

**ASSESSMENT OF THE FEASIBILITY OF CO-
PRODUCTION OF FUCOXANTHIN, OMEGA-3 FATTY
ACIDS AND BIOETHANOL FROM MARINE
MICROALGAE**

R.G.M.M. Premarathne

198093G

Degree of Master of Science

Department of Chemical and Process Engineering

University of Moratuwa

Sri Lanka

June 2021

**ASSESSMENT OF THE FEASIBILITY OF CO-
PRODUCTION OF FUCOXANTHIN, OMEGA-3 FATTY
ACIDS AND BIOETHANOL FROM MARINE
MICROALGAE**

R.G.M.M. Premarathne

198093G

Thesis submitted in partial fulfillment of the requirements for the degree
Master of Science in Chemical and Process Engineering

Department of Chemical and Process Engineering

University of Moratuwa


Sri Lanka

June 2021

DECLARATION OF THE CANDIDATE & SUPERVISORS

I declare that this is my own work and this thesis does not incorporate without acknowledgement any material previously submitted for a Degree or Diploma in any other University or institute of higher learning and to the best of my knowledge and belief it does not contain any material previously published or written by another person except where the acknowledgement is made in the text.

Also, I hereby grant to University of Moratuwa the non-exclusive right to reproduce and distribute my thesis, in whole or in part in print, electronic or other medium. I retain the right to use this content in whole or part in future works (such as articles or books).

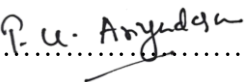
Signature: 

Date: 11.06.2021

The above candidate has carried out research for the Master's Thesis under our supervision.

Supervisor (PI):


Dr. H.L.T.U. Ariyadasa,
Department of Chemical and Process Engineering,
University of Moratuwa, Sri Lanka

Signature of the supervisor: 

Date: 11.06.2021

Supervisor:

Emeritus Professor R.A. Attalage,
University of Moratuwa, Sri Lanka

Signature of the supervisor: 

Date: 11-June-2021

DEDICATION

Dedicated to my parents, family and teachers who have supported me unconditionally.

ACKNOWLEDGEMENTS

I would like to thank my supervisors, the principal investigator Dr. Thilini Ariyadasa and co-supervisor Prof. R.A. Attalage, who provided insight, expertise and guidance for successful completion of the research project. I am also grateful to Prof. Chandima Jayasuriya, Prof. P.G. Rathnasiri and Dr. Manisha Gunasekara who provided their valuable insight to improve the scientific merit of the project.

I would like to express my gratitude towards all the staff and colleagues of the Department of the Chemical and Process Engineering, University of Moratuwa, especially Mr. B. Karunathilaka and Ms. Indika Athukorala from the Microbiology laboratory, for their unwavering support throughout the project duration.

Furthermore, I would like to thank Mr. Eshantha Salgado from Lion Brewery Ceylon PLC for providing the yeast strain for fermentation experiments and Ms. Dinusha Martino for her assistance in chromatographic analysis.

ABSTRACT

The marine microalga *Tisochrysis lutea* is renowned for its ability to synthesize fucoxanthin and docosahexaenoic acid (DHA), which are nutritionally valuable high-value compounds. Although numerous studies in literature have assessed fucoxanthin and DHA production by *T. lutea*, very few have evaluated the feasibility of comprehensively utilizing biomass for co-production of these metabolites within the framework of biorefineries. To this end, the current study focused on the synthesis of fucoxanthin and DHA by cultivation of *T. lutea* under two different initial nitrate concentrations (1x: 882 μM , 3x: 2,646 μM) and three different illuminance levels (LL: 3,750 lux; ML: 7,500 lux; HL: 11,250 lux). The maximum fucoxanthin yield of 8.80 ± 0.30 mg/L (14.43 ± 0.52 mg/g) and DHA yield of 7.08 ± 0.02 mg/L (11.90 ± 0.14 mg/g) were achieved in the 3x HL culture at the end of 16 days of cultivation. Thereafter, a biphasic solvent extraction procedure using ethanol/n-hexane/water (10:9:1 v/v/v) was utilized for co-extraction of $97.96 \pm 0.54\%$ fucoxanthin and $74.11 \pm 1.49\%$ DHA from 3x HL biomass, and products were separated into two fractions. Fermentation of the residual biomass obtained from co-extraction resulted in a bioethanol yield of 48.49 ± 0.58 mg/g. Thus, results showcase the efficacy of the developed co-extraction procedure and the biorefinery potential of *T. lutea*.

TABLE OF CONTENTS

Declaration of the candidate & supervisors	i
Dedication	ii
Acknowledgements.....	iii
Abstract	iv
Table of contents.....	v
List of figures	vi
List of tables	vii
List of abbreviations	viii
1. Introduction	1
1.1. Research gap	2
1.2. Hypotheses.....	3
1.3. Objectives	3
2. Literature Review.....	5
2.1. Fucoxanthin	5
2.2. Omega-3 fatty acids	5
2.3. Microalgal strains for co-production of omega-3 fatty acids and fucoxanthin.....	6
2.4. <i>Tisochrysis lutea</i> as a producer of DHA and fucoxanthin.....	8
2.5. Production of bioethanol from residual microalgal biomass	9
3. Materials and methods	11
4. Results and Discussion.....	21
4.1. Biomass production of <i>T. lutea</i> under different initial nitrate concentrations and illuminance levels	21

4.2. Accumulation of DHA under different initial nitrate concentrations and illuminance levels	23
4.3. Accumulation of fucoxanthin under different initial nitrate concentrations and illuminance levels	26
4.4. Co-extraction of fucoxanthin and DHA from <i>T. lutea</i>	29
4.5. Bioethanol production from residual biomass	31
5. Conclusion	33
6. Publications	34
References	35

LIST OF FIGURES

Figure 2-1: The chemical structure of fucoxanthin [14]	5
Figure 2-2: Structures of omega-3 fatty acids, EPA and DHA [32]	6
Figure 3-1: Biomass production in <i>T. lutea</i> cultures cultivated under various initial nitrate concentrations and illuminance levels	13
Figure 3-2: Calibration curve used for spectrophotometric determination of nitrate concentration	14
Figure 3-3: Crude extracts of fucoxanthin (left) and microalgal lipids (right)	15
Figure 3-4: Color development in sugar hydrolysates upon the addition of phenol-sulfuric reagent for determination of carbohydrate concentration	17
Figure 3-5: Calibration curve used for spectrophotometric determination of carbohydrate concentration	18
Figure 3-6: Seed cultures of <i>S. cerevisiae</i> prepared for fermentation of residual <i>T. lutea</i> biomass (left) and fermentation apparatus (right)	19
Figure 4-1: Biomass concentrations of <i>T. lutea</i> grown under different illuminance levels (low light LL: 3,750 lux; medium light, ML: 7,500 lux; and high light, HL: 11,250 lux)	

and initial nitrate concentrations (1x: 882 μ M; 3x: 2,646 μ M) over the 16-day cultivation period (a) 1x cultures (b) 3x cultures	21
Figure 4-2: Variation of nitrate concentration in the culture media under different illuminance levels (low light LL: 3,750 lux; medium light, ML: 7,500 lux; and high light, HL: 11,250 lux) and initial nitrate concentrations (1x: 882 μ M; 3x: 2,646 μ M) over the 16-day cultivation period (a) 1x cultures (b) 3x cultures	22
Figure 4-3: Docosahexaenoic acid (DHA) concentrations of <i>T. lutea</i> grown under different illuminance levels (low light LL: 3,750 lux; medium light, ML: 7,500 lux; and high light, HL: 11,250 lux) and initial nitrate concentrations (1x: 882 μ M; 3x: 2,646 μ M) over the 16-day cultivation period (a) 1x cultures (b) 3x cultures.....	24
Figure 4-4: Variation of cellular docosahexaenoic acid (DHA) content of <i>T. lutea</i> grown under different illuminance levels (low light LL: 3,750 lux; medium light, ML: 7,500 lux; and high light, HL: 11,250 lux) and initial nitrate concentrations (1x: 882 μ M; 3x: 2,646 μ M) over the 16-day cultivation period (a) 1x cultures (b) 3x cultures	25
Figure 4-5: Fucoxanthin concentrations of <i>T. lutea</i> grown under (low light LL: 3,750 lux; medium light, ML: 7,500 lux; and high light, HL: 11,250 lux) and initial nitrate concentrations (1x: 882 μ M; 3x: 2,646 μ M) over the 16-day cultivation period (a) 1x cultures (b) 3x cultures.....	27
Figure 4-6: Variation of cellular fucoxanthin content of <i>T. lutea</i> grown under different illuminance levels (low light LL: 3,750 lux; medium light, ML: 7,500 lux; and high light, HL: 11,250 lux) and initial nitrate concentrations (1x: 882 μ M; 3x: 2,646 μ M) over the 16-day cultivation period (a) 1x cultures (b) 3x cultures	28
Figure 4-7: Time-variation of fucoxanthin and docosahexaenoic acid (DHA) recovery during co-extraction with 10:9:1 (v/v/v) ethanol/n-hexane/water.....	29

LIST OF TABLES

Table 2-1: Microalgal species capable of synthesizing both fucoxanthin and omega-3 fatty acids	7
--	---

Table 2-2: Summary of selected studies on the production of DHA and fucoxanthin using <i>T. lutea</i>	8
Table 3-1: Composition of f/2-Si medium [50].....	11
Table 3-2: Culture conditions utilized for cultivation of <i>T. lutea</i>	12
Table 3-3: Mobile phase flow in HPLC analysis of fucoxanthin	16
Table 4-1: Final carbohydrate concentrations and of cellular carbohydrate content of <i>T. lutea</i> grown under different illuminance levels (low light LL: 3,750 lux; medium light, ML: 7,500 lux; and high light, HL: 11,250 lux) and initial nitrate concentrations (1x: 882 µM; 3x: 2,646 µM)	32

LIST OF ABBREVIATIONS

PUFA – Polyunsaturated fatty acid

EPA – Eicosapentaenoic acid

DHA – Docosahexaenoic acid

WHO – World Health Organization

CCAP – The Culture Collection of Algae and Protozoa

PTFE – Polytetrafluoroethylene

LEDs – Light emitting diodes

1x – Standard nitrate concentration of f/2-Si media

3x – Three times the standard nitrate concentration of f/2-Si media

LL – Low light; 3,750 lux

ML – Medium light; 7,500 lux

HL – High light; 11,250 lux

FAMES – Fatty acid methyl esters

GC-FID – Gas chromatography with flame ionization detection

HPLC – High-performance liquid chromatography