EVALUATION OF DIFFERENT SOURCES OF WASTEWATER TO CULTIVATE SOME SELECTED ALGAL STRAINS

By

MERIHAN GAMAL ABDEL-RAOUF

B.Sc. Agric. Sci. (Agric. Microbiology), Fac. of Agric., Ain Shams Univ., 2010

A Thesis Submitted in Partial Fulfillment Of The Requirements for the Degree of

MASTER OF SCIENCE in Agricultural Sciences (Agricultural Microbiology)

Department of Agricultural Microbiology Faculty of Agriculture Ain Shams University

ABSTRACT

Merihan Gamal Abdel-Raouf: Evaluation of Different Sources of Wastewater to Cultivate Some Selected Algal Strains Unpublished M.Sc. Thesis, Department of Microbiology, Faculty of Agriculture, Ain Shams University, 2021.

The purpose of the present study was to assess the growth of microalgae on varied wastewater as a low-cost media for maximizing biomass production and their chemical composition and use producing biomass as feed additive to silkworm. In the current study, nine microalgae were cultivated on four variations of wastewater (agricultural wastewater, industrial dyes wastewater, whey and crystal wastewater) and compared to the synthetic media. The results indicated that the tested microalgae could not grow in crystal wastewater and weak with sterilized whey while, the ability of microalgae to grow in sterilized (synthetic medium, agricultural wastewater and industrial dyes wastewater) and gave greater biomass and chlorophyll (a) than in non-sterilized after three weeks of the incubation period but contamination occurred with non-sterilized whey. Out of the tested nine microalgae, four microalgae were selected which gave the highest significant values of dry weight, biomass productivity and chlorophyll (a) content ,The selected microalgae showed the highest significant values of the chemical composition, i.e., total carbohydrate, protein and lipid when cultivated in agricultural wastewater more than those cultivated in both industrial dyes wastewater and synthetic broth media, Among four microalgae, two microalgae were chosen where the chemical composition contents ranged from 1.17 to 1.21-fold and 1.03 to 1.06-fold of carbohydrate, 1.06 to 1.09-fold and 1.88 to 1.93-fold of protein and 1.41 to 1.52-fold and 1.76 to 1.90-fold of lipid more than those of other microalgae, respectively.

The agriculture wastewater was inoculated with a single culture of *A. oryzae* HSSASE6 (KT277789) or *S. platensis* NIES-39 (A00800) individually with 10 % of inoculum size, which more preferred than was

inoculated with consortium culture. Results also showed that *A. oryzae* HSSASE6 (KT277789) was more efficient strain for giving biomass and productivity in agricultural wastewater than *S. platensis* NIES-39 (A00800) (about 28% more) .

Optimal custom(factorial) statistical design was performed to screen out the factors contributing to produce *A. oryzae* HSSASE6 biomass in agricultural wastewater using submerged culture technique the maximal *A. oryzae* HSSASE6 biomass (1.693 g/L) were achieved during run number 4 with pH (7.0), and agitation speed (100 rpm), incubation period (21d), incubation temperature degree (35°C), respectively .then has been done maximization of *A. oryzae* biomass production in agricultural wastewater using response surface methodology (RSM), the best growth was (1.948g/l) when used agitation speed (125rpm) and incubation temperature degree (40°C).

Phytotoxicity analysis revealed to faba bean seeds, results showed that control and wastewater after cultivated algae (50%) achieved germination ratio 100%.

Application of *A. oryzae* biomass produced after cultivated in agricultural wastewater as feed additive to silkworms (*Bombyx mori* L.) The mean pupal weights increased by 23.9% after using 12 mg of this alga compared with control. The maximum number of eggs (549 eggs) was recorded after using 12 mg d. w. alga /10 ml water. The greatest fresh cocoon weight was observed at 12 mg of *A. oryzae* HSSASE6 (1.72 g), The cocoon shell ratio was 20.38 and 21.51 % when larvae were fed on mulberry leaves treated with 6 mg and 12 mg *A. oryzae*. The mean length of silk filament was 1123.5 m, while 0.23 g, for weight and 1.842 denier for sizes of silk filament produced from larvae fed on mulberry leaves treated with 12 mg of *A. oryzae* /10 ml water.

Keywords: Biomass production, Microalgae, Cyanobacteria, Agricultural wastewater, Industrial dyes wastewater, Silkworm, optimal custom(factorial) and Response surface methodology.

CONTENTS

	Page
LIST OF TABLES	V
LIST OF FIGURES	IX
LIST OF PHOTOS	XIII
LIST OF ABBREVIATIONS	XIV
1. INTRODUCTION	1
2. REVIEW OF LITERATURE	4
2.1. Identification of Wastewater	4
2.2. Wastewater Treatment systems	5
2.3. Types of wastewaters	6
2.4. Cultivation of Algae on Wastewater	8
2.5. Microalgae	9
2.5.1. Overview and classification	9
2.5.2. Culture mode	10
2.5.3. Mass Production of Microalgae	11
2.6. Role of Algae in the Life	11
2.6.1. CO ₂ capture and produce O ₂	12
2.6.2. In modern agriculture	13
2.6.3. The role of algae in bioremediation	13
2.6.4. Algae used in nutrition worldwide	14
2.6.4.1. Food	14
2.6.4.2. High value products	15
2.6.4.3. Feed additives	16
2.6.5. Biofuel production	17
2.7. Interesting microalgae	18
3. MATERIALS AND METHODS	19
3.1. Materials	19
3.1.1. Sampling	19
3.1.1.1. Samples of Wastewater	19
3.1.1.2. Chemical composition of wastewater samples	19
3.1.2. Microalgae used	20

	Page
3.1.3. Seeds used	20
3.1.4. Silkworm	20
3.1.5. Culture media	21
3.2. Methods	25
3.2.1. Maintenance of microalgae cultures	25
3.2.1.1. Stock agar slants	25
3.2.1.2. Liquid culture	25
3.2.2 Cultivation of microalgae isolates and strains on synthetic	
media	26
3.2.3. Cultivation of microalgae isolates and strains on	
wastewater samples	26
3.2.4. Selection of the most efficient isolates and strains grow	
in the wastewater and determination intracellular	
contents	27
3.2.4.1. Growth curves and kinetics for the selected microalgae	
in different wastewater	27
3.2.4.2. Determination of the intracellular contents to the	
selected microalgae	27
3.2.5. Effect of single and consortia microalgae strains on	
biomass production in agricultural wastewater	28
3.2.6. Optimization of A. oryzae HSSASE6 biomass	
production in agriculture wastewater using statistical	
design expert (SDE)	28
3.2.6.1. Screening of most significant fermentation parameters	
using optimal custom (factorial) design	28
3.2.6.2. Maximization of A. oryzae HSSASE6 biomass	
production in agricultural wastewater using response	
surface methodology (RSM) by optimal custom design	31
3.2.7. Phytotoxicity assay	33

	Page
3.2.8. Application of A. oryzae HSSASE6 biomass produced	
after cultivated in agricultural wastewater as feed additive	
to silkworms (Bombyx mori L.)	33
3.2.8.1 Rearing and feed additives used	33
3.2.9. Analytical procedures	34
3.2.9.1. Wastewater analysis	34
3.2.9.2. Microalgae	35
3.2.9.3. Intracellular contents extraction and assay	36
3.2.9.4. Criteria used for evaluating the tested feed additive	36
3.2.10. Statistical analysis	38
4. Results and Discussion	39
4.1 Chemical composition of wastewater samples before	
cultivation of microalgae	39
4.2 Cultivation of microalgae isolates and strains in synthetic	
media	40
4.3 Cultivation of microalgae isolates and strains in	
wastewater and whey as growth medium	48
4.3.1 Agricultural wastewater as a medium	48
4.3.2 Industrial dyes wastewater as a medium	56
4.3.3 Glass industrial wastewater as a medium	63
4.3.4 Whey as a medium	64
4.4 Selection of the most efficient isolates capable to grow in	
the wastewater	70
4.5 Growth curves and kinetics for the selected microalgae in	
different wastewater	71
4.6 Intracellular contents accumulation	75
4.7 Effect of single and consortia microalgae strains in	
biomass production	78
4.8 Optimization of <i>A. oryzae</i> HSSASE6 in agriculture	
wastewater using statistical design expert (SDE)	80

	Page
4.8.1 Screening of most significant fermentation parameters	
using optimal custom(factorial) design	80
4.8.2 Maximization of A. oryzae biomass production in	
agricultural wastewater using response surface	
methodology (RSM) by optimal custom design	86
4.9 Phytotoxicity assay	92
4.10 Application of A. oryzae HSSASE6 biomass produced	
after cultivated in agricultural wastewater as food	
additive to silkworms (Bombyx mori L.)	93
4.10.1 Biological parameters	93
4.10.1.1 Weights of produced pupae	93
4.10.1.2 Number of deposited eggs per mated female moth	96
4.10.1.3 Produced cocoons	97
4.10.2 Silk filament	101
5.SUMMARY	104
6.REFERENCES	109
ARABIC SUMMARY	

LIST OF TABLES

Table No.		Page
1	sources of collected wastewater samples.	19
2	Coded and actual level of four variables investigated regarding <i>A. oryzae</i> HSSASE6 biomass production as determined by optimal custom(factorial) design.	29
3	Evaluation of the most significant factors affected <i>A</i> . <i>oryzae</i> HSSASE6 biomass production after cultivated in agriculture wastewater using optimal custom(factorial)	
	design.	30
4	Coded and actual level of two variables investigated regarding <i>A. oryzae</i> HSSASE6 biomass production as determined using response surface methodology (RSM)	
	by optimal custom design.	31
5	Optimization the most significant factors for <i>A. oryzae</i> HSSASE6 biomass after cultivated in agriculture	
	wastewater using response surface methodology (RSM)	32
6	by optimal custom design. Chemical composition of wastewater samples before	32
U	cultivation of microalgae.	39
7	Cell dry weight (g/L) of microalgae grown in non-sterilized synthetic broth media during 4 weeks under	3)
	static culture.	41
8	Cell dry weight (g/L) of microalgae grown in sterilized synthetic broth media during 4 weeks under static culture.	42
9	Chlorophyll a (mg/L) of microalgae grown in non-sterilized synthetic media broth during 4 weeks under	
	static culture.	44
10	Chlorophyll a (mg/L) of microalgae grown in sterilized	
11	synthetic media broth during 4 weeks under static culture. Cell dry weight (g/L) of microalgae grown in non-	45

Table		Page
No.		
	sterilized agricultural wastewater during 4 weeks under static culture.	48
12	Cell dry weight (g/L) of microalgae grown in sterilized	
	agricultural wastewater during 4 weeks under static	
	culture.	49
13	Chlorophyll a (mg/L) of microalgae grown in non-	
	sterilized agricultural wastewater during 4 weeks under	
	static culture.	52
14	Chlorophyll a (mg/L) of microalgae grown in sterilized	
	agricultural wastewater ring 4 weeks under static culture.	53
15	Cell dry weight (g/L) of microalgae grown in non-	
	sterilized dyes wastewater during 4 weeks under static	
	culture.	56
16	Cell dry weight (g/L) of microalgae grown in sterilized	
	industrial dyes wastewater during 4 weeks under static	
	culture.	57
17	Chlorophyll a (mg/L) of microalgae grown in non-	
	sterilized industrial dyes wastewater during 4 weeks	
	under static culture.	60
18	Chlorophyll a (mg/L) of microalgae grown in sterilized	
	industrial dyes wastewater during 4 weeks under static	
	culture.	61
19	Cell dry weight (g/L) of microalgae isolates grown in	
	sterilized whey as by- product growth medium during 4	
	weeks under static culture.	65
20	Chlorophyll a (mg/L) of microalgae isolates grown in	
	sterilized whey as media broth during 4 weeks under	
	static culture.	67

Table		Page
No.		
21	Summarized the biomass and Chlorophyll a and	
	Productivity and (Chlorophyll/Biomass) on different	
	wastes media.	70
22	Optimal custom(factorial) design matrix and the actual	
	values of A. oryzae HSSASE6 biomass production in	
	agricultural wastewater.	81
23	Statistical analysis of variance (ANOVA) of optimal	
	custom(factorial) design for A.oryzae HSSASE6 biomass	
	production.	82
24	Optimal custom (response surface methodology) design	
	matrix and the actual values of A. oryzae HSSASE6	
	biomass production in agricultural wastewater using	
	submerged culture.	87
25	Statistical analysis of variance (ANOVA) of optimal	
	custom (response surface methodology) design for A.	
	oryzae HSSASE6 biomass production.	88
26	Phytotoxicity of agricultural wastewater on bean (Vicia	
	faba) seeds germination before and after cultivation of A .	
	oryzae HSSASE6.	92
27	Mean weights of B. mori pupae (g) fed as fifth instar	
	larvae on one daily meal of mulberry leaves treated with	
	different concentrations of A. oryzae HSSASE6 strain	
	and the other daily meals on untreated leaves.	94
28	Mean numbers of deposited eggs laid by mated female B .	
	mori moth fed as fifth larval instars on one daily meal of	
	mulberry leaves treated with different concentrations of	
	A. oryzae HSSASE6 strain and the other daily meals on	
	untreated leaves.	96
29	Dimension of <i>B. mori</i> cocoons produced by larvae fed as	
	fifth instars on one daily meal of mulberry leaves treated	

Table		Page
No.		
	with different concentrations of A. oryzae HSSASE6 and	
	the other daily meals on untreated leaves.	98
30	Mean weights of fresh cocoon and cocoon shell (g) and	
	cocoon shell ratio produced by B. mori larvae fed as fifth	
	instar on one daily meal of mulberry leaves treated with	
	different concentrations of A. oryzae HSSASE6 and the	
	other daily meals on untreated leaves.	99
31	Mean length (m), weight (g) and size (denier) of silk	
	filament reeled from cocoons produced by full grown B .	
	mori larvae fed as fifth instars on one daily meal of	
	mulberry leaves treated with different concentrations of	
	A. oryzae and the other daily meals on untreated leaves.	101

LIST OF FIGURES

Fig.		Page
No.		
1	Biomass productivity (g/L/w) of microalgae grown in	
	non-sterilized (A) and sterilized (B) synthetic broth	
	media during 4 weeks under static culture.	43
2	Chlorophyll (a) yield coefficient relative to biomass	
	(YCh/B) (mg/g/L) of the most efficient microalgae	
	after grown in non-sterilized (A) and sterilized (B)	
	synthetic broth media after 3 weeks of incubation	
	period under static culture.	47
3	Biomass productivity (g/L/w) of microalgae grown in	
	non-sterilized (A)and sterilized (B) agricultural	
	wastewater during 4 weeks under static culture.	51
4	Chlorophyll a yield coefficient relative to biomass	
	(YCh/B) (mg/g/L) of the most efficient microalgae	
	after grown in non-sterilized (A) and sterilized (B)	
	agricultural wastewater after 3 weeks of incubation	
	period under static culture.	55
5	Biomass productivity (g/L/w) of microalgae grown in	
	non-sterilized (A)and sterilized (B) industrial dyes	
	wastewater during 4 weeks under static culture.	59
6	Chlorophyll a yield coefficient relative to biomass	
	(YCh/B) (mg/g/L) of the most efficient microalgae	
	after grown in non-sterilized (A) and sterilized (B)	
	industrial dyes wastewater after 3 weeks of incubation	
	period under static culture.	62
7	Biomass productivity (g/L/w) of microalgae grown in	
	sterilized Whey as media broth during 4 weeks under	
	static culture.	66

	P
Chlorophyll <i>a</i> yield coefficient relative to biomass	
(YCh/B) (mg/g/L) of microalgae isolates after grown	
in sterilized whey as media broth after 3 weeks of	
incubation period under static culture.	
Sigmoidal growth curves of the most efficient	
microalgae in wastewater and synthetic medium	
(control) during 28days of incubation periods under	
static culture.	
Correlation coefficient between the cell dry weight	
(growth) at log phase and incubation periods (d) of	
the most efficient microalgae in wastewater and	
synthetic medium (control).	
Specific growth rate (μ) , doubling time (td) and	
multiplication rate (MR) of the most efficient	
microalgae in wastewater and synthetic medium	
(control) during 21days of incubation periods under	
static culture.	
Carbohydrate content presence in the selected	
microalgae strains.	
Protein content presence in the selected microalgae.	
Lipid content presence in the selected microalgae.	
Effect of single culture of <i>A. oryzae</i> HSSASE6 and <i>S.</i>	
platensis NIES-39 at different concentrations on	
biomass production during cultivation in agricultural	
wastewater.	
Effect of mixed cultures (A. oryzae: S. platensis) at	
different concentrations on biomass production during	
cultivation in agricultural wastewater.	

	Page
The actual and predicted values of optimal custom (factorial) design for <i>A. oryzae</i> HSSASE6 biomass production.	83
The model graph at one-factor level for A.	
oryzaeHSSASE6biomass.	84
The model graph at interaction between two factors	0.5
for A. oryzae HSSASE6 biomass.	85
Pareto graph showing contribution effect % of	
different variables on A. oryzae HSSASE6 biomass	0.0
production in agricultural wastewater.	86
The actual and predicted values of optimal custom (response surface methodology) design for A anyzag	
(response surface methodology) design for <i>A. oryzae</i> HSSASE6 biomass production.	89
The model graph at one-factor level (A) and	09
nteraction between two factors (B) for A. oryzae	
HSSASE6biomass.	90
Three-dimensional response surface and two-	70
dimensional contour plots showing the effect of	
incubation temperature and agitation speed on algae	
dry weight.	91
Mean weights of B. mori pupae (g) fed as fifth instar	
larvae on one daily meal of mulberry leaves treated	
with different concentrations of <i>A. oryzae</i> HSSASE6	
strain and the other daily meals on untreated leaves.	94
Mean numbers of deposited eggs laid by mated	
female B. mori moth fed as fifth larval instars on one	
daily meal of mulberry leaves treated with different	
concentrations of A. oryzae HSSASE6 strain and the	
other daily meals on untreated leaves.	97

Fig.		Page
No.		
26	Dimension of B. mori cocoons produced by larvae fed	
	as fifth instars on one daily meal of mulberry leaves	
	treated with different concentrations of A. oryzae	
	HSSASE6 and the other daily meals on untreated	
	leaves.	98
27	Mean weights of fresh cocoon and cocoon shell (g)	
	produced by B. mori larvae fed as fifth instar on one	
	daily meal of mulberry leaves treated with different	
	concentrations of A. oryzae HSSASE6 and the other	
	daily meals on untreated leaves.	100
28	Mean length (m) of silk filament reeled from cocoons	
	produced by full grown B. mori larvae fed as fifth	
	instars on one daily meal of mulberry leaves treated	
	with different concentrations of A.oryzae	
	HSSASE6and the other daily meals on untreated	
	leaves.	102

LIST OF PHOTOS

Photo		Page
No.		
1	Compartion weights of <i>B. mori</i> pupae (g) fed as fifth instar larvae on one daily meal of mulberry leaves treated with different concentrations of <i>A. oryzae</i> HSSASE6 strain and the other daily meals on	
	untreated leaves.	98
2	Comparison weights of fresh cocoon (g) produced by <i>B. mori</i> larvae fed as fifth instar on one daily meal of mulberry leaves treated with different concentrations of <i>A. oryzae</i> HSSASE6 and the other	
	daily meals on untreated leaves.	100

LIST OF ABBREVIATION

ABBREVIATION Mean

AWW Agricultural wastewater

DWW Dyes wastewater

d day

g/l gram per liter

 $\begin{array}{cc} mg/l & milligram \ per \ liter \\ \mu g/l & microgram \ per \ liter \end{array}$

min minutes
nm Nanometer

RSM response surface methodology

S m-control Synthetic medium

W week