



Effect of Different Environmental Factors and Radiation on Bioremediation of Acid Mine Drainage by Sulfate Reducing Bacteria

THESIS
Submitted for Ph.D. Degree in
Microbiology

By

Naglaa Mohamed Abdelaal

M.Sc. (Microbiology 2011)
Lecturer Assistant- National Center for Radiation Research
and Technology (NCRRT)

Microbiology Department,
Faculty of Science,
Ain Shams University.
(2020)



Effect of Different Environmental Factors and Radiation on Bioremediation of Acid Mine Drainage by Sulfate Reducing Bacteria

Thesis

Submitted for the Award of the Degree of Doctor of Philosophy in Microbiology

By

Naglaa Mohamed Abdelaal

M.Sc. (Microbiology 2011)

Lecturer Assistant- National Center for Radiation Research and Technology (NCRRT)

Supervisors

Prof. Dr. Fawkia Mohamed El-Beih

Professor of Microbiology, Microbiology Department, Faculty of Science, Ain Shams University.

Prof Dr. Loutfy Abdel- Rehim Ali Moosa

Professor of Microbiology, National Center for Radiation Research and Technology (NCRRT).

Prof. Dr. Khaled Zakaria EL- Baghdady

Professor of Microbiology, Microbiology Department, Faculty of Science, Ain Shams University.

Dr. Abeer Emam Mohamed Zakaria

Associate professor of Microbiology, National Center for Radiation Research and Technology (NCRRT).

**Microbiology Department,
Faculty of Science,
Ain Shams University.
(2020)**

Approval Sheet



Effect of Different Environmental Factors and Radiation on Bioremediation of Acid Mine Drainage by Sulfate Reducing Bacteria

By

Naglaa Mohamed Abdelaal

*Lecturer Assistant- National Center for Radiation Research
and Technology (NCRRT)*

Supervisors

Prof. Dr. Fawkia Mohamed El-Beih

*Professor of Microbiology, Microbiology Department,
Faculty of Science, Ain Shams University.*

Prof. Dr. Khaled Zakaria EL- Baghdady

*Professor of Microbiology, Microbiology Department,
Faculty of Science, Ain Shams University.*

Prof. Dr. Loutfy Abdel- Rehim Ali Moosa

*Professor of Microbiology, National Center for Radiation
Research and Technology (NCRRT).*

Dr. Abeer Emam Mohamed Zakaria

*Associate professor of Microbiology, National Center for
Radiation Research and Technology (NCRRT).*

Examination committee

Prof. Dr. Zeinab Mohamed Hassan Kheirallah

Professor of Microbiology, Botany Department, Faculty of Girls, Ain Shams University.

Prof. Dr. Mohamed Hany Abdelaal Mubasher

Professor of Microbiology, Faculty of Science, Cairo University.

Prof. Dr. Khaled Zakaria EL- Baghdady

Professor of Microbiology, Faculty of Science, Ain Shams University.

Prof. Dr. Loutfy Abdel- Rehim Ali Moosa

*Professor of Microbiology, National Center for Radiation Research and Technology
(NCRRT).*

Date / / 20

University Council approved / / 20

Approval date / / 20

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

Declaration

**This dissertation has not been
previously submitted for any degree
at this or at any other university**

Naglaa Mohamed Abdelaal

ACKNOWLEDGEMENT

*I would like to express my profound gratitude to **Prof. Dr. Fawkia Mohamed El-Beih**, Assistant Professor of Microbiology, Microbiology Department, Faculty of Science, Ain Shams University for continuous support and encouragement interest.*

*I am really greatly indebted to **Dr. Khaled Zakaria EL- Baghdady**, Associate Professor of Microbiology, Microbiology Department, Faculty of Science, Ain Shams University for his effective cooperation and following actively the work through providing theoretical information and practical help.*

*I would like also to thank deeply **Dr. Abeer Emam Zakaria**, Associate Professor of Microbiology, National Center for Radiation Research and Technology for suggesting the point, providing the protocol of the work, valuable advice and her kind supervision during all stages of this work.*

*I would like to acknowledge with deep gratitude **Prof. Dr. Loutfy Abdel Rahim Aly Moussa**, Professor of Microbiology, Microbiology Department, National Center for Radiation Research and Technology for continuous guidance, criticism and active help to achieve this work.*

*I would like to express my deep appreciation and thanks to **Prof. Dr. Abdel Fattah Helal**, former Vice Chairman of Atomic Energy Authority.*

Deep thanks to Microbiology Department, Faculty of Science, Ain Shams University.

I wish to thank my colleagues at Microbiology Department, NCRRT for their continuous cooperation and encouragement.

My deep acknowledgment should be directed to the members of Central Laboratory for Elemental and Isotopic Analysis, Nuclear Research Center, Atomic Energy Authority of Egypt.

*Finally, a very special thanks to **My Mother** who has stood by me all through my study and her continues support, advices and prayers; and great thanks to my lovely daughters; **Lojain and Jody** for their patience and love.*

DEDICATION

To

My Father's Soul, Brothers, Family

And a special dedication to

My Mother and

My sweetie Daughters;

Lojain& JOdy

Naglaa M. Adelaal



CONTENTS

SUBJECT	PAGE
List of tables	
List of figures	
List of abbreviation	
ABSTRACT	
INTRODUCTION	1-2
AIM OF THE WORK	3
1. LITRATURE REVIEW	4-41
1.1.Heavy metals: definitions and classification	5
1.2.Bioavailability of metals in the environment	7
1.3.Heavy metals pollution	8
1.4.Acid mine drainage formation (AMD)	10
1.5.Environmental Impact of AMD and industrial Wastewaters	11
1.6.Heavy metal toxicity	12
1.7.Health effects of some heavy metals in contaminated wastewaters	13
1.7.1. Iron	13
1.7.2. Zinc	13
1.7.3. Cupper	14
1.7.4. Cobalt	15
1.7.5. Nickle	15



SUBJECT	PAGE
1.8. Heavy metal remediation methods	16
1.8.1. Reverse Osmosis	16
1.8.2. Electrochemical methods	17
1.8.3. Ultrafiltration	18
1.8.4. Ionic Exchange	18
1.8.5. Chemical Precipitation	19
1.8.6. Foam Flotation	20
1.8.7. Coagulation-Flocculation	20
1.8.8. Bioremediation	21
1.9. Microbial heavy metal remediation	22
General mechanism of Microbial metal resistance	22
Exopolymer binding	22
Siderophore complexation	23
Biosurfactants complexation	25
Precipitation by metal reduction	25
1.10. Sulfate reduction biotechnology	27
1.11. Habitat of SRB	29
1.12. Phylogeny of SRB	30
1.13. Physiology of SRB	33
1.14. Factors affecting metal removal efficiency of SRB	35



SUBJECT	PAGE
1.14.1. Effect of environmental pH	36
1.14.2. Effect of temperature	37
1.14.3. Effect of sulfate (SO_4^{-2}) concentration	37
1.14.4. Effect of gamma irradiation	38
Effect of ionizing radiation on living cells	39
Direct action of ionizing radiation	40
Indirect effect of ionizing radiation	41
2. MATERIAL AND METHODS	42-58
2.1.MATERIAL	42-46
2.1.1. Samples	42
2.1.2. Culture medium	42
2.1.3. Chemical reagent	44
Metal solution	44
Copper reagent	44
Ethidium bromide solution	44
TE buffer pH 7.5	44
10x Loading buffer	44
TBE electrophoresis buffer	45
0.1 M Tris-HCl buffer	45



SUBJECT	PAGE
2.1.4. Equipment	45
Spectrophotometer	45
Gamma radiation source	45
Energy Dispersive X-ray (EDX)	46
Inductive Coupled Plasma device (ICP)	46
2.2. METHODS	47-58
2.2.1. Collection of environmental samples	47
2.2.2. Screening for sulfate reducing bacteria	47
2.2.3. Preparation of inocula	48
2.2.4. Enumeration of SRB	48
2.2.5. Estimation of sulfide concentration produced by SRB	49
2.2.6. Examination of SRB growth at different pH	51
2.2.7. Selection of acid- metal tolerant SRB consortia	51
2.2.7.1. Metal tolerance of SRB consortia	51
Screening of SRB tolerance against individual metals	51
Tolerance and metal removal potentials of SRB growing on metal mixture	52
Estimation of the residual heavy metals and sulfide precipitates	52
Metal tolerance of SRB at different metal mixtures (100, 125 mg/l of each metal)	53
2.2.7.2. Acid tolerance of SRB consortia	53
SRB growth at different pH values	53
Acid adaptation	54



SUBJECT	PAGE
2.2.8. Isolation and identification of the most potential isolate	54
2.2.9. Comparative study of the metal removal efficiency rates of the acidic adapted and non-adapted SRB strain at different pH values	55
2.2.10. Factors affecting metal removal efficiency of the acid adapted isolate	56
2.2.10.1. Temperature	56
2.2.10.2. Sulfate Concentration	56
2.2.10.3. Type of inoculum	57
2.2.10.4. gamma irradiation	57
2.11. Treatment of Abou-Rawach metal contaminated wastewater by the selected isolate	58
3. RESULTS	59-150
3.1 Screening of the most promising acid and heavy metals tolerant isolate	59
3.1.1. Screening of SRB	59
3.1.2. Selection of high heavy metal tolerant SRB consortia	61
3.1.2.1 Screening of SRB consortia at different individual metal concentrations	61
3.1.2.2. Tolerance of SRB consortia at different concentrations to mixture of metals	63
3.1.2.3. The heavy metal removal efficiency of SRB consortia at 75 mg/l of metal mixture	87
3.1.2.4 Estimation of residual metals concentration by ICP technique	87



SUBJECT	PAGE
3.1.2.5. Qualitative analysis of metal sulfide precipitates	89
3.1.2.6. Screening of SRB metal tolerance at 100 and 125 mg/l metal mixture concentrations	93
3.1.3. Acid tolerance of SRB consortia	94
3.2. Identification of S7 isolate	96
3.3. Comparative study of the metal removal efficiency rates of the acid-adapted and non-adapted <i>Citrobacter amalonaticus</i> S7 at different pH values	98
3.4. Factors affecting metal removal efficiency of local isolated acid-adapted <i>Citrobacter amalonaticus</i> S7	110
3.4.1. Effect of Temperature	110
3.4.2 Effect of inoculum type	119
3.4.3 Effect of different sulfate concentrations	127
3.4.4 Effect of gamma irradiation	138
3.5 Bioremediation of Abou-Rawach metal contaminated wastewater by <i>Citrobacter amalonaticus</i> S7	148
4. DISCUSSION	151-175
5. SUMMARY	176-182
5. CONCLUSION	183
6. REFERENCES	184-215
7. ARABIC SUMMARY	V-VI



LIST OF TABLES

SUBJECT	PAGE
Table (1): Sites of samples collections.	40
Table (2): Operating condition of ICP-OES.	43
Table (3): The SRB counts in the collected samples.	58
Table (4): The tolerance of SRB consortia to different individual heavy metal concentrations.	60
Table (5-a): The growth rate of AS7 consortia at different concentrations of heavy metals mixture.	62
Table (5-b): The growth rate of AS23 consortia at different concentrations of heavy metals mixture.	63
Table (5-c): The growth rate of AS19 consortia at different concentrations of heavy metals mixture.	64
Table (5-d): The growth rate of AS10 consortia at different concentrations of heavy metals mixture.	65
Table (5-e): The growth rate of AS12 consortia at different concentrations of heavy metals mixture.	66
Table (5-f): The growth rate of AS3 consortia at different concentrations of heavy metals mixture.	67



SUBJECT	PAGE
Table (5-g): The growth rate of AS21 consortia at different concentrations of heavy metals mixture.	68
Table (5-h): The growth rate of AS6 consortia at different concentrations of heavy metals mixture.	69
Table (5-i): The growth rate of SRB of sample 5 at different concentrations of heavy metals mixture.	70
Table (5-j): The growth rate of AS11 consortia at different concentrations of heavy metals mixture.	71
Table (6-a): The sulfide production activity of AS7 consortium at different concentrations of heavy metals mixture.	73
Table (6-b): The sulfide production activity of AS23 consortium at different concentrations of heavy metals mixture.	74
Table (6-c): The sulfide production activity of AS19 consortium at different concentrations of heavy metals mixture.	75
Table (6-d): The sulfide production activity of AS10 consortium at different concentrations of heavy metals mixture.	76
Table (6-e): The sulfide production activity of AS12 consortium at different concentrations of heavy metals mixture.	77
Table (6-f): The sulfide production activity of AS3 consortia at different concentrations of heavy metals mixture.	78