BIOLOGICAL TREATMENT OF AGRICULTURAL DRAINS WATER

Submitted By Marwa Mohamed Reda Mahmoud Afifi

B.Sc. of Agricultural Sciences (Dairy Sciences & Technology), Faculty of Agriculture,

Ain Shams University, 1999

M. Sc. in Agricultural Sciences (Dairy Sciences & Technology), Faculty of Agriculture,

Ain Shams University, 2009

A thesis submitted in Partial Fulfillment Of

The Requirement for the Doctor of Philosophy Degree

In

Environmental Sciences

Department of Environmental Agricultural Sciences
Institute of Environmental Studies and Research
Ain Shams University

APPROVAL SHEET BIOLOGICAL TREATMENT OF AGRICULTURAL DRAINS WATER

Submitted By

Marwa Mohamed Reda Mahmoud Afifi

B.Sc. of Agricultural Sciences (Dairy Sciences & Technology), Faculty of Agriculture,

Ain Shams University, 1999

M. Sc. in Agricultural Sciences (Dairy Sciences & Technology), Faculty of Agriculture,

Ain Shams University, 2009

A thesis submitted in Partial Fulfillment

Οt

The Requirement for the Doctor of Philosophy Degree

In

Environmental Sciences

Department of Environmental Agricultural Sciences

This thesis Towards a Doctor of Philosophy Degree in Environmental Science Has been Approved by:

Name

Signature

1-Prof. Dr. Wafaa Mohamed El-Sayed Haggag

Head of Agricultural & Biological Research Division National Research Center

2-Prof. Dr. Mohamed El Sayed El Nennah

Prof. of Soils & Water

Faculty of Agriculture

Ain Shams University

3-Prof. Dr. Hesham Ibrahim El Kassas

Prof. of Soils & Water Environment

Dean, Institute of Environmental Studies & Research

Ain Shams University

4-Prof. Dr. Maryam Mohamed Mostafa Abd El Motolb

Prof. of Agriculture Water Management, Deputy Director of Central Laboratory for Environmental Quality Monitoring, National Water Research Center

5-Prof. Dr. Abo El-Kahir Badwy El-Sayed

Prof. & Head of Algae Biotechnology Unit, Head of Fertilization Technology Department, Agriculture & Biological Division, National Research Center

BIOLOGICAL TREATMENT OF AGRICULTURAL DRAINS WATER

Submitted By

BIOLOGICAL TREATMENT OF AGRICULTURAL DRAINS WATER

Submitted By

Marwa Mohamed Reda Mahmoud Afifi

B.Sc. of Agricultural Sciences (Dairy Sciences & Technology), Faculty of Agriculture,

Ain Shams University, 1999

M. Sc. in Agricultural Sciences (Dairy Sciences & Technology), Faculty of Agriculture,

Ain Shams University, 2009

A thesis submitted in Partial Fulfillment

Of

The Requirement for the Doctor of Philosophy Degree In Environmental Sciences

Department of Environmental Agricultural Sciences This thesis Towards a Doctor of Philosophy Degree in Environmental Science

Under The Supervision of:

1-Prof. Dr. Hesham Ibrahim El Kassas

Prof. of Soils & Water Environment

Dean, Institute of Environmental Studies & Research Ain Shams University

2-Prof. Dr. Maryam Mohamed Mostafa Abd El Motolb

Prof. of Agriculture Water Management, Deputy Director of Central Laboratory for Environmental Quality Monitoring, National Water Research Center

3-Prof. Dr. Abo El-Khair Badawy El-Sayed

Prof. & Head of Algae Biotechnology Unit, Head of Fertilization Technology Department, Agriculture & Biological Division, National Research Centre

ACKNOWLEDGMENTS

My profound gratitude and appreciation to **Prof. Dr. Hesham EL Kasass**, Dean of Institute of Environmental Studies and Research, Ain Shams University, for his Valuable advice, generous assistance and encouragement of my abilities. Wards could not express my appreciation.

I am sincerely Thankful and indebted to **Prof. Dr. Maryam**Mohamed Mostafa, Prof of Agriculture Water Management, Head of

Quality and Information Department, Central Laboratory for

Environmental Quality Monitoring, National Water Research Center, for

giving every possible help, encouragement, invaluable expertise. I also

thank her for her patience and time in putting my final thesis.

I would like to express my gratitude to **Prof. Dr. Abo El- Kahir Badwy El- Sayed**, Head of Fertilization Technology Department, Head of Algae Biotechnology Unit, National Research Center (NRC), for continuous supporting, and planning for the experimental work and sincere advice, providing me with all the facilities I need, guidance, and constructive supervision. Thank you for making me able to get this thesis done.

All Thanks are to Algal Biotechnology staff members (NRC) for their helpful with me throughout the current practical work.

Finally, I want to thank my family due to their support me throughout the creation of this thesis.

ABSTRACT

Marwa Mohammad Reda Mahmoud Afifi: Biological Treatment of Agricultural Drains Water. Unpublished *Ph.D* Thesis, Agriculture Science Department, Institute of Environmental Studies and Research, Ain Shams University, 2017.

Against the backdrop of scarce water resources coupled with the increasing demand on water for agricultural expansion, the reuse of drainage water has become an important element of Egypt's national water policy. Discharging untreated sewage and chemical wastes directly into drains has become an alarming problem and with the growing population, water bodies would no longer cope with the increasing pollution loads. Therefore, biological treatment of drainage water became one of the most promising solutions to solve the water scarcity problem.

Aiming at the biological treatments of drains water to be useful in agriculture processes as well as producing the green algal biomass, the present work was achieved. Water samples were collected from EL-Salam canal from different sites including Bahr El-Bakar and Bahr Hadous to draw the contamination line through chemical and biological analyses .

Highest results of chemical composition and microbial load in Bahr El-Bakar drain was found in Om EL-Resh site compare to other nutrients in other sites that mostly lies within the permissible limits making water in suitable for irrigation purposes. From the presented results Om- EL Resh location seems to be the most salt contaminate water and more suitable for many algal species growth that able to drastically removing such nutrients .

Treatment drains water by algae has two stages: the first stage; growth curve of algal species including *Chlorella vulgaris* and *Spirulina platensis* was determined under recommended growth media of tape water. Several nutritional modifications were performed to enhance elements absorption and removal by algae through enhancing algal growth. Experiments were including the nitrogen sources (urea & nitrate) and potassium starvation for both algae species parallel to determination of nutrients removal. It could be concluded that urea stimulates algal growth as well as nutrients removal by both examined algae with some

surpasses response in *Chlorella* rather than *Spirulina*. The second stage; drains water were used to grow the proper alga (*Chlorella vulgaris*). Growth was performed using original wastewater and as it was enriched by nitrogen or phosphorous and both of them by the same amount of BG-11 growth medium.

Urea as well as phosphorous enrichment increased algal growth using Om El-Resh wastewater, but mixed enriching with urea and phosphorous added an extra enhancing effect. Microbial load was completely disappeared after 48 hours of algal incubation with different wastewater .

Om El-Resh treated water by algae represented the most polluted load which in turn gave the highest algal growth rate and selected to use it in castor bean irrigation . Besid use of Om El-Resh treated water by alga in castor irrigation; other water sources were used including Om El-Resh original wastewater, Om El-Resh wastewater containing the grown alga, ground water and fresh water.

In filed, Germination rat of castor seeds was found to be as low as. Thus, seedling was performed in artificial soil. No fertilizer compounds were added to the grown castor plants. Yield and yield components of castor bean were determined .

Growth and yield were run in the following order of Om El-Resh wastewater containing the grown alga, Om El-Resh treated water, Om El-Resh original wastewater, ground water and then fresh water. Seeds and other yield components gave the same order. Oil content was drastically affected by water type use and recorded as 9.09, 34.9, 29.65, 31.89 and 42.71 % with fresh water, ground water, original wastewater, treated wastewater and wastewater containing the grown alga, respectively.

The most potent fatty acid present in castor oil is the hydroxy fatty acid; concerning fatty acid composition of castor seed oil, ricinoleic acid represented over 87% of the total fatty acid composition. Other fatty acids present were linoleic (5.28-12.03%), oleic (0.0–4.33%), palmitic (1.36–3.86%), stearic (1.18-1.73%) and γ -Linoleic (0.0-2.2%). The unsaturated fatty acids content was 97.5% of the total fatty acids composition in oil produced from plants irrigated with wastewater containing the alga biomass.

Key Words: Wastewater Treatment; Algae; Irrigation; Castor oil.

CONTENTS

Item	Page
LIST OF TABLES	viii
LSIT OF FIGURES	xi
1. INTODUCTION	1
2. REVIEW OF LITRATURE	6
2.1. Water Resources and Wastewater problems	6
2. 2. Drains Re-use in Agricultural	10
2.3. Algae Production	15
2. 4. Wastewater treatment by algae and their beneficial uses	18
2.4.1. Nutrient removal as a result of wastewater treatment by algae	18
2. 4.2. Wastewater treatment by algae with pathogenic removed beneficial	24
2.5. Castor Cultivation under wastewater treatment by algae and oil Production	27
2.6. Plant oil as renewable energy feedstock	33
3. MATERIALS AND METHODS	37
3. 1. Study area description	37
3. 2. Water samples	38
3.2. 1. Surveyed samples	38
3.2.2. Selected sample for study	39
3.3. Soil sample	40
3. 4. Analyses	40
3. 4.1. Water Analyses	40
3. 4.1.1. Chemical Analyses	40

3. 4.1.2. Bacteriological Analyses	43
3. 4.2. Soil analysis	43
3. 5. Algae species and growth condition	43
3. 5.1. Algae species	43
3. 5.2. Nutrients solution	44
3. 5. 3. Growth Units	46
3. 5.3.1. Indoor growth unit	46
3. 5.3.2. Outdoor growth unit	47
3.6. Laboratory Experiments of alga growth profile	49
3.6.1.Tap water of alga growth profile	49
3.6.1.1. Nitrogen source	49
3.6.1.2. Potassium starvation	49
3.7. Drains water of algae growth profile	50
3.7.1. Growth with original drains water (Treat.1)	50
3.7.2. Growth under urea nitrogen enriched drains water (Treat. 2)	50
3.7.3. Growth under phosphorous enriched drains water (Treatment 3)	50
3.7.4. Growth under nitrogen and phosphorous enriched drains water (Treatment 4)	50
3.8. Growth parameters of alga	51
3.8.1. Dry weight	51
3.8.2. Growth analysis	51
3.8.2.1.Growth rate	51
3.8.2.2. Doubling time	52
3.8.2.3. Degree of multiplications	52

3.8.2.4. Percentage increase	52
3.9. Nutrients removing by examined algae	52
3.10. Scaling up	53
3.11. Castor cultivation	53
3.12. Castor oil	54
3.12.1. Extraction, determination	54
3.12.2. Fatty acids methyl ester identification	54
4. RESULTS AND DISCUSSION	55
4.1. Water quality of Bahr El-Bakar Drain (BBD) and Bahr Hadous Drain(BHD)	55
4.1.1. Physico-chemical properties	55
4.1.1.1. Acid reaction (pH)	55
4.1.1.2.Electric conductivity (E.C)	56
4.1.1.3. Total Dissolved Solids (TDS)	56
4.1.1.4. Total Alkalinity	57
4.1.1.5. Total Nitrogen	58
4.1.1.6. Biological Oxygen Demand (BOD)	58
4.1.1.7. Chemical Oxygen Demand (COD)	59
4.1.1.8. Cations	60
4.1.1.9. Anions	61
4.1.1.10. Heavy metals	62
4.1.2. Bacteriological analysis	65
4.2. Algal growth curve with recommended growth media	67

4.2.1. Effect of nitrogen sources	67
4.2.1.1 Effect of nitrogen sources on alga dry weight accumulation	67
4.2.1.2. Growth analysis of different nitrogen sources grown algae	69
4.2.1.3. Effect of nitrogen sources on media reaction (pH)	70
4.2.1.4. Effect of nitrogen sources on salinity margin	72
4.2.1.5. Effect of nitrogen sources on nutrient removal	74
4.2.1.5.1. Effect of nitrogen sources on nitrogen removal	74
4.2.1.5.2. Effect of nitrogen sources on phosphorous removal	75
4.2.1.5.3. Effect of nitrogen sources on potassium removal	76
4.2.1.5.4. Effect of nitrogen sources on sodium removal	77
4.2.1.5.5. Effect of nitrogen sources on calcium removal	79
4.2.2. Effect of potassium starvation on algal growth curve	82
4.2.2.1. Effect of potassium starvation on alga dry weight accumulation	82
4.2.2.2. Growth analysis of potassium starved grown algae	83
4.2.2.3. Effect of potassium starvation on nutrient removal	85
4.2.2.3.1. Effect of potassium starvation on nitrogen removal	85
4.2.2.3.2 Effect of potassium starvation on phosphorous removal	86
4.2.2.3.3. Effect of potassium starvation on sodium removal	87
4.2.2.3.4. Effect of potassium starvation on calcium removal	88
4.3. Drains water quality affecting algal growth	91
4.3.1. Water quality of study area	91

4.3.1.1. Acid reaction (pH) of study area	91
4.3.1.2. Electric conductivity (E.C) of study area	91
4.3.1.3. Total Dissolved Solids (TDS) of study area	91
4.3.1.4. Total Alkalinity of study area	92
4.3.1.5. Total Nitrogen of study area	92
4.3.1.6. Biological Oxygen Demand (BOD) of study area	93
4.3.1.7. Chemical Oxygen Demand (COD) of study area	93
4.3.1.8. Cations of study area	94
4.3.1.9. Anions of study area	95
4.3.1.10. Heavy metals of study area	96
4.3.1.11 Bacteriological analysis of study area	97
4.4. Algal growth curve with different wastewater sources	98
4.4.1. Growth under different drains water sources (Treatment 1)	99
4.4.1.1. Dry weight under different drains water sources	99
4.4.1.2. Growth analysis under different drains water sources	100
4.4.2.Growth under different drains water enriched by urea nitrogen (Treatment 2)	102
4.4.2.1. Dry weight under different drains water enriched by urea	102
nitrogen	
4.4.2.2 Growth analysis under different drains water enriched by urea	103
nitrogen	

4.4.3.Growth under different drains water sources enriched by phosphorous (Treatment 3)	104
4.4.3.1. Dry weight under different drains water sources enriched by phosphorous	104
4.4.3.2 Growth analysis under different drains water sources enriched by phosphorous	106
4.4.4.Growth under different drains water sources enriched by urea nitrogen and phosphorous (Treatment 4)	107
4.4.4.1.Dry weight under different drains water sources enriched by urea nitrogen and phosphorous	107
4.4.4.2.Growth analysis under different drains water sources enriched by urea nitrogen and phosphorous	108
4.4.5. Media reaction (pH) status of different drains water as affected by Chlorella vulgaris under nutrient enrichment	109
4.4.6. Electric conductivity (EC) status of different drains water as affected by <i>Chlorella vulgaris</i> under nutrient enrichment	112
4.4.7 Nutrient removal under different wastewater conditions by <i>Chlorella vulgaris</i>	114
4.4.7.1Nitrogen removal under different wastewater conditions by Chlorella vulgaris	114
4.4.7.2.Phosphorous removal under different wastewater conditions by <i>Chlorella vulgaris</i>	117
4.4.7.3.Potassium removal under different wastewater conditions by Chlorella vulgaris	120
4.4.7.4.Sodium removal under different wastewater conditions by <i>Chlorella vulgaris</i>	123

4.4.7.5Calcium removal under different wastewater conditions by <i>Chlorella vulgaris</i>	125
4.4.8.Biological oxygen demand (BOD) and chemical oxygen demand (COD)	126
4.4.9. Coliform Bacteria under different wastewater conditions by <i>Chlorella vulgaris</i>	130
4.5. Castor cultivation	132
4.5.1. Soil and water analyses	132
4.5.2. Seedling ratio	133
4.5.3 Seedling and transplanting	134
4.5.4. Growth characteristics	135
4.5.5. Fresh and dry weights of castor plant as affected by different irrigation water sources.	137
4.5.6. Yield and oil content	140
4.5.7. Fatty acids methyl ester profile	142
5. SUMMARY	145
6. CONCLUSION AND RECOMMENDATION	156
7. REFERENCES	158
ARABIC SUMMARY	1

LIST OF TABLES

No.	Title	Page
1	Castor world production	32
2	Chemical composition of BG-II macronutrients solution used for <i>Chlorella vulgaris</i> growth.	45
3	Chemical composition of Zarrouk macronutrients solution used for <i>Spirulina platensis</i> growth.	45
4	Chemical composition of A5 micronutrients solution	46
5	Technical specifications of open plate unit (Outdoor growth unit)	47
6	Physico-chemical properties of Bahr El-Baqar Drain (BBD) and Bahr Hadous Drain (BHD) water samples.	60
7	Cations and anions of Bahr El Bakar Drain (BBD) and Bahr Hadous Drain (BHD) water samples.	62
8	Heavy metals of BBD water samples	64
9	Heavy metals of BHD water samples	64
10	Bacterial indicators of Bahr El Bakar Drain (BBD) and Bahr Hadous Drain (BHD) water samples.	66
11	Growth analysis of <i>Chlorella vulgaris</i> and <i>Spirulina platensis</i> grown under nitrate and urea nitrogen	70
12	Initial, maximum and rate of nutrients-absorption (mg.l-1) by nitrate and urea grown <i>Chlorella vulgaris</i> and <i>Spirulina platensis</i>	81
13	Growth analysis of <i>Chlorella vulgaris</i> and <i>Spirulina platensis</i> grown under free potassium medium	85
14	Initial, maximum and rate of nutrients-absorption (mg.l ⁻¹) by free potassium grown <i>Chlorella vulgaris*</i> and <i>Spirulina platensis**</i>	89

No.	Title	Page
15	Initial, maximum and rate of nutrients-absorption (mg.l ⁻¹) by sodium replaced potassium grown <i>Chlorella vulgaris</i> and <i>Spirulina platensis</i>	89
16	. Physico-chemical properties of study area	94
17	Cations and anions of study area	95
18	Heavy metals of study area	96
19	Bacterial indicators of study area	98
20	Growth analysis of <i>Chlorella vulgaris</i> grown under different water drains	101
21	Growth analysis of <i>Chlorella vulgaris</i> grown under different water drains enriched by urea nitrogen	104
22	Growth analysis of <i>Chlorella vulgaris</i> grown under different water drains enriched by phosphorous	106
23	Growth analysis of <i>Chlorella vulgaris</i> grown under different water drains enriched by urea nitrogen and phosphorous	109
24	Media reaction status of different drains water as affected by Chlorella vulgaris under nutrient enrichment	111
25	Electric conductivity (EC) status of different drains water as affected by Chlorella vulgaris under nutrient enrichment	113
26	Initial, maximum absorption and rate of nitrogen absorption (mg.l ⁻¹) by Chlorella vulgaris as affected by wastewater sources and nutrient enrichment	116
27	Initial, maximum absorption and rate of phosphorous absorption (mg.l ⁻¹) by <i>Chlorella vulgaris</i> as affected by wastewater sources and nutrient enrichment	118