

**INVESTIGATION OF THE EFFECT OF
PRETREATMENT METHODS ON LIPID YIELD AND
FATTY ACID PROFILE OF LOCALLY ISOLATED
MICROALGAE SPECIES**

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Degree of Master of Philosophy

Department of Chemical and Process Engineering
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University of Moratuwa
Sri Lanka

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DECLARATION

I declare that this is my own work and this thesis/dissertation 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. I retain the right to use this content in whole or part in future works (such as articles or books).

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Date: 23/02/2024

DEDICATION

To my father who never saw this adventure

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ABSTRACT

The introduction of a novel chemical cell disruption method with economic and environmental feasibility to increase the lipid extraction yield is of paramount importance to enhance the commercialization drive of microalgae biofuels. Recently, researchers have suggested a novel chemical cell disruption method, electro-Fenton's process (EFP), for microalgal biodiesel production. The present study demonstrates the feasibility of employing EFP incorporating a sacrificial steel anode as a novel approach for microalgal cell disruption.

Primarily in the current research, *Chlorella* sp. has been selected after screening four locally available microalgal species and it was identified as *Chlorella homosphaera* using molecular identification. Moreover, the effect of chlorophyll removal on the FAME profile and quality of biodiesel produced using *C. homosphaera* were evaluated where chlorophyll removal significantly improved the biodiesel quality while reducing the resulted lipid yield.

Selected process parameters of the electrolytic cell were optimized using the electro-generation of H₂O₂ prior to the EFP experiments. Subsequently, the electrolytic cell with the optimized reactor parameters was employed in microalgal cell disruption via EFP incorporating a sacrificial steel anode where two process parameters, namely reaction time and the biomass concentration, were optimized. Moreover, results were compared with wet (WT) and dry (DR) lipid extraction methods without cell disruption. The EFP showed a significant improvement in lipid yield over the WT method and comparatively higher biodiesel quality than WT and DR methods. Finally, the results obtained for optimized EFP were analyzed comparatively with four conventional mechanical methods. According to the results, EFP contributed to the production of biodiesel with comparatively improved quality than that of mechanical cell disruption methods. Thus, the results of the present study demonstrate that the EFP could be a promising method for industrial-scale applications owing to the ability to produce high-quality biodiesel compared to conventional mechanical methods.

Keywords: microalgae, lipid yield, chlorophyll removal, electro-Fenton's process, chemical cell disruption

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LIST OF ABBREVIATIONS

Abbreviation	Description
(ANOVA)	Analysis of variance
(AU)	Autoclaving
(BLAST)	Basic Local Alignment Search Tool
(BB)	Bead beating
(BBM)	Bold's Basal Medium
(BBD)	Box-Behnken design
(CCD)	Central composite design
(CN)	Cetane number
(DU)	Degree of unsaturation
(ρ)	Density
(DNA)	Deoxyribonucleic acid
(DR)	Dry lipid extraction without cell disruption
(EFP)	Electro-Fenton's process
(EIA)	Energy Information Administration
(EPSs)	Extracellular polymeric substances
(FAME)	Fatty acid methyl ester
(FA)	Fatty acids
(FID)	Flame ionization detector
(FFA)	Free fatty acid
(GC)	Gas chromatography
(GHG)	Greenhouse gas
(HHV)	Higher heating value
(OH \cdot)	Hydroxyl free radical
(IV)	Iodine value
(L \cdot)	Lipid free radical
(MTBE)	Methyl tert-butyl ether
(MUFA)	Monounsaturated fatty acids
(NCBI)	National Center for Biotechnology Information
(NRSn)	Non-radical species
(OD ₇₅₀)	Optical density at 750 nm

(OS)	Oxidative stability
(LO ₂ H)	Peroxides
(LO ₂ •)	Peroxy radical
(PBR)	Photobioreactor
(PCR)	Polymerase chain reaction
(PUFA)	Polyunsaturated fatty acids
(ROS)	Reactive oxygen species
(RSM)	Response surface methodology
(SV)	Saponification value
(SFA)	Saturated fatty acids
(3D)	Three-dimensional
(TAGs)	Triacylglycerols
(US)	Ultrasonication
(LH)	Unsaturated lipid molecules
(v)	Viscosity
(WH)	Water bath heating
(WT)	Wet lipid extraction without cell disruption
(2-EE)	2-ethoxyethanol