

سامية محمد مصطفى



شبكة المعلومات الجامعية

بسم الله الرحمن الرحيم



سامية محمد مصطفى



شبكة المعلومات الجامعية



شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



سامية محمد مصطفى



شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



سامية محمد مصطفى



شبكة المعلومات الجامعية



بعض الوثائق الأصلية تالفة



سامية محمد مصطفى



شبكة المعلومات الجامعية



بالرسالة صفحات لم ترد بالأصل



**BIOTECHNOLOGICAL STUDY ON HEAVY METALS
ACCUMULATION BY SELECTED FRESH WATER
BACTERIA USING BIOFILM SYSTEMS**

*Thesis
Submitted in Partial Fulfillment
For the Degree of Master of Science*



*In
Biotechnology*

Dr. Mohiy Eldin Abdel-Samir

Elh
E. ElBestawy

Prof.

By
Sameh Reyad Abdallah Ayoub
(B.Sc. Biochemistry, 1993)
Faculty of Science
University of Alexandria

Department of Biotechnology
Institute of Graduate Studies and Research
University of Alexandria

2004 2003

B
15220

SUPERVISORS

Dr. Mohamed Hisham A. El-Masry

*Assoc. Prof. of Microbiology and Molecular Biology
Dept. of Biotechnology
Institute of Graduate Studies and Research
University of Alexandria*

Dr. Salah Ahmed Sheweita

*Assoc. Prof. of Biochemistry
Dept. of Biotechnology
Institute of Graduate Studies and Research
University of Alexandria*

Dr. Ebtesam Abd El-Hameid El-Bestawy

*Assoc. Prof. of Environmental Sciences
Dept. of Environmental Studies
Institute of Graduate Studies and Research
University of Alexandria*

APPROVED SHEET

Thesis Title

*"Biotechnological study on heavy metals accumulation by
selected fresh water bacteria using biofilm systems"*

EXAMINATION COMMITTEE

Prof. Mohey El-Dein Abd El-Samei Farag

ME Abdel-Samei.....

Prof. Fahmy Mahmoud El-Sharkawy

FMA.....

Dr. Mohammad Hisham Ahmad El-Masry

MH El-Masry.....

Dr. Ebtesam Abd El-Hameed El-Bestawy

E. El-Bestawy.....

Date:

15/ 1 /2004

ACKNOWLEDGMENT

I would like to express my great appreciation to Dr. Mohamed Hisham El-Masry, Associate Prof. of Microbiology and Molecular Biology, Department of Biotechnology, Institute of Graduate Studies and Research (IGSR), University of Alexandria, for devoting much of his time and effort in supervising this thesis, his guidance, and useful criticism during the preparation of this work.

I wish to express my sincere thanks to Dr. Salah Sheweta, Associate Prof. of Biochemistry, Department of Biotechnology, IGSR, University of Alexandria, for his support, supervision, encouragement and help throughout this study.

I would like to express my deep gratitude to Dr. Ebtesam El-Bestawy, Associate Prof. of Environmental sciences, Department of Environmental Studies, IGSR, University of Alexandria, for her devoting and unlimited time during this study, her continuous advice, encouragement, and her valuable assistance during the preparation of the thesis.

I also would like to express my deepest thanks and appreciation to Dr. Fatma Abou Shouk, Head of Alexandria Regional Branch Office (Alex. RBO), Egyptian Environmental Affairs Agency (EEAA), for her continuous support, encouragement, providing all facilities, that let this thesis come true.

A lot of thanks are also to Dr. Eatedal Abd El-Kreem for her assistance, and also to all colleagues in EEAA who facilitate my work during the course of this thesis.

To...

**My beloved
mother**

CONTENTS

Contents	Page
	i
List of Tables	iv
List of Figures	vi
List of Abbreviations	vii
SUMMARY	1
1. INTRODUCTION AND AIM OF THE WORK	5
2. REVIEW OF LITERATURE	7
2.1. Heavy metals	7
2.1.1 Metals terminology	7
2.1.2. Methods of determination	8
2.2. Heavy metals and the environment	9
2.2.1. Metals in the terrestrial environment	9
2.2.2. Metals in the aquatic environment	10
2.3. Microbe - Metal interaction	12
2.3.1. Intracellular accumulation	12
2.3.2. Cell wall binding	12
2.3.3 Metal - siderophore interaction	12
2.3.4. Extracellular mobilization / immobilization of metals	13
2.3.5. Extracellular polymer - metal interaction	13
2.3.6. Transformation and volatilization of metals	14
2.4. Essentiality and toxicity of metals for microorganisms	14
2.4.1. Essentiality	14
2.4.2. Toxicity	15
2.5. Heavy metal and bioaccumulation	18
2.6. Biofilm system	21
2.6.1. Biofilm system component	22
2.6.2. Biofilm initiation and development	24
2.6.3. Particle detachment	26
2.6.3.1. Physical causes of detachment	26
2.6.3.2. Chemical causes of detachment	27
2.6.3.3. Biological causes of detachment	27

	Page
2.6.4. Advantage of Bacterial Attachment	27
2.6.5. Laboratory model of biofilm fermentor	28
2.6.5.1. Substratum	29
2.6.5.2. Biofilm sampling	29
2.6.5.3. Steady - state biofilms	29
2.7. Mutagenesis as a tool for metal removal enhancement	30
2.7.1. Chemical mutagenesis	30
2.7.2. Alkylation using nitrosamines	31
 3. MATERIALS AND METHODS	 32
3.1. Biofilm system development	32
3.1.1. Microorganisms	32
3.1.2. Sterilization	32
3.1.3. System construction	33
3.1.4. Determination of population dynamics	35
3.1.5. Biofilm formation	35
3.2. Wild biofilm efficiency for heavy metal removal	36
3.2.1. Preparation of Heavy metal synthetic solution	36
3.2.2. Operation conditions of the continuous treatment using the biofilm	36
3.2.3. Determination of heavy metals	37
3.2.4. Application of the biofilm system in the treatment of contaminated wastewater	38
3.3. Determination of biofilm efficiency for reducing organic load	39
3.3.1. Determination of biochemical oxygen demand (BOD)	39
3.3.2. Determination of Chemical Oxygen Demand (COD)	39
3.3.3. Determination of total solids (TS)	40
3.3.4. Determination of total dissolved solids (TDS)	40
3.3.5. Determination of total suspended solids (TSS)	40
3.3.6. Determination of grease and oil (G & O)	40
3.4. Chemical mutation	41
3.4.1. Induction of chemical mutation	41
3.4.2. Selection of mutants exhibiting high metal resistance	41
3.4.3 Using the mutants strains in the biofilm system	42

	Page
4. RESULTS	43
4.1. Biofilm formation	43
4.1.1. Bacterial growth and population dynamics	43
4.1.2. Bacterial adhesion on the supporting material	46
4.1.3. Detecting the bacterial adhesion using scanning electron microscope	49
4.2. Efficiency of Heavy metals removal using a biofilm of parent bacterial consortium	49
4.2.1. Optimization of metals removal at different pH levels	51
4.2.1.1. Iron	51
4.2.1.2. Cadmium	53
4.2.1.3. Lead	53
4.2.1.4. Copper	56
4.2.1.5. Zinc	58
4.2.2. Optimization of metals removal using different flow rates	60
4.2.2.1. Iron	61
4.2.2.2. Cadmium	61
4.2.2.3. Lead	66
4.2.2.4. Copper	66
4.2.2.5. Zinc	66
4.2.3. Effect of using bacterial consortium instead of individual bacteria on heavy metals removal	74
4.3. Application of the proposed biofilm system for the treatment of wastewater contaminated with heavy metal	78
4.3.1. Treatment of raw wastewater	78
4.3.2. Treatment of primary treated wastewater	80
4.4. Effect of mutation on enhancing the removal of heavy metals by the tested bacteria	82
4.5. Evaluation of organic load reduction using the biofilm filter	84
5. DISCUSSION	87
6. REFERENCES	92
ARABIC SUMMARY	

LIST OF TABLES

<u>Table #</u>	Page
1. Sources and the environmental level of metals investigated in the present study	11
2. Population dynamics of the bacterial consortium in the medium used for the biofilm formation	44
3. Total viable count (TVC) and contribution % of the individual bacteria after detachment procedures.....	47
4. Removal efficiency % of Fe under different pH values and the lowest flow rate of 15 ml/hr.....	52
5. Removal efficiency % of Cd under different pH values and the lowest flow rate of 15 ml/hr.....	54
6. Removal efficiency % of Pb under different pH values and the lowest flow rate of 15 ml/hr.....	55
7. Removal efficiency % of Cu under different pH values and the lowest flow rate of 15 ml/hr.....	57
8. Removal efficiency % of Zn under different pH values and the lowest flow rate of 15 ml/hr.....	59
9. Effect of flow rate on Iron removal efficiency at pH 7.....	62
10. Effect of flow rate on Cadmium removal efficiency at pH 7.....	64
11. Effect of flow rate on Lead removal efficiency at pH 7.....	67
12. Effect of flow rate on Copper removal efficiency at pH 7.....	69
13. Effect of flow rate on Zinc removal efficiency at pH 7.....	71
14. Removal efficiency % of the investigated metals using individual and mixed bacterial cultures biofilm.....	75
15. Removal efficiency % of the investigated metals from untreated wastewater of WWTP using the biofilm system at a flow rate of 25ml/hr, pH 7 and 2.5 hr exposure time.....	79

	Page
16. Removal efficiency % of the investigated metals from the primary treated wastewater of WWTP using the biofilm system at a flow rate of 25ml/hr, pH 7 and 2.5 hr exposure time.....	81
17. Effect of mutation on the removal of heavy metal using the proposed biofilm system at a flow rate of 25ml/hr, pH7 and 2.5 hr exposure time	83
18. Degradation and / or removal efficiency % of wastewater organic load using bacteria and bacteria-free filter at a flow rate of 25ml/hr, pH7 and 2.5 hr exposure time.....	85