BIOREMEDIATION OF HEAVY METAL POLLUTED WATER USING IMMOBILIZED FRESHWATER GREEN MICROALGA, *BOTRYOCOCCUS* sp.

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A thesis submitted in
Fulfilment of the requirement for the award of the
Degree of Master of Master of Science

Faculty of Science, Technology and Human Development
Universiti Tun Hussein Onn Malaysia

MAY 2015

DEDICATION

This work is dedicated to God Almighty, my source of Hope and Strength. My dearly beloved parents, my siblings, nieces and nephews, cousins, and my fiancé.

ACKNOWLEDGEMENTS

My big thanks and appreciation goes to God Almighty for His sufficient grace, provision and protection all through the period of my study and stay in Malaysia. For making my study a great success, I am grateful Lord and I owe it all to You my Maker.

My appreciation goes to my wonderful and ever cheerful supervisor, Dr. Hazel Monica Matias Peralta for her endless support throughout my study period. She was not just a supervisor but a mother outside home, she made me feel at home at all times, approachable and always willing to guide and teach. I call her my mentor and role model. Thank you so much for making my work flexible and for your support and understanding. I truly and sincerely appreciate your effort and time spent to make this work a huge success, God bless you and your family.

My next appreciation goes to my co-supervisor, Dr. Norshuhaila Mohamed Sunar for her guidance, direction, support and understanding through the period of my study. Thank you for your friendly and approachable nature. I would love to say a big thank you to Mrs Siti Nazmilah bt Misrin and Mr Ishak bin Ayub of the Microbiology Laboratory, Mr Hazrin, Mr Faizal, Mr Redzwan, Mrs Nadia, Mrs Fazliana and Mrs Mahfuzah for making the laboratory work smooth and easy due to your ever lending support and availability throughout the period of this research.

My warm appreciation goes to my biggest brother, Dr. Onalo Ugbede whom with the help of God made my coming to study in Malaysia a reality. He was not just a brother in Malaysia but he became a father, a friend, a confidant and a source of encouragement. Thank you so much for your resources, time, energy and support spent to make my programme a success, God bless you real good sir.

My heartfelt gratitude goes to my dad, Chap. Major General I.O Onalo and my mum DCNS R.A Onalo (JP), thank you for always loving me, providing for me and always being there for me as lovely parents, God bless you. Next to my lovely and beautiful sisters, Mrs Loveth Uneku Abalaka, Mrs Anita Ojoma Onalo, Mrs Olaide Onalo, Mrs Adeola Onalo and Salama. My lovely brothers; Mr Ogbadu Abalaka, Dr. Onalo Ugbede, Mr Daniel Onalo, Mr Victor Onalo and Mr Idoko

Onalo. My cute nephews and nieces; Ojima, Bobo, Nimi, Numa, Chenemi, Bobo 2, Duke, Dutches, Adasa, baby Oj and baby Zoe. Aunty loves you all and God bless you. Also the family of Alhaji N.M Ibrahim, thank you for your love and encouragements, God bless you. To all the Hope brothers and sisters, thank you for your endless love for the kingdom, for loving us and for keeping the fire burning. More of God's grace upon you all. To Hospital chapel brethren, thank you for your prayers, God bless you. To my friends and colleagues, I remain grateful to you all.

Finally, my lovely appreciation goes to my fiancé; Dr. Ojonojima Ajanya. You are indeed the true definition of "LOVE". Thank you for your care, love, encouragements, patience, understanding and always been there for me. Thank you also for always trying your best in making me feel loved and happy at all times. God Almighty will reward you beautifully and bless you richly and will make all your desires to come true.

ABSTRACT

Heavy metal containing wastewater are regarded as highly toxic to the aquatic environment and to life in general due to their bio-accumulating, cytotoxic, mutagenic and carcinogenic effects on life. Bioremediation is the use of biological materials (e.g. microalgae) in the removal of toxic compounds from the environment such as the heavy metals which is considered more cost effective and environmentally friendly when compared to the physical and chemical methods. The present study was undertaken to check for the heavy metal bioremoval efficiency of free and immobilized *Botryococcus* sp. Four heavy metals were studied and the free cells efficiently reduced Chromium which is equivalent to 94%, followed by Copper (45%), Arsenic (9%) and Cadmium (2%). For the immobilized biomass, the highest ($P<0.05$) removal efficiency was recorded in the highest biomass concentration (i.e. 15 beads/ml) for Cadmium, Arsenic and Chromium at 76%, 68% and 67%. Whereas, the highest ($P<0.05$) removal of copper was observed in the blank alginate beads at 84%. The positive control (free cells) recorded the highest ($P<0.05$) reduction for biological oxygen demand (BOD) whereas, the 15 beads/ml gave the highest ($P<0.05$) reduction for control gave the highest ($P<0.05$) reduction for the Chemical oxygen demand (COD). In the LD50 experiment, immobilized biomass harvested from the bioremoval study experiment were used on fishes for toxicity testing. A total of 100% mortality was recorded in the positive control after 24 hours whereas, 3% mortality was observed in negative control and in the 10 beads/ml treatment after 72 hours. No mortality was found in any other treatment after a period of 96 hours. The results obtained from this study suggests that, immobilized cells of *Botryococcus* sp. is efficient in the bioremoval of heavy metals from contaminated waters and also have great potential in the biotransformation of toxic compounds to less-toxic forms.

ABSTRAK

Logam berat yang terkandung dalam air sisa dianggap sangat bertoksik kepada persekitaran dan kehidupan akuatik yang disebabkan oleh kesan bio-accumulating, cytotoxic, mutagenic dan carcinogenic dalam kehidupan. Bioremediasi adalah merupakan penggunaan bahan biologi (eg. mikroalga) dalam penyingkiran bahan toksik seperti logam berat secara lebih efektif dari segi kos serta mesra alam jika dibandingkan dengan kaedah konvensional. Kajian ini telah dijalankan untuk menilai kecekapan penyingkiran logam berat dengan menggunakan *Botryococcus* sp. Empat logam berat telah dikaji menggunakan free sel yang mana Kromium dikurangkan sebanyak 94%. Diikuti oleh Copper (45%), Arsenik (9%) dan Kadmium (2%). Manakala untuk biojisim bergerak, yang paling tinggi (P <0.05) penyingkiran dicatatkan pada kepekatan biojisim yang tertinggi (iaitu 15 manik/ml) dan untuk Kadmium, Arsenik serta Kromium masing-masing pada 76%, 68% dan 67%. Manakala, yang tertinggi (P <0.05) penyingkiran kuprum diperhatikan dalam manik alginat kosong pada 84%. Kawalan positif (sel percuma) merekodkan jumlah tertinggi (P<0.05) pengurangan permintaan oksigen biologi (BOD) manakala, 15 manik/ml memberi nilai tertinggi (P<0.05) pengurangan untuk memberi kawalan yang paling tinggi (P<0.05) pengurangan permintaan oksigen kimia (COD). Dalam eksperimen LD50, biomas bergerak dituai dari eksperimen kajian bioremoval digunakan pada ikan untuk ujian ketoksikan. Sebanyak 100% kematian dicatatkan pada kawalan positif selepas 24 jam sedangkan, 3% kematian diperhatikan dalam kawalan negatif dan dalam 10 manik / rawatan ml selepas 72 jam. Tiada kematian didapati dalam mana-mana rawatan yang lain selepas tempoh 96 jam. Keputusan yang diperolehi daripada kajian ini menunjukkan bahawa, sel-sel *Botryococcus* sp. bergerak. cekap dalam menyingkirkan logam berat daripada air yang tercemar dan juga mempunyai potensi besar dalam bio-transformasikan sebatian toksik kepada bentuk yang kurang bertoksik.

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LIST OF SYMBOLS

%	-	Percentage
Mg/L	-	Milligram per Litre
^{o}C	-	Degree Celsius
rpm	-	Rounds per Minute
psi	-	Per Square Inch
g	-	Grams
ml	-	Millilitre
L	-	Litre
g/L	-	Grams per Litre
Cells/Square	-	Cells per Square
Cells/ml	-	Cells per Millilitre
Beads/ml	-	Beads per Millilitre
dH_20	-	Distilled Water

LIST OF ABBREVIATIONS

TN	-	Total Nitrogen
PO_4^{3-}	-	Orthophosphate
TOC	-	Total Organic Carbon
TC	-	Total Carbon
TC	-	Inorganic Carbon
TS	-	Total Solids
DO	-	Dissolved Oxygen
TDS	-	Total Dissolved Solids
TSS	-	Total Suspended Solids
AAS	-	Atomic Absorption Spectrophotometer
As	-	Arsenic
Cd	-	Cadmium
Cr	-	Chromium
Cu	-	Copper
<	-	Less than
NaCl	-	Sodium Chloride
$K_2Cr_2O_7$	-	Potassium dichromate

$AgSO_4$	-	Silver Sulphate
$HgSO_4$	-	Mercuric Sulphate
H_2SO_4	-	Sulphuric Acid
CO_2	-	Carbon di Oxide
H_2O	-	Water
SEM	-	Scanning Electron Microscopy
LD50	-	Median Lethal Dose
UTHM	-	Universiti Tun Hussein Onn Malaysia
FSTPi	-	Faculti Sains, Teknology dan Pembangunan Insan

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Increase in human activities such as industrialization and civilization has resulted in the issue of enviornmental pollution and in most cases the pollution of water bodies. This issue has for long been a great problem that requires adequate attention. Pollution according to Abdel-Raouf et al. (2012) is referred to as a non-natural occurrence usually associated with elevated concentrations of naturally existing materials or synthetically made substances that are released into a particular environment. Heavy metals are environmental pollutants of inorganic origin with atomic weights of about 63.5 to 200.6 g/mol (Srivastava & Majumder, 2008). Heavy metals are known to be emptied into freshwaters and their resources via industrial effluents (Tripathi, 2014).

They tend mainly to pollute surface water and groundwater due to natural events or human activities. They are carcinogenic, mutagenic and cytotoxic and therefore, pose a great and deadly effect on the environment and on living things (Moo-Young, 2011). The release of both organic and inorganic contaminants into the environment by either industrial activities or other form of anthropogenic practices has led to the case of environmental pollution (Mouchet, 1986; Lim *et al*., 2010).

Heavy metal discharge into the environment has rather become a rapid practice owing to the increasing trend in technology and also their use in different industrial processes (Tripathi, 2014). According to Vidal (2001), environmental quality has a lot to do with the quality of life on earth as this go hand in hand. Recently, the problem of environmental pollution has been accepted as a general or global issue as the evaluated contaminated areas is significant (Cairney, 1993).

Among the major new technologies that have appeared since the 1960s, bioremediation applications have attracted a great deal of attention and interest. The application of microbial metabolic potential (bioremediation) is accepted as an environmentally benign and economical measure for the decontamination of polluted environments. Bioremediation methods are generally categorized into ex situ and in situ bioremediation. The bioremediation of heavy/toxic metals is focused on the reduction in the level or amount of hazardous wastes as the immoderate accumulation of these metallic ions can be highly toxic to plants, aquatic life and the environment as a whole (Singh *et al.*, 2002). Mohee & Mudhoo, (2012), stated that the limited achievements in using these biological materials in the process of bioremediation have recently been ascribed to the reduction in biological system productivity and diversity under environmental conditions. Considering the many adverse effects that accompany environmental pollution by heavy metals comes a better and an effective remedial approach, a process known as bioremediation.

Bioremediation is a branch of biotechnology that utilizes biological processes to render harmless the environmental contaminants (Boopathy, 2000). There are a number of agents used for bioremediation out of which are the bacteria, fungi and the microalgae but the microalgae presenting unique characteristics out of which is their ability to distinguish between the essential and the non-essential substances that are present in the environment. In addition, these set of organisms are single celled and as a result they do not mutate. Microalgae are unicellular and colonial organisms that are highly diversified and are made up of the eukaryotic protists and the prokaryotic blue-green algae (cyanobacteria). These set of organisms are known to be outstanding in assessing environmental quality and are thus, considered unique (Day *et al*, 1999).

Microorganisms, especially the microalgae present a relatively great size for heavy metal ion binding at an eco-friendly rate of production and have been therefore, applied in the bioremoval of metallic ions from contaminated

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