

# بسم الله الرحمن الرحيم





شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



شبكة المعلومات الجامعية

# جامعة عين شمس

التوثيق الالكتروني والميكروفيلم

# قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها على هذه الأفلام قد أعدت دون أية تغيرات



# يجب أن

تحفظ هذه الأفلام بعيدا عن الغبار

في درجة حرارة من ٢٥-١٥ مئوية ورطوية نسبية من ٢٠-١٠ في درجة حرارة من ٢٥-١٥ مئوية ورطوية نسبية من ٢٠-١٤. To be Kept away from Dust in Dry Cool place of 15-25- c and relative humidity 20-40%

# بالرسالة صفحات لم ترد بالاصل

بعض الوثائـــق الأصليــة تالفـه

# STUDIES ON DEGRADATION PROPERITIES OF ACTINOMYCETES INHABITING GRAVEL BED HYDROPONIC (GBH) SYSTEM USED FOR INDUSTRIAL EFFLUENT TREATMENT

Thesis Submitted By

# SAHAR AHMED EL-SHATOURY

B.Sc. (Botany), M.Sc. (Microbiology), Suez Canal University

For The Fulfilment of The Requirements For The Degree of Doctor of Philosophy



Botany Department Faculty of Sciences Suez Canal University 2001 بندالهالحمرالعي

# **Approval Sheet**

### TITLE:

# STUDIES ON DEGRADATION PROPERTIES OF ACTINOMYCETES INHABITING GRAVEL BED HYDROPONIC (GBH) SYSTEM USED FOR INDUSTRIAL EFFLUENT TREATMENT

A. Deweda

By:

Sahar Ahmed El-Shatoury, B.Sc., M.Sc.

### THE SUPERVISION COMMITTEE:

Prof. Dr. Ahmed Dewedar Professor of Microbiology Botany Department Faculty of Science Suez Canal University Ismailia – Egypt

Dr. Julian Mitchell

Senior Lecturer in Genetics School of Biological Sciences University of Portsmouth, UK

Dr. Magdi Bahgat

Assistant Professor of Microbiology Faculty of Environmental Sciences Suez Canal University El-Arish- Egypt Intian Mitchell

Magdi Bolgel

# CONTENTS

Declaration Acknowledgments List of Figures List of Micrographs List of Tables List of Appendices Abbreviations	Pages I II III IV V V VI
PART I	
CHAPTER 1 Introduction and literature review	
1.1 Nature of the industrial wastewater and its impact on the environment	1
1.2 Approaches to the treatment of industrial wastewater 1.2.1 Non-biological approaches for the treatment of industrial wastes	2 2
1.2.2 Bioremediation of industrial wastewater	3
1.3 Gravel Bed Hydroponic (GBH) systems	5
1.4 Application of constructed wetlands for industrial effluent treatment	6
1.4.1 Removal mechanisms in constructed wetlands	7
1.4.2 Fate of polycyclic aromatic hydrocarbons (PAH) in wastewater	
treatment systems	7
1.5 General aspects of polycyclic aromatic hydrocarbons (PAH)	
biodegradation pathways	8
1.6 Factors influencing the biodegradation of polycyclic aromatic	
hydrocarbons (PAH) in wastewater treatment systems	9
1.6.1 Physical and Chemical factors	9
1.6.2 Biological factors	13
1.7 Actinomycetes in wastewater treatment systems	16
1.8 Approaches to the characterisation and identification of actinomycetes	17
1.8.1 Traditional and advanced systems for actinomycetes taxonomy	17
1.8.2 Numerical classification of actinomycetes	19
1.9 Mechanisms of Polycyclic Aromatic Hydrocarbons (PAH) degradation	
by actinomycetes	20
1.10 Problems of the pesticide 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane	
(DDT) in Egypt	22
1.11 Microbial degradation of the pesticide DDT	23
1.12 Industrial wastewater at 10 <sup>th</sup> Ramadan city	24
1.13 Objectives of the study	25
CHAPTER 2 Materials and methods	
2.1 Media, buffers and reagents	27
2.1.1 Media	27
2.1.2 Buffers and Reagents	30
2.2 Study site	31

2.3	Sampling procedure	32
	2.3.1 Water samples	32
	2.3.2 Gravel-root matrix samples	32
2.4	Microbiological analysis	33
	2.4.1 Preparation of biofilm suspension	33
	2.4.2 Enumeration of actinomycetes and heterotrophic eubacteria from	
	the GBH system	33
	2.4.3 Maintenance of actinomycetes isolates	33
2.5	Physico-chemical analysis	34
	2.5.1 pH	34
	2.5.2 Dissolved Oxygen (DO) mg/l	34
	2.5.3 Biochemical Oxygen Demand (BOD) mg/l	34
	2.5.4 Chemical Oxygen Demand (COD) mg/l	35
	2.5.5 Inorganic nitrogen species	35
	2.5.6 Total suspended solids (TSS) mg/l	36
2.6	Characterisation of actinomycetes	36
	2.6.1 Macroscopic characterisation	36
	2.6.2 Microscopic characteristics	37
	2.6.3 Soluble pigment production	37
	2.6.4 Melanin production	37
	2.6.5 Degradation of organic compounds	38
	2.6.6 Growth tests	38
	2.6.7 Utilisation of compounds as sole carbon sources	38
	2.6.8 Utilisation of compounds as sole nitrogen sources	38
	2.6.9 Antibiotic resistance	39
	2.6.10 Detection of 2,6-diamino-pimelic (DAP) acid in whole	
	cell hydrolysate	39
	2.6.11 Detection of mycolic acids	40
	2.6.12 Detection of characteristic sugars in the whole cell hydrolysate	41
2.7	Degradation studies	42
	2.7.1 Screening for degradation abilities of actinomycetes isolates	42
	2.7.2 Culture conditions for DDT degradation experiments	42
	2.7.3 Construction of percolating gravel microcosms	42
	2.7.4 Measurement of DDT concentration by UV spectrophotometry	43
	2.7.5 Measurement of DDT concentration by gas chromatography-	
	mass spectrum detection (GC/MS)	43
	2.7.6 DDT extraction procedure	44
2.8	Molecular biology techniques	45
	2.8.1 Plasmid detection by alkaline lysis	45
	2.8.2 Rapid plasmid screening method	46
	2.8.3 Plasmid detection using Qiagen Mega Plasmid Isolation Kit	47
	2.8.4 Pulsed field gel electrophoresis (PFGE) analysis	47
2.9	Data analysis	50
	2.9.1 Statistical data analysis	50
	2.9.2 Numerical classification	50

# PART II

CHAPTER 3 Factors influencing the distribution of actinomycetes population in GBH system for industrial wastewater treatm	ıent
3.1 Introduction	52
3.2 Result	53
3.2.1 Selection of actinomycetes isolation medium	53
3.2.2 Impact of replication of samples	54
3.2.3 Distribution of microbial populations in the biofilm along the	
GBH bed	55
3.2.4 Physico-chemical characters of the GBH bed	56
3.2.5 Interactions between actinomycetes population and other	
variables in the GBH bed	58
3.3 Discussion	60
CHAPTER 4 Numerical taxonomy and biodiversity of actinomycetes associated with the GBH system for industrial wastewater treatn	nent
4.1 Introduction	62
4.2 Results	64
4.2.1 Characterisation of taxa	64
4.2.2 Numerical classification of actinomycetes from the GBH system	77
4.2.3 Distribution of actinomycetes taxa along the GBH system	80
4.3 Discussion	81
CHAPTER 5 Degradation abilities of actinomycetes isolated from the GBH system at 10 <sup>th</sup> Ramadan City	
5.1 Introduction	82
5.2 Experimental results	85
5.2.1 Chatechol degradation by actinomycetes isolated from the GBH	63
system	85
5.2.2 Degradation of selected Polycyclic Aromatic Hydrocarbons	65
(PAH) by actinomycetes in minimal medium	85
5.2.3 Toxicity of the studied PAH to the degrading actinomycetes	87
5.2.4 Degradation of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane	0/
(DDT) pesticide by selected actinomycetes strains	88
5.2.5 DDT degradation by <i>Ndp</i> -11 in percolating gravel microcosms	. 93
5.2.6 Plasmid Screen for DDT degrading isolates	95
5.3 Discussion	96

# PART III

# **General Discussion**

I. Ecology of actinomycetes and heterotrophic eubacteria	
in the GBH system at 10 <sup>th</sup> Ramadan City	99
II. Factors influencing the distribution actinomycetes population	
in the GBH system at 10th Ramadan City	101
III. Numerical taxonomy and biodiversity of actinomycetes	
in the GBH system at 10 <sup>th</sup> Ramadan City	102
IV. Degradation of Polycyclic Aromatic Hydrocarbons (PAH) by	
actinomycetes inhabiting the GBH system at 10th Ramadan City	105
V. Detection of DDT metabolic products produced by the isolates	
Nocardiopsis dassonvillei 11 and Streptomyces cyaneus 85 using GC/MS	108
VI. DDT degradation in percolating gravel microcosm	109
VII. Screening for plasmids in the DDT degrading isolates	111
Conclusion and Recommendations	113
Summary	114
References	
Appendices	
Arabic summary	

# **Declaration**

I hereby certify that the work submitted in this thesis is my own and has not been presented previously, or separately, for any other degree.

Signed Sahar El-Shatoury

Sahar El-Shatoury

### **ACKNOWLEDGMENTS**

I am very grateful to my supervisor, Prof. A. Dewedar, Assistant Secretary General- Association of Arab Universities, for suggesting the topic of the research and providing all the facilities to complete it. His wide experience and continuous advice has helped me during this work.

I would like to thank Dr. J. Mitchell, Senior lecturer in genetics – Portsmouth Univ., who gave me the opportunity to complete this research in his laboratory – School of Biological Sciences, Portsmouth University. My deepest thanks to him for his invaluable supervision, advice and patience throughout the research and thesis preparation.

My thanks to Dr. M. Bahgat, Assist. Prof. – Faculty of environmental science, Suez Canal Univ., for his help, advice and encouragement.

I am grateful to members of Botany Dept., Faculty of Science, SCU and School of Biological Sciences, Portsmouth Univ.; whose experience and cheerful accompany helped me throughout my research. Particular thanks to Dr. R. Greenwood – Head of School of biological Sciences, for facilitating gas chromatography / mass spectrometry analysis. I similarly express my thanks to Dr. S. Moss - School of biological Sciences, for providing the facilities to perform the electron microscopy work.

I would like to acknowledge the Mission Dept., Ministry of Higher Education, for kindly funding this work.

My special and sincere thanks to my dear parents; who encourage me throughout my education and whose support is behind any success I have gained. I will always be grateful to my family, without their care and cheerful support this work would have never been achieved.

### LIST OF FIGURES

- 1.1 Ring cleavage of aromatic compounds.
- 1.2 Proposed bacterial degradation pathway for DDT.
- 1.3 Distribution of industries at 10<sup>th</sup> Ramadan City and volumes of discharged waste effluent.
- 1.4 Outlet pipe from oxidation pond 2 at 10<sup>th</sup> Ramadan City.
- 2.1 The established Gravel Bed Hydroponic (GBH) system at 10<sup>th</sup> Ramadan City.
- 2.2 The design of the percolating gravel microcosm.
- 3.1 Counts of actinomycetes on different selective media.
- 3.2 Counts of actinomycetes in nine gravel-root matrix sub-samples.
- 3.3 Mean counts of actinomycetes isolated from the gravel-root matrix of the GBH bed
- 3.4 Mean counts of heterotrophic bacteria isolated from the gravel-root matrix of the GBH bed.
- 3.5 Physico-chemical characters of the GBH bed
- 3.6 BOD: COD ratio for wastewater received by the GBH bed at 10<sup>Th</sup> Ramadan City during the study period.
- 3.7 Plot of interaction between different variables in the GBH system with actinomycetes count.
- 4.1 A dendrogram showing clustering of the actinomycetes from the industrial GBH system.
- 4.2 Proportion of actinomycetes genera in the GBH bed.
- 4.3 Distribution of actinomycetes genera along the GBH bed.
- 5.1 Percentage of actinomycetes showing degradation ability and tolerance on selected polycyclic aromatic hydrocarbons.
- 5.2 Distribution of the actinomycetes genera showing abilities to degrade the target polycyclic aromatic hydrocarbons.
- 5.3 DDT degradation by *Nocardioides sp.* 7, *Nocardiopsis sp.* 11 and *Streptomyces sp.* 85 in M56 minimal medium amended with 100 ppm DDT.
- 5.4 DDT degradation by *Nocardiopsis sp.* 11 and *Streptomyces sp.* 85 in M56 minimal medium amended with 100 ppm DDT.