

بسم الله الرحمن الرحيم



-Caron-





شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم





جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

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



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغيار



BIOFUEL PRODUCTION FROM ALGAE

By

FATMA MOHAMED IBRAHIM NASR

B.Sc. Agric. Sci. (Biochemistry), Fac. Agric., Cairo Univ., 2011

THESIS

Submitted in Partial Fulfillment of the Requirement for the Degree of

MASTER OF SCIENCE

In

Agricultural Sciences (Microbiology)

Department of Agricultural Microbiology
Faculty of Agriculture
Cairo University
EGYPT

2021

Format Reviewer

Vice Dean of Graduate Studies

SUPERVISION SHEET

BIOFUEL PRODUCTION FROM ALGAE

M.Sc. Thesis
In
Agricultural Sci. (Agricultural Microbiology)

 $\mathbf{B}\mathbf{y}$

FATMA MOHAMED IBRAHIM NASR

B.Sc. Agric. Sci. (Biochemistry), Fac. Agric., Cairo Univ., 2011

SUPERVISION COMMITTEE

Dr. MAHMOUD.W. SADIK

Professor of Microbiology, Fac. Agric., Cairo University

Dr. SAYED ABDEL KADER FAYED

Professor of Agriculture Biochemistry, Fac. Agric., Cairo University

Dr. NASHWA ABD ELALIM HASSAN FETYAN

Head Researcher in Soils, Water and Environment Research Institute, Agricultural Research Center

DEDICATION

This work is dedicated to My family, Aspecial feeling to my Dad and my Mom for their words of encouragement and pray and their support along the period of my life. My dear Brothers, My dear husband and my lovely sons. with my love and respect.

ACKNOWLEDGEMENT

I would like to express sincere gratitude and thanks to **Dr. Mohammed Zakaria Sedik,** Professor of Agricultural Microbiology, Faculty of Agriculture, Cairo University for his efforts to complete the research plan. My deepest thanks and gratefulness are due to **Dr. Mohmoud. W. Sadik** Professor of agricultural Microbiology, Fac. Agric., Cairo University for his help and supervision of this work.

My deepest thanks are also extended to Prof. **Dr. Nashwa A. H. Fetyan**, Professor of agricultural microbiology, Soils, Water and Environment Research Institute, Agricultural Research Center for her great guidance encouragement, throughout the work in the thesis.

Special thanks are extended to **Dr. Sayed abdelkader** Professor of Agricultural Biochemistry, Faculty of Agriculture, Cairo University. For, excellent guidance, honest cooperation of this work.

I would like to express my greatest appreciation to Prof. **Dr. Abo El-Khair B. El-Sayed,** Professor of Algal Biotechnology, Head of Algal Biotechnology Unit, National Research Centre (NRC), for suggesting and planning the work, guidance, revision, continuous help and encouragement.

I am also extremely thankful to **Dr.Mohamed Hassoub**, **Amr Mostafa**, **Mostafa Zohair**, **Aya and Mohamed Sami**r for their great help and co-operation in the work and solving the problems I faced.

The work as supported by a grant from the Academy of Scientific Research and Technology (ASRT), Master scholarship, Scientist for Next Generation (SNG) program

Name of Candidate: Fatma Mohamed Ibrahim Nasr Degree: M.Sc.

Title of Thesis: Biofuel production from algae

Supervisors: Dr. Mahmoud Wafik Ahmed Sadik

Dr. Sayed Abdel kader Fayed

Dr. Nashwa Abd elalim Hassan Fetyan

Department: Agricultural Microbiology Approval: 10/4/2021

ABSTRACT

In response to the world energy crisis, micro algal biodiesel production has received much interest in an effort to search for sustainable development. Beside, algae nutrition seems to be the most limiting factor concerning proper growth and economy cost. The main figure in this respect is carbon nutrition. Growth was performed using F2 growth medium for inoculum preparation and sub-culturing; while artificial growth medium was applied in order to enhance both dry weight and lipid productivities. The major properties of the produced biodiesel was investigated, moreover the residual defatted biomass of Nannochloropsis oculata alga was used as a fermentation feedstock for bioethanol production after hydrolysis under varying conditions of acid concentration and/or constant enzyme dosage.

Results showed that a high nutritional composition of bagasse extract as an alternative source of organic carbon (98.9% of total cell carbon) was obtained by cultures grown with full F2 growth medium enriched by 10% of bagasse extract. Chemical composition reveled the relatively high content of carbohydrates (26.6%) and oils (11.9%) on the expense of protein content (32.8%) and the maximum figure of ash content (2%) goes back to sodium ions.

Results of bioethanol production from defatted biomass of *N. oculata* indicated optimal conditions for hydrolysis process were 30 hours using a commercial enzyme that includes two stages: liquefaction process using diluted sulphoric acid (3.0% v/v) at 121°C for 15 minute followed by incubation in commercially available hydrolytic enzymes α-amylase 1000 IU /g at 95°C with a pH of 6, while for the scarification process using commercially available enzyme mixtures contain multiple enzyme activities, mainly exoglucanase, endoglucanase, hemi-cellulose, and beta-glucosidase. The hydrolysis was carried out by incubating the mixture at 60 °C, pH 5.5, for 24 hrs. gave a maximum yield of sugar (232.39 mg.g-1) for defatted biomass cultivated vegetative and multi factor stresses conditions. The fermentation of hydrolysate using Saccharomyces cerevisiae gives ethanol yield of 0.26 g.g-1 reducing sugar in the highest yield scenario observed during current study.

Key words: *Nannochloropsis oculata*, Bagasse, Biodiesel, Fuel properties, Defatted microalgal biomass, Hydrolysate, Bioethanol.

CONTENTS

Page	
INTRODUCTION	1
REVIEW OF LITERATURE	4
1. Energy crisis	4
2. Global warming and climate change	
3. Energy in Egypt	
4. Types of Energy Resources	
5. Renwable Energy	
a. Bioenergy	
b. Biofuels from Lignocellulosic Biomass	13
6. Microalgae	18
7. Growth dynamic	19
a. Lag or induction phase	19
b. Exponential phase or Log phase	20
c. Stationary phase	20
d. Death or crash phase	20
8. Chemical composition of microalgae	21
9. Nannochlorpsis oculata	22
10. Factors affecting growth of microalgae	
a. Light	24
b. Temparature	
c. Media rection(PH)	
d. Salinity	
e. Aeration and turbulence	
f. nutrients	
11. Mode of cultivation	
12. Use of agricultural wastes in algae nutrition	
13. Cultivation techniques of microalgae	
a. Open systems	
b. Closed systems (photo bioreactors)	
14. Harvesting of microalgae	
a. Flocculation	
b. Centrifugation	39
c. Biomass filtration	
15. Products from microalgae	
a. Biodisel production from alage	40

b. Bioethanol production from microalgae	42
16. Strengths of microalgae as abiofuel feedstock	
17. Advantages and disadvantages of microalgae as biofuel	
feedstock	45
MATERIALS AND METHODS	48
1. Microalgae	48
2. Growth medium	
3. Inoculums preparation	49
4. Growth conditions	
5. Growth units	50
a. Laboratory growth units	50
b. Outdoor large scale cultivation unit	
(Zigzag-Shape photobioreactor)	52
6. Analysis and preparation of of sugar cane bagasse	
aqueous extract (BE)	53
7. Experiments	53
a. Effect of bagasse aqueous extract concentration on	
Nannochloropsis oculata growth	54
b. Effect of nitrogen deficiency on Nannochloropsis oculata	
growth	54
c. Effect of bagasse extract concentrations and stress factors	
on Nannochloropsis oculata growth	54
8. Outdoor growth	55
9. Harvesting and drying of biomass	55
10. Growth parameters	55
a. Dry weight	55
b. Total chlorophyll	56
c. Total carotenoids	56
11. Oil extraction and determination	57
12. Determination of Fatty acid methyl ester profile	59
a. Preparation of fatty acid methyl esters	59
b. Gas-Liquid Chromatographic analysis of fatty acids	
methyl esters	59
13. Biodiesel production	60
a. Oil extraction from large biomass60	
c. Transesterification	60
d. Evaluation of biodiesel properties	61
14. Chemical analysis of outdoor produced algae	62
15. Growth analysis	63

16. Ethanol production form defatted biomass	63
a. Acid hydrolysis and preparation of defatted biomass	
hydrolysates	63
b. Enzyme hydrolysis and preparation of defatted biomass	
hydrolysates	64
c. Ethanol production	65
17. Analytical procedures	
18. Statistical analysis	67
RESULTS AND DISCUSSION	68
1. Bagasse chemical composition	
2. Effect of Bagasse extract concentration on N. oculata	
growth	69
a. Dry weight	69
b. Chlorophyll	
c. Carotenes	73
3. Effect of BE on N. oculata growth under N deficiency	74
a. On algae dry weight	74
b. On algae total chlorophyll	78
c. On algae total carotenes	80
4. Effect of induction on N. oculata growth	
5. Outdoor cultivation and yield	84
6. Chemical composition of outdoor grown N. oculata	85
7. Effects of Bagasse extract and stress factors on fatty acids	
profile and Lipid Productivity of N. oculata	87
8. Prediction of biodiesel properties	
9. Bioethanol production from defatted <i>Nanochoropsis oculata</i>	
biomass	93
a. Effect of acid hydrolysis on total carbohydrates and total	
reduced sugar content	94
b. Effect of enzymatic hydrolysis on total carbohydrates and	
total reduced sugar content	95
10. Sugar composition of the polysaccharides	
11. Ethanol productivity	
SUMMARY	107
REFERENCES	111

LIST OF TABLES

No	Title	Page
1.	Growth modes of algae (microalgae) cultivation	34
2.	Chemical composition of F2 medium	49
3.	General specification of Zigzag-shape photobioreactor	52
4.	Some chemical analyses of of bagasse extract (BE); Fagrowth medium and artificial sea water (ASW)	
5.	Growth kinetics of outdoor grown Nanochloropsis oculate with bagasse extract(BE)	
6.	Chemical composition of outdoor produced Nannochloropsis oculata	
7.	GC/MS chromatogram of fatty acids methyl ester of Nannochloropsis oculata grown under vegetative and multifactor stresses conditions(BE , NaCl and nitrogen deficiency)	i n
8.	Some fuel properties of <i>N. oculata</i> grown under vegetative and multi factor stresses conditions (BE, NaCl and nitroget deficiency)	n
9.	Sugar composition % (weight 10%) of the polysaccharide isolated from <i>N. oculata</i> microalgae biomass pretreated with acid hydrolysis (AH) and acid, enzymehydrolysis(AEH)	d e

LIST OF FIGURES

N	o. Title F	Page
1.	Renewable energy resources	9
2.	Xylan and glucomannan, the two most abundant hemicellulose's building blocks in softwoods	14
3.	The structure and the inter- and intra-chain hydrogen bonding pattern in cellulose	
4.	Example of lignin structure from softwood	15
5.	Growth phases of microalgae culture	20
6.	Flexible biofuels production from microalgae	44
7.	Photo of Nannochloropsisoculata	48
8.	Laboratory growth units (tubes) 2.5 L	51
9.	Vertical tubular photobioreactor 14L	51
10.	Zigzag-Shape photobioreactor with 1000L	53
11.	Soxhlet apparatus used for extraction	58
12.	Dry weight (g.l ⁻¹) of N oculata grown under different BE concentrations extract combined with same nutrient concentrations	
13.	Total chlorophyll (mg.l ⁻¹) of <i>Nannochloropsis oculata</i> grown under different bagasse extract concentrations combined with same nutrient concentrations	
14.	Carotene (mg.l ⁻¹) of <i>Nannochloropsis oculata</i> grown under different bagasse extract concentrations combined with same nutrient concentrations	
15.	Dry weight (g.1 ⁻¹) of <i>Nannochloropsis oculata</i> grown under 10% bagasse extract	
16.	Chlorophyll of <i>Nannochloropsis oculata</i> grown under 10% bagasse extract	79

N	o. Title I	Page
17	. Carotene of <i>Nannochloropsis</i> oculata grown under 10% bagasse extract	80
18	Dry weight (g.l ⁻¹); total chlorophyll (%) and total carotenes (%) of <i>N. oculata</i> grown under stress of 2.0% salinity, nitrogen deficiency, 25% F2 medium and 10% bagasse extract.	
19	. Effect acid hydrolysis (AH) treatment on total carbohydrates and Reducing sugars mg.g of <i>N. Oculata</i> defatted biomass	
20	Effect of acid, enzyme hydrolysis(AEH) treatment on total carbohydrates and Reducing sugars mg.g of <i>N. Oculata</i> defatted biomass	
21	.(A-D): HPLC chromatograms of monosaccharaides of N . Oculata extracts from acid hydrolysis (A) , (B) and acid , enzyme hydrolysis (C),(D)	
22	. HPLC chromatograms of hydroxyl methyl furfural (HMF): (A) standard and (B) sample of <i>N. Oculata</i> extracts from acid enzyme hydrolysis	
23	. Fermentation with acid hydrolysate of <i>N. oculata</i> hydrolysate using, <i>S. cerevisiae</i>	
24	. Fermentation with acid , enzyme hydrolysate of <i>N. oculata</i> hydrolysate using <i>S. cerevisiae</i>	