are grazing areas for ruminant. Also, new information has surfaced that molybdenum at levels above the toxic levels for ruminants (2 to 200 ppm) is found at several waste oil lubricant dumping sites in Malaysia. Alarmingly, these sites are also grazing areas for free wandering cows presenting a greater threat. In addition, molybdenum recycling from industrial waste waters can also take advantage of the bacterial reduction process as both a recycling activity and a reduction of metal content from the wastewater before discharge (Blokhin *et al*., 2000; AhmadPanahi *et al.*, 2014; Halmi *et al.*, 2014a; Shukor and Shukor, 2015). Taking these issues into consideration, isolation of cyanide-degrading bacteria with high molybdenum tolerance can be used to remediate these sites or recycle molybdenum from wastewater. The conversion of soluble molybdenum to insoluble molybdenum-blue (reduction product) as was demonstrated in Tyrol Austria present a real and possible remediation technology using microorganisms (Neunhäuserer *et al.*, 2001). Bioremediation is generally cost effective and provides an alternative to the existing physicochemical methods of heavy metals removal. Some physicochemical techniques such as evaporation, chemical precipitation, filtration, ion exchange, membrane technology, reverse osmosis and electrochemical treatment are used to treat industrial wastes. However, these methods remain ineffective at lower heavy metals concentrations, are laborious and often expensive (Rosca *et al.*, 2015). Similarly, the solubility of heavy metal salts in wastewater made their effective removal via physical technique a complex process.

Several of the ways to improve reduction and further understand the mechanism of reduction are studied at the molecular level. For instance, the genome of the molybdenum resistant *Bacillus subtilis* LM 4–2 have been sequenced, and 116 redox protein-coding genes have been identified (You *et al.,* 2015). Also, a 100 KDa protein has been shown to be a potential identity of the Mo-reducing enzyme in *Serratia* sp. strain DrY5 (Shukor *et al.,* 2014). However, the protein was not sequenced, and the true identity of the enzyme remains unknown. Furthermore, modelling of Mo-reduction and molybdenum inhibition kinetics have revealed important constants of growth and inhibition that can be used for further modelling of molybdenum reduction (Othman *et al.,* 2013; Halmi *et al.,* 2016). Thus, this study focused on screening previously isolated cyanide-degrading bacterium for molybdenum-reducing capability and to optimize molybdenum reduction in this bacterium using OFAT and RSM. Other studies that need to be carried out include the kinetics of reduction, the effect of heavy metals on reduction, the possibility of molybdenum reduction in the presence of cyanide and the purification of the molybdenum-reducing enzyme from this bacterium including the enzyme characteristics. These studies are not only very important fundamentally but important for further understanding the capability and limitations of this bacterium in the future bioremediation or bioremoval of molybdenum.

## 1.3 Hypothesis

Cyanide-degrading isolate can efficiently reduce molybdate to molybdenum-blue via enzymatic reaction; that kinetic studies of the molybdenum reduction process can yield important constants and limitation of the system; that further optimization through RSM can increase the reduction efficiency and permit the production of adequate amount of enzyme to enable the purification of Mo-reducing enzyme.

## 1.4 Objectives

This research is aimed at characterizing the molybdenum-reducing property of previously isolated cyanide-degrading bacteria. With this in mind the objectives of this study are as follows;

1. To screen for the best molybdenum-reducer from previously isolated cyanide-degrading bacteria and identify the best isolate using molecular phylogenetic methods.
2. To characterize the Mo-reducing properties of the best cyanide-degrading isolate using one-factor-at-a-time (OFAT) and response surface method (RSM).
3. To carry out primary and secondary modelling of the kinetics process for molybdenum reduction.
4. To purify and characterize the Mo-reducing enzyme from this bacterium.

## REFERENCES


AbdEl-Mongy, M.A., Shukor, M.S., Hussein, S., Ling, A.P.K., Shamaan, N.A. and Shukor, M.Y. (2015). Isolation and characterization of a molybdenum-reducing, phenol- and catechol-degrading *Pseudomonas putid*a strain amr-12 in soils from Egypt. *Scientific Study & Research Chemistry & Chemical Engineering, Biotechnology, Food Industry* 16, 353–369.

Abo-Shakeer, L.K.A., Ahmad, S.A, Shukor, M.Y. and Syed, M. (2013). Isolation and characterization of a molybdenum-reducing *Bacillus pumilus*strain lbna. *Journal of Environmental Microbiology and Toxicology* 1, 9–14.

Ahmad, S.A., Shamaan, N.A., Arif, N.M., Koon, G.B., Shukor, M.Y.A. and Syed, M.A. (2012). Enhanced phenol degradation by immobilized *Acinetobacter* sp. strain AQ5NOL 1. *World Journal of Microbiology and Biotechnology* 28, 347–352.

Ahmad, S.A., Shukor, M.Y., Shamaan, N.A., Mac Cormack, W.P. and Syed, M.A. (2013). Molybdate reduction to molybdenum blue by an antarctic bacterium. *BioMed Research International* 1–10.

Ahmad, W.A., Zakaria, Z.A., Zakaria, Z. and Surif, S. (2009). Hexavalent chromium reduction at different growth phases of *Acinetobacter haemolyticus*. *Environmental Engineering Science* 26, 1275–1278.

AhmadPanahi. H., Hosseinzadeh, M., Adinehlo, H., Moniri, E. and Manoochehri, M. (2014). Removal of molybdenum from environmental sample by adsorption using modified aniline-formaldehyde with salicylic acid. *World Applied Sciences Journal* 30, 1892–1898.

Aiba, S., Shoda, M. and Nagatani, M. (1968). Kinetics of product inhibition in alcohol fermentation. *Biotechnology and Bioengineering* 10(6), 845–864.

Akaike, H. (1974). New look at the statistical model identification. *IEEE Transactions on Automatic Control* 19, 716–723.

Alloway, B.J. (1995) 'Heavy metals in soils trace metals and metalloids in soils and their bioavailability.' (BJ Alloway, Ed.). (Springer Dordrecht Heidelberg New York London) doi:10.1007/9789400744707.

Amoozegar, M.A., Ghasemi, A., Razavi, M.R. and Naddaf, S. (2007). Evaluation of hexavalent chromium reduction by chromate-resistant moderately halophile, *Nesterenkonia* sp. strain MF2. *Process Biochemistry* 42, 1475–1479.

Amor, L., Kennes, C. and Veiga, M.C. (2001). Kinetics of inhibition in the biodegradation of monoaromatic hydrocarbons in presence of heavy metals. *Bioresource Technology* 78, 181–185.

Angelo, A.J.S. and Kuck, J.C. (1977). Effects of cyanide on peanut lipoxygenase. *Lipids* 12, 682–683.

Annadurai, G., Ling, L.Y. and Lee, J. (2008). Statistical optimization of medium components and growth conditions by response surface methodology to enhance phenol degradation by *Pseudomonas putida*. *Journal of Hazardous Materials* 151, 171–178.

Ariff, A.B., Rosfarizan, M., Ghani, B., Sugio, T. and Karim, M.I.A. (1997). Molybdenum reductase in *Enterobacter cloacae*. *World Journal of Microbiology and Biotechnology* 13, 643–647.

Babák, L., Šupinová, P. and Burdychová, R. (2012). Growth models of *Thermus aquaticus* and *Thermus scotoductus*. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis* 60, 19–26.

Balusu, R., Paduru, R.R., Kuravi, S.K., Seenayya, G. and Reddy, G. (2005). Optimization of critical medium components using response surface methodology for ethanol production from cellulosic biomass by *Clostridium thermocellum* SS19. *Process Biochemistry* 40, 3025–3030.

Baranyi, J. (1995). Mathematics of predictive food microbiology. *International Journal of Food Microbiology* 26, 199–218.

Baranyi, J. and Roberts, T.A. (1994). A dynamic approach to predicting bacterial growth in food. *International Journal of Food Microbiology* 23, 277–294.

Barceloux, D.G. (1999). Copper. *Journal of Toxicology Clinical Toxicology* 37, 217–230.

Berssnyi, A., Berta, E., Kadar, I., Glavits, R., Szilagyi, M. and Fekete, S.G. (2008). Effects of high dietary molybdenum in rabbits. *Acta Veterinaria Hungarica* 56, 41–55.

Bhatia, S.C. (2006). *Environmental Chemistry*. *CBS Publishers and Distributors* (1st Ed.) New Delhi (India) pp 549

Blokhin, A.A., Kopyrin, A.A., Boev, A.A. and Kirillova, M.K. (2000). Removal of molybdenum(VI) from tungstate solutions with weakly basic anion exchangers. *Russian Journal of Applied Chemistry* 73, 404–408.

Bolker, B.M. (2008). 'Ecological Models and Data in R.' *Princeton University Press: Princeton,* New Jersey, pp 395.

Boon, B. and Laudelout, H. (1962). Kinetics of nitrite oxidation by Nitrobacter winogradskyi. *The Biochemical Journal*, 85, 440–447.

Bopp, L.H. and Ehrlich, H.L. (1988). Chromate resistance and reduction in *Pseudomonas fluorescens* strain LB300. *Archives of Microbiology* 150, 426–431.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72 (1-2), 248–254

Buchanan, R.L. (1993). Predictive food microbiology. *Trends in Food Science and Technology* 4, 6–11.

Buchanan, R.L., Whiting, R.C. and Damert, W.C. (1997). When is simple good enough: A comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves. *Food Microbiology* 14, 313–326.

Burnham, K.P. and Anderson, D.R. (2002). 'Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach.' *Springer Science & Business Media*, pp 488.

Camakaris, J., Voskoboinik, I. and Mercer, J.F. (1999). Molecular mechanisms of copper homeostasis. *Biochemical and Biophysical Research Communications* 261, 225–232.

Campbell, A.M., Del Campillo-Campbell. A. and Villaret, D.B. (1985). Molybdate reduction by *Escherichia coli* K-12 and its chl mutants. *Proceedings of the National Academy of Sciences of the United States of America* 82, 227–231.

Campillo-campbell, A. and Villaret, D.B. (1985). Correction: Molybdate reduction by *Escherichia coli* K-12 and its chl mutants. *Proceedings of the National Academy of Sciences* 82, 3532–3532.

Cappuccino, J.G. and Sherman, N. (2005). Microbiology: A Laboratory Manual 7th Edition. Pearson Education, Inc, San Francisco. 45-47.

Cervantes, C., Ji, G., Ramírez, J.L. and Silver, S. (1994). Resistance to arsenic compounds in microorganisms. *FEMS Microbiology Reviews* 15, 355–367.

Chai, B., Wu, Y., Liu, P., Liu, B. and Gao, M. (2011). Isolation and phosphate-solubilizing ability of a fungus, *Penicillium* sp. from soil of an alum mine. *Journal of Basic Microbiology* 51, 5–14.

Clesceri, L.S., Greenberg, A.E. and Trussel, R.R., (1989). Standard methods for the examination of wastewater. *American Public Health Association, Port City Press*, Baltimore, Maryland. pp 4-166

Counotte, G.H.M. and Prins, R.A. (1979). Calculation of *Km* and *Vmax* from substrate concentration versus time plot. *Applied and Environmental Microbiology* 38, 758–760.

Davis, G.K. (1991). Molybdenum. In Merian, E. (ed) Metals and their compounds in the environment, occurrence, analysis and biological relevance. New York: VCH Weinheim. pp. 1089-1100

Dawson, R.M.C., Elliot, D.C. and Elliot, W.H., (1969). Data for biochemical research", *Clarendon Press*, Oxford.

Debbie Furber (2009). Is molybdenum lurking in your forages? https://www.canadiancattlemen.ca/2009/05/14/is-molybdenum-lurking-in-your-forages/

Deeb, B.E. and Altalhi, A.D. (2009). Degradative plasmid and heavy metal resistance plasmid naturally coexist in phenol and cyanide assimilating bacteria. *American Journal of Biochemistry and Biotechnology* 5, 84–93.

Deutscher, M.P. (2009). Setting Up a Laboratory. *Methods in Enzymology* (1st ed., Vol. 463). Elsevier Inc.

Devereux, R. and Wilkinson, S.S. (2004). Amplification of ribosomal RNA sequences. *Molecular Microbial Ecology Manual* 3, 509–522.

Dey, S. and Paul, A.K. (2012). Optimization of cultural conditions for growth associated chromate reduction by *Arthrobacter* sp. SUK 1201 isolated from chromite mine overburden. *Journal of Hazardous Materials* 213, 200–206.

Dey, S. and Paul A.K. (2014). Reduction of hexavalent chromium by immobilized viable cells of *Arthrobacter* sp . SUK 1201. *Bioremediation Journal* 18, 1–11.

Dinarvand, M., Rezaee, M., Masomian, M., Jazayeri, S.D., Zareian, M., Abbasi, S. and Ariff, A.B. (2013). Effect of C / N ratio and media optimization through response surface methodology on simultaneous productions of intra- and extracellular inulinase and invertase from *Aspergillus niger* ATCC 20611. *BioMed Research International*, 2013, 1–13.

Dixit, R., Malaviya, D., Pandiyan, K., Singh, U.B., Sahu, A., Shukla, R., Singh, B.P., Rai, J.P., Sharma, P.K., Lade, H. and Paul, D. (2015). Bioremediation of heavy metals from soil and aquatic environment: An overview of principles and criteria of fundamental processes. *Sustainability* 7, 2189–2212.

Ebrahimpour, A., Rahman, R.N.Z.R.A., Ean, D.H., Basri, M. and Salleh, A. (2008). A modeling study by response surface methodology and artificial neural network on culture parameters optimization for thermostable lipase production from a newly isolated thermophilic *Geobacillus* sp. strain AMR. *BMC Biotechnology* 8, 1–15.

Elekwachi, C.O., Andresen, J., Hodgman, T.C. (2014). Global use of bioremediation technologies for decontamination of ecosystems. *Journal of Bioremediation & Biodegradation* 05, 1–9.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolutuion* 39 (4), 783-791.

Fujikawa, H. (2010). Development of a new logistic model for microbial growth in foods. *Biocontrol Science* 15, 75–80.

Gaines, J.M., Carty, N.L., Colmer-Hamood, J.A. and Hamood, A.N. (2005). Effect of static growth and different levels of environmental oxygen toxA and ptxR expression in the *Pseudomonas aeruginosa* strain PAO1. *Microbiology* 151, 2263–2275.

Garbisu, C., Alkorta, I., Llama, J. and Serra, J.L. (1998). Aerobic chromate reduction by *Bacillus subtilis*. *Biodegradation* 9, 133–141.

Ghani, B., Takai, M., Hisham, N.Z., Kishimoto, N., Ismail, A.K.M., Tano, T. and Sugio, T. (1993). Isolation and characterization of a $Mo^{6+}$-reducing bacterium. *Applied and Environmental Microbiology* 59, 1176–1180.

Gibson, A.M., Bratchell, N. and Roberts, T.A. (1987). The effect of sodium chloride and temperature on the rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. *Journal of Applied Bacteriology* 62, 479–490.

Glenn, J.L. and Crane, F.L. (1956). Studies on metalloflavoproteins v. the action of silicomolybdate in the reduction of cytochrome c by aldehyde oxidase. *Biochimica Et Biophysica Acta* 22, 111–115.

Gluszcz, P., Petera, J. and Ledakowicz, S. (2011). Mathematical modeling of the integrated process of mercury bioremediation in the industrial bioreactor. *Bioprocess and Biosystems Engineering* 34, 275–285.

Gompertz, B. (1825). On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies. *Philosophical Transactions of Royal Society of London* 115, 513–585.

Gupta, A., Joia, J., Sood, A., Sidhu, C. and Kaur, G. (2016). Microbes as potential tool for remediation of heavy metals: A Review. *Journal of Microbial & Biochemical Technology* 8, 364–372.

Gusmanizar, N., Halmi, M., Rusnam, M., Rahman, M., Shukor, M., Azmi, N. and Shukor, M.Y. (2016). Isolation and characterization of a molybdenum-reducing and azo-dye decolorizing *Serratia marcescens* strain neni-1 from Indonesian soil. *Journal of Urban and Environmental Engineering* 10, 113–123.

Halmi, M.I.E. (2014). A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Malaysia.

Halmi, M.I.E., Abdullah, S.R.S., Johari, W.L.W., Ali, M.S.M., Shaharuddin, N.A., Khalid, A. and Shukor, M.Y. (2016). Modelling the kinetics of hexavalent molybdenum ($Mo^{6+}$) reduction by the *Serratia* sp. strain MIE2 in batch culture. *Rendiconti Lincei* 27, 653–663.

Halmi, M.I.E., Ahmad, S.A., Syed, M.A., Shamaan, N.A. and Shukor, M.Y. (2014a). Mathematical modelling of the molybdenum reduction kinetics in *Bacillus pumilus* strain Lbna. *Bulletin of Environmental Science and Management* 2, 24–29.

Halmi, M.I.E., Wasoh, H., Sukor, S., Ahmad, S.A., Yusof, M.T. and Shukor, M.Y. (2014b). Bioremoval of molybdenum from aqueous solution. *International Journal of Agriculture and Biology* 16, 848–850.

Halmi, M.I.E., Zuhainis, S.W., Yusof, M.T., Shaharuddin, N.A., Helmi, W., Shukor, Y., Syed, M.A. and Ahmad, S.A. (2013). Hexavalent molybdenum reduction to Mo-blue by a sodium-dodecyl-sulfate- degrading *Klebsiella oxytoca* strain dry14. *BioMed Research International* 2013, 1–8. Article number 384541.

Hamid, A.A., Usman, L.A., Elaigwu, S.E. and Zubair, M.F. (2010). Environmental and health risk of bush burning. *Advances in Environmental Biology* 4(2), 241–249.

Hamitouche, A.E., Bendjama, Z., Amrane, A., Kaouah, F. and Hamane, D. (2012). Relevance of the Luong model to describe the biodegradation of phenol by mixed culture in a batch reactor. *Annals of Microbiology* 62(2), 581–586.

Han, K. and Levenspiel, O. (1988). Extended monod kinetics for substrate, product, and cell inhibition. *Biotechnology and Bioengineering* 32, 430–437.

Haywood, S., Dincer, Z., Jasani, B. and Loughran, M.J. (2004) Molybdenum-associated pituitary endocrinopathy in sheep treated with ammonium tetrathiomolybdate. *Journal of Comparative Pathology* 130, 21–31.

Hettiarachchi, G.M., Pierzynski, G.M. and Ransom, M.D. (2000). In situ stabilization of soil lead using phosphorus and manganese oxide. *Environmental Science and Technology* 34, 4614–4619.

Hoffman, D.R., Okon, J.L., Sandrin and T.R. (2005). Medium composition affects the degree and pattern of cadmium inhibition of naphthalene biodegradation. *Chemosphere* 59, 919–927.

Hong, H.B., Nam, I.H., Kim, Y.M., Chang, Y.S., Schmidt, S. (2007). Effect of heavy metals on the biodegradation of dibenzofuran in liquid medium. *Journal of Hazardous Materials* 140, 145–148.

Hora, A. and Shetty, V. (2014). Inhibitory and stimulating effect of single and multi-metal ions on hexavalent chromium reduction by *Acinetobacter* sp. Cr-B2. *World Journal of Microbiology and Biotechnology* 30, 3211–3219.

Huang, L. (2013). Optimization of a new mathematical model for bacterial growth. *Food Control* 32, 283–288.

Ibrahim, Y., Abdel-Mongy, M., Shukor, M.S., Hussein, S., Ling, A.P.K. and Shukor, M.Y. (2015). Characterization of a molybdenum-reducing bacterium with the ability to degrade phenol, isolated in soils from Egypt. *Biotechnologia: Journal of Biotechnology, Computational Biology and Bionanotechnology* 96, 234–245.

Ibrahim, S., Shukor, M.Y., Khalil, K.A., Halmi, M.I.E., Syed, M.A. and Ahmad, S.A. (2015). Application of response surface methodology for optimising caffeine-degrading parameters by *Leifsonia* sp. strain SIU. *Journal of Environmental Biology* 36, 1215–1221.

Ilyin, I., Rozovskaya, O., Travnikov, M., Varygina, M. (2015). Heavy metals: Analysis of long-term trends, country-specific research and progress in mercury regional and global modelling. *EMEP Status report* 3, 1-70

Jan, A.T., Azam, M., Ali, A., Haq, Q.M.R. (2013). Prospects for exploiting bacteria for bioremediation of metal pollution. *Critical Reviews in Environmental Science and Technology* 519–560.

Jeter, M.A. and Davis, G.K. (1953). The effect of dietary molybdenum upon growth, hemoglobib, reproduction and lactation of rats. *The Journal of Nutrition* 217–220.

Johnsen, A.R., Binning, P.J., Aamand, J., Badawi, N. and Rosenbom, A.E. (2013). The Gompertz function can coherently describe microbial mineralization of growth-sustaining pesticides. *Environmental Science and Technology* 47, 8508–8514.

Jukes, T.H. and Cantor, C.R. (1969). Evolution of protein molecules. In 'Mammalian Protein Metabolism' (Ed. HN Munro.) Academic Press, New York pp. 21–132.

Kaplan, D. (2013). Absorption and adsorption of heavy metals by microalgae. *Handbook of Microalgal Culture: Applied Phycology and Biotechnology* 602–611.

Karamba, K.I., Ahmad, S.A., Zulkharnain, A., Syed, M.A., Khalil, K.A., Shamaan, N.A., Dahalan FA, Shukor MY (2016). Optimisation of biodegradation conditions for cyanide removal by *Serratia marcescens* strain AQ07 using one-factor-at-a-time technique and response surface methodology. *Rendiconti Lincei* 27, 533–545.

Karamba, K.I., Shukor, M.Y., Syed, M.A., Zulkharnain, A. and Adeela, N. (2015). Isolation, screening and characterisation of cyanide-degrading *Serratia marcescens* strain AQ07. *Journal of Chemical and Pharmaceutical Sciences* 8, 401–406.

Kargar, M., Khorasani, N., Karami, M., Rafiee, G. and Naseh, R. (2011). Study of aluminum, copper and molybdenum pollution in groundwater sources surrounding (Miduk) Shahr-E-Babak copper complex tailings dam. *World Academy of Science, Engineering & Technology* 52, 278–282.

Khayat, M.E., Abd Rahman, M.F., Shukor, M.S., Ahmad, S.A., Shamaan, N.A. and Shukor, M.Y. (2016). Characterization of a molybdenum-reducing *Bacillus* sp. strain khayat with the ability to grow on SDS and diesel. *Rendiconti Lincei* 27, 547–556.

Khuri, I.A. and Mukhopadhyay, S. (2010). Response surface methodology. *Advance Review WIREs computational statistics, John Wiley & Sons Inc* 2, 128–149.

King, R.B., Long, K. and Sheldon, J.K. (1992). 'Practical Environmental Bioremediation: The Field Guide (2nd Edition), *Lewis Publisher, Florida*, pp 208.

Lee, J.D. (1977). 'Concise Inorganic Chemistry.' (3rd Edition),*Van Reinhold Co., New York*, pp 325.

Lee, K. and Park, G. (2006). A global robust optimization using Kriging based approximation model. *JSME International Journal Series C* 49, 779–788.

Levine, V.E. (1924). The reducing properties of microorganisms with special reference to selenium compounds. *Journal of Bacteriology* X, 217–262.

Li, P. and Tao, H. (2015). Cell surface engineering of microorganisms towards adsorption of heavy metals. *Critical reviews in microbiology* 51(2), 140-149.

Liau, S.Y., Read, D.C., Pugh, W.J., Furr, J.R. and Russell, A.D. (1997). Interaction of silver nitrate with readily identifiable groups: Relationship to the antibacterial action of silver ions. *Letters in Applied Microbiology* 25, 279–283.

Lim, H.K., Syed, M.A. and Shukor, M.Y. (2011). Reduction of molybdate to molybdenum blue by *Klebsiella* sp. strain hkeem. *Journal of Basic Microbiology* 51, 1–10.

Lim, H.K., Syed, M.A. and Shukor, M.Y. (2012). Reduction of molybdate to molybdenum blue by *Klebsiella* sp. strain hkeem. *Journal of Basic Microbiology* 52, 296–305.

Lloyd, J.R. (2003). Microbial reduction of metals and radionuclides. *FEMS Microbiology Reviews* 27, 411–425.

López, S., Prieto, M., Dijkstra, J., Dhanoa, M.S. and France, J. (2004). Statistical evaluation of mathematical models for microbial growth. *International Journal of Food Microbiology* 96, 289–300.

Luong, J. H. (1987). Generalization of monod kinetics for analysis of growth data with substrate inhibition. *Biotechnology and Bioengineering* 29(2), 242–248.

Lyubimov, A.V., Smith, J.A., Rousselle, S.D., Mercieca, M.D., Tomaszewski, J.E., Smith, A.C. and Levine, B.S. (2004). The effects of tetrathiomolybdate (TTM, NSC-714598) and copper supplementation on fertility and early embryonic development in rats. *Reproductive Toxicology* 19, 223–233.

Majak, W., Steinke, D., Mcgillivray, J. and Lysyk, T. (2004). Clinical signs in cattle grazing high molybdenum forage. *Rangeland Ecology & Management* 57, 269–274.

Mansur, R., Gusmanizar, N., Dahalan, F.A., Masdor, N.A., Ahmad, S.A., Shukor, M.S., Roslan, M.A.H. and Shukor, M.Y. (2016). Isolation and characterization of a molybdenum-reducing and amide-degrading *Burkholderia cepacia* strain Neni-11 in soils from west Sumatera, Indonesia. *Journal - Institute of Integrative Omics and Applied Biotechnology* 7, 28–40.

Margush, T. and McMorris, F.R. (1981). Consensus n-trees. *Bulletin of Mathematical Biology* 43, 239–244.

Masdor, N., Shukor, M.S., Khan, A., Halmi, M.I.E., Abdullah, S.R.S., Shamaan, N.A. and Shukor, M.Y. (2015) Isolation and characterization of a molybdenum-reducing and SDS- degrading *Klebsiella oxytoca* strain Aft-7 and its bioremediation application in the environment. *BIODIVERSITAS* 16, 238–246.

Masood, F. and Malik, A. (2011). Hexavalent chromium reduction by *Bacillus* sp . strain FM1 isolated from heavy-metal contaminated soil. *Bulletting of Environmental Contamination and Toxicology* 86, 114–119.

Meyer, M., Pesch, R., Schröder, W., Steinnes, E. and Uggerud, H.T. (2014). Spatial patterns and temporal trends of heavy metal concentrations in moss and surface soil specimens collected in Norway between 1990 and 2010. *Environmental Sciences Europe* 26, 1–18.

Miller, J.K., Moss, B.R., Bell, M.C. and Sneed, N.N. (1972). Comparison of $^{99}$Mo metabolism in young cattle and swine. *Journal of Animal Science* 34(5), 846–850.

Miyazaki, K., Wintrode, P.L., Grayling, R.A., Rubingh, D.N. and Arnold, F.H. (2000). directed evolution study of temperature adaptation in a psychrophilic enzyme. *Journal of Molecular Biology* 297, 1015–1026.

Monod, J. (1949). The growth of bacterial cultures. *Annual Review of Microbiology* 3(1), 371–394.

Motulsky, H.J. and Ransnas, L.A. (1987). Fitting curves to data using nonlinear regression: a practical and nonmathematical review. *The FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 1, 365–374.

Mulchandani, A., Luong, J.H.T. and Groom, C. (1989). Substrate inhibition kinetics for microbial growth and synthesis of poly-fl-hydroxybutyric acid by *Alcaligenes eutrophus* ATCC 17697:f. *Applied Microbiology and Biotechnology* 30, 11–17.

Neunhäuserer, C., Berreck, M. and Insam, H. (2001). Remediation of soils contaminated with molybdenum using soil amendments and phytoremediation. *Water, air, and soil pollution* 128, 85–96.

Nickzad, A., Mogharei, A., Monazzami, A., Jamshidian, H. and Vahabzadeh, F. (2012). Biodegradation of phenol by *Ralstonia eutropha* in a kissiris-immobilized cell bioreactor. *Water Environmental Research* 84(4), 626-634.

Olaniran, A.O., Balgobind, A. and Pillay, B. (2013). Bioavailability of heavy metals in soil: impact on microbial biodegradation of organic compounds and possible improvement strategies. *International Journal of Molecular Sciences* 14(5), 10197–10228.

Opperman, D.J., Piater, L.A., Van Heerden, E. (2008). A novel chromate reductase from *Thermus scotoductus* SA-01 related to old yellow enzyme. *Journal of Bacteriology* 190, 3076–3082.

Osborne, F.H. and Ehrlich, H.L. (1976). Oxidation of arsenite by a soil isolate of *Alcaligenes*. *Journal of Applied Biotecnology* 41, 295–305.

Othman, A.R., Abu Zeid, I.M., Rahman, M.F., Ariffin, F. and Shukor, M.Y. (2015). Isolation and characterization of a molybdenum-reducing and orange G-decolorizing *Enterobacter* sp. strain Zeid-6 in soils from Sudan. *Bioremediation Science and Technology Research* 3, 13–19.

Othman, A.R., Bakar, N.A., Halmi, M.I.E., Johari, W.L.W., Ahmad, S.A., Jirangon, H., Syed, M.A. and Shukor, M.Y. (2013) Kinetics of molybdenum reduction to molybdenum blue by *Bacillus* sp. strain A.rzi. *BioMed Research International* 2013, 1–9. Article number 371058.

Padhir, A.R. and Modi, H.A. (2013). Optimization of lipase production by *Saccharomonospora azurea* ap 11 / 18 using plackett-burman design and response surface methodology. *International Journal of Bio-Technology and Research (IJBTR)* 3, 59–66.

Page, R.D.M. (1996). On consensus, confidence, and 'total evidence'. *Cladistics* 12, 83–92.

Pandey, R. and Singh, S.P. (2002). Effects of molybdenum on fertility of male rats. *BioMetals* 15, 65–72.

Park, C.H., Keyhan, M., Wielinga, B. and Fendorf, S. (2000). Purification to homogeneity and characterization of a novel *Pseudomonas putida* chromate reductase. *Applied and Environmental Microbiology* 66, 1788–1795.

Pitt, M.A. (1976). Review Molybdenum Toxicity: Interactions between copper, molybdenum and sulphate. *Agents and Actions* 6, 758–769.

Prapulla, S.G., Jacob, Z., Chand, N., Rajalakshmi, D. and Karanth, N.G. (1992). Maximization of lipid production by *Rhodotorula gracilris* CFR- 1 using response surface methodology. *Biotechnology and Bioengineering* 40, 965–970.

Prive, G.G. (2007). Detergents for the stabilization and crystallization of membrane proteins. *Methods* 41, 388–397.

Rahman, M.F.A., Shukor, M.Y., Suhaili, Z., Mustafa, S., Shamaan, N.A. and Syed, M.A. (2009). Reduction of Mo(VI) by the bacterium *Serratia* sp. strain DRY5. *Journal of Environmental Biology* 30, 65–72.

Rajagopalan, K.V. (1988). Molybdenum : An Essential Trace Element in Human Nutrition. *Annual Review of Nutrition* 8, 401–427.

Richards, F.J. (1959). A flexible growth function for empirical use. *Journal of Experimental Botany* 10, 290–300.

Ricker, F.J. (1979). Growth rates and models. 'Fish Physiol.' (Eds WS Hoar, JR Brett, DJ Randall) Bioenergetics and Growth. pp. 677–743.

Robinson, M.F., McKenzie, J.M., Tomson, C.D. and van Rij, A.L. (1973). Metabolic balance of zinc, copper, cadmium, iron, molybdenum and selenium in young New Zealand women. *The British Journal of Nutrition* 30, 195–205.

Rosca, M., Hlihor, R.M., Cozma, P., ComĂniţĂ, E.D., Simion, I.M. and Gavrilescu, M. (2015). Potential of biosorption and bioaccumulation processes for heavy metals removal in bioreactors. *The 5th IEEE International Conference on E-Health and Bioengineering Conference, EHB* 31–34.

Ross, D.A. (2004). The toxicology of mercury. *The New England Journal of Medicine* 350(9), 945–947.

Ross, T., McMeekin, T.A. (1994). Predictive microbiology. *International Journal of Food Microbiology* 23, 241–264.

Runnells, D.D. (1976). Wastewaters in the vadose zone of arid regions: Geochemical interactions. *Ground Water* 14, 374–385.

Sabullah, M.K., Rahman, M.F., Ahmad, S.A., Sulaiman, M.R., Shukor, M.S., Shamaan, N.A. and Shukor, M.Y. (2016). Isolation and characterization of a molybdenum-reducing and glyphosate-degrading *Klebsiella oxytoca* strain saw-5 in soils from sarawak. *Agrivita* 38, 1–13.

Sahinkaya, E. and Dilek, B.D. (2007). Modeling chlorophenols degradation in sequencing batch reactors with instantaneous feed-effect of 2,4-DCP presence on 4-CP degradation kinetics. *Biodegradation* 18, 427–437.

Said, W.A. and Lewis, D.L. (1991). Quantitative assessment of the effects of metals on microbial degradation of organic chemicals. *Applied and Environmental Microbiology* 57, 1498–1503.

Saitou, N., and Nei, M. (1987). The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4(4), 406–425.

Sandrin, T.R., Chech, A.M. and Maier, R.M. (2000). A rhamnolipid biosurfactant reduces cadmium toxicity during naphthalene biodegradation. *Applied and Environmental Microbiology* 66, 4585–4588.

Sandrin, T.R. and Maier, R.M. (2003). Impact of metals on the biodegradation of organic pollutants. *Environmental Health Perspectives* 111, 1093–1101.

Sani, R.K., Peyton, B.M. and Brown, L.T. (2001). Copper-induced inhibition of growth of *Desulfovibrio desulfuricans* G20: assessment of its toxicity and correlation with those of zinc and lead. *Applied and Environmental Microbiology* 67(10), 4765–4772.

Sarubbo, L.A., Rocha, R.B., Luna, J.M., Rufino, R.D., Santos, V.A. and Banat, I.M. (2015). Some aspects of heavy metals contamination remediation and role of biosurfactants. *Chemistry and Ecology* 7540, 1–17.

Schroeder, H.A., Frost, D.V. and Balass, J.J. (1970). Essential metals in man: selenium. *Journal of Chronic Diseases* 23, 227–243.

Scopes, R.K. (1994). 'Protein Purification: Principle and practice.' (CR Cantor, Ed.). (Springer Science+Business Media New York: New York)

Sebenik, R.F., Burkin, A.R. and Dorfler, R.R. (2013). Molybdenum and molybdenum compounds. (Neitherland) doi:10.1002/14356007.a16_655.

Shen, H. and Wang, Y. (1995). Simultaneous chromium reduction and phenol degradation in a coculture of *Escherichia coli* ATCC 33456 and *Pseudomonas putida* DMP-1. *Applied and Environmental Microbiology* 61, 2754–2758.

Shukor, M.Y. (2014). Short Communications: Revisiting the role of the electron transport chain in molybdate reduction by Enterobacter cloacae strain 48. *Indian Journal of Biotechnology* 13, 404–407.

Shukor, Y., Adam, H., Ithnin, K., Yunus, I., Shamaan, N.A. and Syed, A. (2007). Molybdate reduction to molybdenum blue in microbe proceeds via phosphomolybdate intermediate. *Journal of Biological Sciences* 7, 1448–1452.

Shukor, M.Y., Ahmad, S.A., Nadzir, M.M.M., Abdullah, M.P., Shamaan, N.A. and Syed, M.A. (2010a). Molybdate reduction by *Pseudomonas* sp. strain DRY2. *Journal of Applied Microbiology* 108, 2050–2058.

Shukor, M.Y., Habib, S.H.M., Rahman, M.F.A., Jirangon, H., Abdullah, M.P.A., Shamaan, N.A. and Syed, M.A. (2008a). Hexavalent molybdenum reduction to molybdenum blue by *S. marcescens* strain Dr. Y6. *Applied Biochemistry and Biotechnology* 149, 33–43.

Shukor, M.Y., Halmi, M.I.E., Rahman, M.F.A., Shamaan, N.A. and Syed, M.A. (2014). Molybdenum reduction to molybdenum blue in *Serratia* sp. strain DRY5 is catalyzed by a novel molybdenum-reducing enzyme. *BioMed Research International* 2014, 1–8. Article ID 853084,

Shukor, M., Hamdan, M.H., Othaman, M.A., Shamaan, N.A. and Syed, M.A. (2009b). Mo (VI) reduction to molybdenum blue by *Serratia marcescens* strain Dr. Y9. *Polish Journal of Microbiology* 58, 141–147.

Shukor, M.S., Khan, A., Masdor, N., Halmi, M.I.E., Abdullah, S.R.S. and Shukor, M.Y. (2016). Isolation of a novel molybdenum-reducing and azo dye decolorizing *Enterobacter* sp. strain Aft-3 from Pakistan. *Chiang Mai University Journal of Natural Sciences* 15, 95–114.

Shukor, M.Y., Lee, C.H., Omar, I., Karim, M.I.A., Syed, M.A. and Shamaan, N.A. (2003). Isolation and characterization of a molybdenum-reducing enzyme in *Enterobacter cloacae* strain 48. *Pertanika Journal of Science and Technology* 11, 261–272.

Shukor, M.Y., Rahman, M.F.A., Shamaan, N.A., Lee, C.H., Karim, M.I.A. and Syed, M.A. (2008b). An improved enzyme assay for molybdenum-reducing activity in bacteria. *Applied Biochemistry & Biotechnology* 144, 293–300.

Shukor, M.Y., Rahman, M.F., Shamaan, N.A. and Syed, M.S. (2009c). Reduction of molybdate to molybdenum blue by *Enterobacter* sp. strain Dr.Y13. *Journal of Basic Microbiology* 49, 43–54.

Shukor, M.Y., Rahman, M.F., Suhaili, Z., Shamaan, N.A., Syed, M.A. (2009a). Bacterial reduction of hexavalent molybdenum to molybdenum blue. *World Journal of Microbiology and Biotechnology* 25, 1225–1234.

Shukor, M.Y., Rahman, M.F., Suhaili, Z., Shamaan, N.A., Syed, M.A. (2010b. Hexavalent molybdenum reduction to Mo-blue by *Acinetobacter calcoaceticus*. *Folia Microbiologica* 55, 137–143.

Shukor, M.Y.A., Shamaan, N.A., Syed, M.A., Lee, C.H. and Karim, M.I.A. (2000). Characterization and quantification of molybdenumm blue production in *Enterobacter cloacae* strain 48 using 12-molybdophophate as reference compound. *Asia Pacific Journal of Molecular Biology and Biotechnology* 8, 167–172.

Shukor, M.S. and Shukor, M.Y. (2014). Statistical diagnostic tests of the Luong model in fitting molybdenum reduction from the bacterium *Bacillus* sp. strain A.rzi. *Journal of Environmental Microbiology and Toxicology* 2, 53–57.

Shukor, M.S. and Shukor, M.Y. (2015). Bioremoval of toxic molybdenum using dialysis tubing. *Chemical Engineering Research Bulletin* 18, 6–11.

Shukor, M.Y. and Syed, M.A. (2010c). Microbiological reduction of hexavalent molybdenum to molybdenum blue. 'Curr. Res. Technol. Educ. Top. Appl. Microbiol. Microb. Biotechnol.' (Ed A Mendez-Vilas) Microbiology Book Series.(Formatex Research Center: Badajoz, Spain)

Shukor, M.Y., Syed, M.A., Lee, C.H., Karim, M.I.A. and Shamaan, N.A. (2002). A method to distinguish between chemical and enzymatic reduction of molybdenum in *Enterobacter cloacae* strain 48. *Malaysian Journal of Biochemistry* 7, 71–72.

Silver, S. and Phung, L.T. (1996). Bacterial Heavy Metal Resistance: New Surprises. *Annual Review of Microbiology* 50, 753–789.

Singh, A. and Prasad, S.M. (2011). Reduction of heavy metal load in food chain : technology assessment. *Review in Environmental Science & Biotechnology* 10, 199–214.

Sinnakkannu, S., Abdullah, A.R., Tahir, N.M. and Abas, M.R. (2004). Degradation of metsulfuron methyl in selected Malaysian agricultural soils. *Fresenius Environmental Bulletin* 13, 258–261.

Soares, E.V. and Soares, H.M.V.M. (2012). Bioremediation of industrial effluents containing heavy metals using brewing cells of *Saccharomyces cerevisiae* as a green technology: A review. *Environmental Science and Pollution Research* 19, 1066–1083.

Soda, S.O., Yamamura, S., Zhou, H., Ike, M. and Fujita, M. (2006). Reduction kinetics of As (V) to As (III) by a dissimilatory arsenate-reducing bacterium, *Bacillus* sp. SF-1. *Biotechnology and Bioengineering* 93, 812–815.

Soo, E.L., Salleh, A., Basri, M., AbdulRahman, R.N.Z.R. and Kamaruddin, K. (2003). Optimization of the enzyme-catalyzed synthesis of amino acid-based surfactants from palm oil fractions. *Journal of Biosience and Bioengineering* 95, 361–367.

Stafford, J.M., Lambert, C.E., Zyskowski, J.A., Engfehr, C.L., Fletcher, O.J., Clark, S.L., Tiwary, A., Gulde, C.M. and Sample, B.E. (2016). Dietary toxicity of soluble and insoluble molybdenum to northern bobwhite quail (*Colinus virginianus*). *Ecotoxicology* 25, 291–301.

Stoll, V.S. and Blanchard, J.S. (1990). Buffers: Principles and Practice. *Methods Enzymology* 182, 24–38.

Sugio, T., Tsujita, Y., Katagiri, T., Inagaki, K. and Tano, T. (1988). Reduction of $Mo^{6+}$ with elemental sulfur by *Thiobacillus ferrooxidans*. *Journal of Bacteriology* 170, 5956–5959.

Sukumar, M. (2010). Reduction of hexavalent chromium by *Rhizopus oryzae*. *African Journal of Environmental Science and Technology* 4, 412–418.

Thompson, J.D., Higgins, D.G. and Gibson, T.J. (1994). Clustal W: Improving the sensitivity of progressive multiple sequence aligment through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22, 4673–4680.

Truex, M.J., Peyton, B.M., Valentine, N.B. and Gorby, Y.A. (1997). Kinetics of U(VI) reduction by a dissimilatory Fe(iii)-reducing bacterium under non-growth conditions. *Biotechnology and Bioengineering* 55, 490-496.

Vimalashanmugam, K. and Viruthagiri, T. (2013). Research article medium optimization for solid state fermentative production of xylanase by *Aspergillus terreus* using central composite design. *Innovative Romanian Food Biotechnology* 13, 18–29.

Ward, G.M. (1978). Molybdenum toxicity and hypocuprosis in ruminants : A review. *Journal of Animal Science* 46, 1078–1085.

Wayman, M. and Tseng, M.C. (1976). Inhibition-threshold substrate concentrations. *Biotechnology and Bioengineering* 18(3), 383–387.

Wheeler, P.A. and Kirchman, D.L. (1986). Utilization of inorganic and organic nitrogen by bacteria in marine systems. *Limnology and Oceanographynology and Oceanography* 31, 998–1009.

World Health Organization, WHO (2011). Molybdenum in Drinking-water Background document for development of WHO Guidelines for Drinking-water Quality.

Wu, S., Hu, C., Tan, Q., Nie, Z. and Sun, X. (2014). Effects of molybdenum on water utilization, antioxidative defense system and osmotic-adjustment ability in winter wheat (*Triticum aestivum*) under drought stress. *Plant Physiology and Biochemistry* 83, 365–374.

Wuana, R.A. and Okieimen, F.E. (2011). Heavy metals in contaminated soils : A review of sources, chemistry, risks and best available strategies for remediation. *International Scholarly Research Network Ecology*, Volume 2011.

Yamaguchi, S., Miura, C., Ito, A., Agusa, T., Iwata, H., Tanabe, S., Tuyen, B.C. and Miura, T. (2007). Effects of lead, molybdenum, rubidium, arsenic and organochlorines on spermatogenesis in fish: Monitoring at Mekong Delta area and in vitro experiment. *Aquatic Toxicology* 83, 43–51.

Yano, T. and Koga, S. (1969). Dynamic behavior of the chemostat subject to substrate inhibition. *Biotechnology and Bioengineering* 11(2), 139–153. http://doi.org/10.1002/bit.260110204.

Yoshimura, K., Ishii, M. and Tarutani, T. (1986). Microdetermination of phosphate in water by gel-phase colorimetry with molybdenum blue. *Analytical Chemistry* 58, 591–594.

Yong, N.K., Oshima, M., Blake, R.C. and Sugio, T. (1997). Isolation and some properties of an iron-oxidizing bacterium *Thiobacillus ferrooxidans* resistant to molybdenum ion. *Bioscience Biotechnology Biochemistry*, *61*(9), 1523–1526.

You, X.Y., Wang, H., Ren, G.Y., Li, J.J., Duan, X., Zheng, H.J. and Jiang, Z.Q. (2015). Complete genome sequence of the molybdenum-resistant bacterium *Bacillus subtilis* strain LM 4–2. *Standards in Genomic Sciences* 10, 127.

Zakaria, Z.A., Zakaria, Z., Surif, S. and Ahmad, W.A. (2007). Hexavalent chromium reduction by *Acinetobacter haemolyticus* isolated from heavy-metal contaminated wastewater. *Journal of Hazardous Materials* 146, 30–38.

Zhai, X., Zhang, Y., Qi, Q., Bai, Y., Chen, X., Jin, L., Ma, X., Shu, R., Yang, Z. and Liu, F. (2013). Effects of molybdenum on sperm quality and testis oxidative stress. *Systems Biology in Reproductive Medicine* 59, 1–5.

van Zwieten, L., Ayres, M.R. and Morris, S.G. (2003). Influence of arsenic co-contamination on DDT breakdown and microbial activity. *Environmental Pollution* 124, 331–339.

Zwietering, M.H., Jongenburger, I., Rombouts, F.M., Van't Riet, K. (1990). Modeling of the bacterial growth curve. *Applied and Environmental Microbiology* 56, 1875–1881.