IMMOBILIZATION OF MICROALGAE *Chlorella vulgaris* FOR BIOMASS RECOVERY AND FATTY ACID METHYL ESTER PRODUCTION

NOOR RAIHANA BINTI ABU SEPIAN

Doctor of Philosophy

UNIVERSITI MALAYSIA PAHANG

SUPERVISOR'S DECLARATION

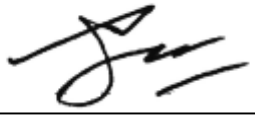
We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Doctor of Philosophy.

(Supervisor's Signature)

Full Name : TS. DR. NUR HIDAYAH MAT YASIN

Position : SENIOR LECTURER

Date : 20/4/2021



(Co-supervisor's Signature)

Full Name : IR. DR. NORAZWINA ZAINOL

Position : ASSOCIATE PROFESSOR

Date : 20/4/2021

STUDENT'S DECLARATION

I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

(Student's Signature)

Full Name : NOOR RAIHANA BINTI ABU SEPIAN

ID Number : PKC16005

Date : 19/4/2021

IMMOBILIZATION OF MICROALGAE *Chlorella vulgaris* FOR BIOMASS RECOVERY AND FATTY ACID METHYL ESTER PRODUCTION

NOOR RAIHANA BINTI ABU SEPIAN

Thesis submitted in fulfillment of the requirements
for the award of the degree of
Doctor of Philosophy

Faculty of Chemical and Process Engineering Technology

UNIVERSITI MALAYSIA PAHANG

APRIL 2021

ACKNOWLEDGEMENTS

In the name of Allah, the Most Gracious and the Most Merciful.

Alhamdulillah. All praises to Allah who bestowed me the perseverance and guidance in completing this study. Being a Ph.D. student as well as a mother of two lovely daughters and one son always giving me challenges which sometimes makes me feel despair in continuing this journey. However, with strong enthusiasm and support from family members, I managed to complete this study within the given time. May this little knowledge bring me closer to Allah and makes me a humble yet beneficial person to society.

The first person that I would like to acknowledge in this section would go to my husband, Saiful Hakim bin Che Mood@Mahyiddin. No words can describe his sacrifices, love, and support to me through thick and thin during my study. My deepest appreciation goes to my parents, Abu sepian bin Mokhtar and Salina binti Ismail, only Allah can repay all the love and sacrifices they give it to me until what I become now. Not to forget here are my parents-in-law, my siblings and siblings in law for their endless love and moral support throughout my study. Thank you for always being there for me.

Next, I express my deepest sense of gratitude to my main supervisor, Ts. Dr. Nur Hidayah Mat Yasin. I deeply appreciated her persistent encouragement, inspiration, and understanding in the period of my study. I wish to thank Ts. Dr. Nur Hidayah for giving me great ideas and offering almost total freedom in research while providing constructive comments and advises to me all the time. Thank you for having faith and confidence in me. I shall remain grateful for all the lessons that I have learned from her. Special thanks also go to my co-supervisor, Assoc. Prof. Ir. Dr. Norazwina Zainol for her valuable guidance and advises to me.

I wish to show my thanks to all the administrative and technical staff of the Faculty of Chemical and Process Engineering Technology, Universiti Malaysia Pahang for their co-operation and kindness in providing me all the essential facilities in completion of my studies. Special appreciation I would like to mention here is to Dr. Noraini Razali and all my friends including Hanani, Kak Kamaliah, Afifah, Kak Norlin and others who directly and indirectly had lent their hand in helping me to complete this study.

Last but not least, I would like to take this opportunity to thank MyBrain15 scholarship and Universiti Malaysia Pahang internal grants [RDU140390; RDU150396; and PGRS170359] for providing me the financial supports.

Noor Raihana binti Abu Sepian
April 2021

ABSTRAK

Mikroalga dikenali sebagai bahan mentah yang berpotensi untuk penghasilan bahan api bio di atas kebolehannya menghasilkan minyak dan tumbuh lebih cepat daripada tanaman konvensional. Namun begitu, saiz mikroalga yang kecil telah menyebabkan kesusahan di proses pengasingan. Oleh itu, kaedah imobilisasi diperkenalkan disebabkan ia tidak memerlukan tenaga dan perbandingan kaedah ini dengan sel kultur bebas dilakukan pada permulaan penyelidikan. Alginat adalah matriks yang paling banyak digunakan tetapi menggunakan alginat sahaja boleh menyebabkan mikroalga pegun pecah. Oleh itu, kombinasi matriks yang baru telah dilaksanakan untuk meningkatkan kestabilan dan pertumbuhan sel. Saringan dan optimuman faktor yang memberi kesan kepada pertumbuhan sel imobilisasi mikroalga telah dilakukan untuk mendapat maksimum nombor pertumbuhan sel. Media Bold Basal (BBM) yang mahal telah mendesak penggunaan efluen kilang kelapa sawit sebagai medium pengkulturan disebabkan ia amat banyak di Malaysia. Mikroalga *Chlorella vulgaris* 211/11B telah diimobilisasikan di dalam tiga gabungan baru matriks dan namanya adalah (1) matriks campuran, $S_AC_{MC}C_A$ (S_A : natrium alginat, C_{MC} : natrium karboksimetil selulosa, C_A : kalsium alginat) (2) S_AC_A dan (3) S_AC_{MC}. Nisbah isi padu matriks ke mikroalga (0.2: 1 - 1: 1) yang sesuai telah diselidiki melalui jumlah pertumbuhan sel. Seterusnya, imobilisasi mikroalga telah disaring dan dioptimumkan menggunakan reka bentuk faktorial berperingkat (FFD) dan reka bentuk komposit berpusat (CCD). Lima faktor yang mempengaruhi bilangan pertumbuhan sel dan kehilangan sel bagi mikroalga pegun *C. vulgaris* termasuk masa pencahayaan, tempoh pengkulturan, kepekatan glukosa, natrium alginat ($NaNO_3$), dan kalsium klorida ($CaCl_2$) telah disaring melalui 16 eksperimen terhasil daripada sistem FFD. Potensi efluen kilang kelapa sawit (POME) sebagai medium kultur telah disiasat melalui perbezaan kepekatan POME (20 % - 100 %) bercampur dengan air suling (DW) dan Media Bold Basal (BBM). Sementara itu, kajian terhadap kinetik dan parameter termodinamik telah dilakukan untuk memahami pengekstrakan imobilisasi mikroalga menggunakan persamaan kadar tindak balas dan persamaan tenaga bebas Gibb's. Mikroalga matriks campuran memaparkan bilangan sel yang tertinggi dengan 1.72×10^9 sel/ml pada hari ke 10 dan 30.43 % hasil pengekstrakan minyak diikuti oleh S_AC_A (0.3:1) (24.29 %), S_AC_{MC} (1:1) (13.00 %), dan S_A (1:1) (6.71 %). Melalui saringan menggunakan FFD, masa pencahayaan dan tempoh pengkulturan telah dikenalpasti sebagai faktor penting untuk kedua-dua respons. Keputusan menunjukkan bahawa FFD adalah alat yang berguna untuk menentukan faktor-faktor penting untuk pertumbuhan sel imobilisasi dan meningkatkan pengeluaran minyak sebanyak 17.27 %. Selepas itu, dua faktor penting tadi telah dioptimumkan menggunakan CCD. Kondisi optimum yang dicadangkan daripada FFD dan CCD adalah 5.03 g/L $NaNO_3$, 1 w/v % $CaCl_2$, 24 h masa pencahayaan, 23.99 g/L glukosa dan 7.96 hari pengkulturan. Pengoptimuman menggunakan CCD telah meningkatkan minyak kepada 51.6 %. Sementara itu, 20 % POME dicampur dengan 80 % air suling sebagai medium kultur untuk mikroalga pegun telah menunjukkan hasil minyak yang agak setanding (38.14 ± 2.2 %) dengan minyak yang diperolehi daripada medium BBM (48.03 ± 3.7 %). Kajian kinetik menunjukkan bahawa nilai kadar pemalar sangat bergantung pada suhu. Kajian termodinamik mendedahkan bahawa pengekstrakan minyak daripada biojisim mikroalga pegun adalah reaksi endotermik, tak boleh berbalik dan spontan. Penggabungan S_A, C_A, dan C_{MC} telah membentuk struktur baru yang sesuai untuk meningkatkan pertumbuhan sel *C. vulgaris* dan meningkatkan pengeluaran minyak berbanding dengan imobilisasi menggunakan matrik tunggal. Selain itu, profil asid lemak metil ester yang diperolehi mempunyai potensi tinggi untuk pengeluaran biodiesel.

ABSTRACT

Microalgae had known to be a promising source for biofuel production as it can produce oil and grown faster than conventional crops. However, the small size of microalgae brings difficulties in the separation process. Thus, the immobilization method was introduced since it does not require energy and a comparison of this method with free cells culture was made initially. Alginate is the most widely used matrix but using alginate alone can cause bead rupture. Thus, a new combination of a matrix was implemented to improve the stability and cell growth. The screening and optimization of the factors affecting the immobilized microalgae cell growth were performed to obtain the maximum number of cell growth. The expensive Bold Basal Medium (BBM) had urged the usage of palm oil mill effluent (POME) as a cultivation medium since it is abundant in Malaysia. The microalgae *Chlorella vulgaris* 211/11B cells were immobilized within three novel combinations of matrices namely (1) mixed matrices, $S_AC_{MC}C_A$ (S_A: sodium alginate, C_{MC}: sodium carboxymethyl cellulose, C_A: calcium alginate) (2) S_AC_A and (3) S_AC_{MC}. The suitable matrix-to-microalgae volume ratios (0.2:1 – 1:1) were determined by evaluating the number of cell growth. Subsequently, the immobilized microalgae were screened and optimized using fractional factorial design (FFD) and central composite design (CCD). Five factors inducing the number of cell growth and cell loss of immobilized *C. vulgaris* including photoperiod, cultivation period, the concentration of glucose, sodium nitrate ($NaNO_3$) and calcium chloride ($CaCl_2$) were screened through 16 experiments generated from the system. The potential of palm oil mill effluent (POME) as a cultivation medium was investigated through different concentrations of POME (20 % - 100 %) mixed with distilled water (DW) and Bold Basal Medium (BBM). Meanwhile, studies on the kinetic and thermodynamic parameters were exhibited to understand the nature of extraction of immobilized microalgae using the reaction rate equation and Gibb's free energy equation. The immobilized microalgae of mixed matrices with a ratio of 0.3:1 exhibited the highest number of cells with 1.72×10^9 cells/mL at day 10 and 30.43 % of oil extraction yield followed by S_AC_A (0.3:1) (24.29 %), S_AC_{MC} (1:1) (13.00 %), and S_A (1:1) (6.71 %). Through the screening process using FFD, photoperiod and cultivation days were identified as the significant factors for both responses. The outcomes indicated that FFD is a convenient tool to determine the important factors for immobilized cell growth and increased lipid production by 17.27 %. Then, the two significant factors were optimized by central composite design (CCD). The suggested conditions obtained from FFD and CCD were 5.03 g/L of $NaNO_3$, 1 w/v % of $CaCl_2$, 24 h of photoperiod, 23.99 g/L of glucose and 7.96 cultivation days. The optimization using CCD had increased the lipid yield to 51.6 %. Meanwhile, 20 % POME mixed with 80 % DW showed a quite comparable lipid yield (38.14 ± 2.2 %) with the lipid obtained from BBM (48.03 ± 3.7 %). The kinetic studies showed that the values of the rate constants relied strongly on temperature. The thermodynamic studies revealed that the lipid extraction of immobilized microalgae biomass is an endothermic, irreversible and spontaneous reaction. The combination of S_A, C_A, and C_{MC} made a new structure that enhanced the growth of *C. vulgaris* cells and increased the lipid production than the immobilization using a single matrix. Moreover, the FAME profile obtained from all the experimental works shows a high prospective for biodiesel production.

TABLE OF CONTENT

REFERENCES

Abdul Halim, Z., Abu Sepian, N. R., & Abd Karim, K. (2017). Potential of Palm Oill Mill Effluent (POME) as Growth Medium for Sulfate-Reducing Bacterium in Microbial Fuel Cell. *Materials Science Forum*, *889*, 184–190. https://doi.org/10.4028/www.scientific.net/MSF.889.184

Abdul Hamid, Z. (2018, October 8). The power of algae. *New Straits Times*, p. 1.

Abdul Samad, K. (2017). *Synergistic ferulic acid production from banana stem waste by co-culture*. Universiti Malaysia Pahang.

Abdul Samad, K., & Zainol, N. (2017a). Effects of agitation and volume of inoculum on ferulic acid production by co-culture. *Biocatalysis and Agricultural Biotechnology*, *10*(January), 9–12. https://doi.org/10.1016/j.bcab.2017.01.010

Abdul Samad, K., & Zainol, N. (2017b). The use of factorial design for ferulic acid production by co-culture. *Industrial Crops and Products*, *95*, 202–206. https://doi.org/10.1016/j.indcrop.2016.10.028

Abdul, W. (2015). Malaysia-Biofuels Annual. *Kuala Lumpur: USDA Foreign Agricultural Service*.

Agency for Natural Resources and Energy. (2017). Key World Energy statistics. *IEA International Energy Agency*. https://doi.org/10.1017/CBO9781107415324.004

Ahmad, A., Bhat, A. H., & Buang, A. (2018). Biosorption of transition metals by freely suspended and Ca-alginate immobilised with *Chlorella vulgaris*: Kinetic and equilibrium modeling. *Journal of Cleaner Production*, *171*, 1361–1375. https://doi.org/https://doi.org/10.1016/j.jclepro.2017.09.252

Ahmad, A., Buang, A., & Bhat, A. H. (2016). Renewable and sustainable bioenergy production from microalgal co-cultivation with palm oil mill effluent (POME): A review. *Renewable and Sustainable Energy Reviews*, *65*, 214–234. https://doi.org/10.1016/j.rser.2016.06.084

Ahmad, A. L., Mat Yasin, N. H., Derek, C. J. C., & Lim, J. K. (2014a). Chemical cleaning of a cross-flow microfiltration membrane fouled by microalgal biomass. *Journal of the Taiwan Institute of Chemical Engineers*, *45*(1), 233–241. https://doi.org/10.1016/j.jtice.2013.06.018

Ahmad, A. L., Mat Yasin, N. H., Derek, C. J. C., & Lim, J. K. (2014b). Comparison of harvesting methods for microalgae *Chlorella* sp. and its potential use as a biodiesel feedstock. *Environmental Technology*, *35*(17), 2244–2253. https://doi.org/10.1080/09593330.2014.900117

Ahmad, A. L., Mat Yasin, N. H., Derek, C. J. C., & Lim, J. K. (2014c). Kinetic studies and thermodynamics of oil extraction and transesterification of *Chlorella* sp. for biodiesel production. *Environmental Technology (United Kingdom)*, *35*(7), 891–897. https://doi.org/10.1080/09593330.2013.855263

Al-Iwayzy, S. H., Yusaf, T., & Al-Juboori, R. A. (2014). Biofuels from the fresh water microalgae *Chlorella vulgaris* (FWM-CV) for diesel engines. *Energies*, *7*, 1829–1851. https://doi.org/10.3390/en7031829

Alam, F., Mobin, S., & Chowdhury, H. (2015). Third generation biofuel from Algae. *Procedia Engineering*, *105* (Icte 2014), 763–768. https://doi.org/10.1016/j.proeng.2015.05.068

Ali, N., Ting, Z., Khan, Y. H., Athar, M. A., Ahmad, V., & Idrees, M. (2014). Making biofuels from microalgae -A review of technologies. *Journal of Food Science and Technology*, *1*(2), 2384–5058.

Almadani, E. A., Harun, F. W., Radzi, S. M., & Muhamad, S. K. (2018). Cu2+ Montmorillonite K10 Clay Catalyst as a Green Catalyst for Production of Stearic Acid Methyl Ester : Optimization Using Response Surface Methodology (RSM). *Bulletin of Chemical Reaction Engineering & Catalysis*, *13*(1), 187–195. https://doi.org/10.9767/bcrec.13.1.1397.187-195

Alphonsus, C. (2018, July 17). Fat Hopes for Bio-Fuel Industry. *Selangor Journal*, p. 1.

Andrade, L. R., Salgado, L. T., Farina, M., Pereira, M. S., Mourão, P. A. S., & Amado Filho, G. M. (2004). Ultrastructure of acidic polysaccharides from the cell walls of brown algae. *Journal of Structural Biology*, *145*(3), 216–225. https://doi.org/10.1016/j.jsb.2003.11.011

Antony, J. (2014). Full Factorial Designs. In J. Antony (Ed.), *Design of Experiments for Engineers and Scientists (Second Edition)* (Second Edi, pp. 63–85). https://doi.org/https://doi.org/10.1016/B978-0-08-099417-8.00006-7

Anuar, M. R., & Abdullah, A. Z. (2016). Challenges in biodiesel industry with regards to feedstock, environmental, social and sustainability issues: A critical review.

Renewable and Sustainable Energy Reviews, *58*, 208–223. https://doi.org/10.1016/j.rser.2015.12.296

Awalludin, M. F., Sulaiman, O., Hashim, R., & Nadhari, W. N. A. W. (2015). An overview of the oil palm industry in Malaysia and its waste utilization through thermochemical conversion, specifically via liquefaction. *Renewable and Sustainable Energy Reviews*, *50*, 1469–1484. https://doi.org/10.1016/j.rser.2015.05.085

Badar, S. N., Mohammad, M., Emdadi, Z., & Yaakob, Z. (2018). Algae and their growth requirements for bioenergy: a review. *Biofuels*, *7269*, 1–19. https://doi.org/10.1080/17597269.2018.1472978

Balat, M., Balat, H., & Oz, C. (2008). Progress in bioethanol processing. *Progress in Energy and Combustion Science*, *34*, 551–573.

Belasco, W. (1997). Algae burgers for a hungry world? The rise and fall of *Chlorella* cuisine. *Technology and Culture*, *38*, 608–634.

Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S., & Escaleira, L. A. (2008). Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta*, *76*(5), 965–977. https://doi.org/10.1016/j.talanta.2008.05.019

Bharte, S., & Desai, K. (2018). Techniques for harvesting, cell disruption and lipid extraction of microalgae for biofuel production. *Biofuels*, *7269*, 1–21. https://doi.org/10.1080/17597269.2018.1472977

Bhujbal, S. V., Paredes-Juarez, G. A., Niclou, S. P., & de Vos, P. (2014). Factors influencing the mechanical stability of alginate beads applicable for immunoisolation of mammalian cells. *Journal of the Mechanical Behavior of Biomedical Materials*, *37*, 196–208. https://doi.org/10.1016/j.jmbbm.2014.05.020

Bligh, E., & Dyer, W. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry*, *37*, 911–917. https://doi.org/10.1139/o59-099

BP, P. I. c. (2011). BP Energy Outlook 2030. In *BP 2011* (Vol. 0383). https://doi.org/10.5555/jan.010a.2013

Chabane, L., Cheknane, B., Zermane, F., Bouras, O., & Baudu, M. (2017). Synthesis and characterization of reinforced hybrid porous beads: Application to the

adsorption of malachite green in aqueous solution. *Chemical Engineering Research and Design*, *120*, 291–302. https://doi.org/10.1016/j.cherd.2016.12.014

Cheah, W. Y., Show, P. L., Juan, J. C., Chang, J. S., & Ling, T. C. (2018). Enhancing biomass and lipid productions of microalgae in palm oil mill effluent using carbon and nutrient supplementation. *Energy Conversion and Management*, *164*(February), 188–197. https://doi.org/10.1016/j.enconman.2018.02.094

Cheirsilp, B., Thawechai, T., & Prasertsan, P. (2017). Immobilized oleaginous microalgae for production of lipid and phytoremediation of secondary effluent from palm oil mill in fluidized bed photobioreactor. *Bioresource Technology*, *241*, 787–794. https://doi.org/10.1016/j.biortech.2017.06.016

Chen, C. Y., Yeh, K. L., Aisyah, R., Lee, D. J., & Chang, J. S. (2011). Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: A critical review. *Bioresource Technology*, *102*(1), 71–81. https://doi.org/10.1016/j.biortech.2010.06.159

Chen, H. (2015). Lignocellulose biorefinery product engineering. In H. B. T.-L. B. E. Chen (Ed.), *Lignocellulose Biorefinery Engineering* (pp. 125–165). https://doi.org/https://doi.org/10.1016/B978-0-08-100135-6.00005-3

Chen, J. J., Li, Y. R., & Lai, W. L. (2014). Application of experimental design methodology for optimization of biofuel production from microalgae. *Biomass and Bioenergy*, *64*, 11–19. https://doi.org/10.1016/j.biombioe.2014.03.056

Chevalier, P., & De la Noüe, J. (1985). Wastewater nutrient re- moval with microalgae immobilized in carrageenan. *Enzyme and Microbial Technology*, *7*, 621–624.

Chisti, Y. (2013). Constraints to commercialization of algal fuels. *Journal of Biotechnology*, *167*(3), 201–214. https://doi.org/10.1016/j.jbiotec.2013.07.020

Chung, H. T., Pau, L. S., Jo-Shu, C., Tau, C. L., & Lan, J. C.-W. (2014). Novel approaches of producing bioenergies from microalgae: A recent review. *Biotechnology Advances*, *33*(6), 1219–1227. https://doi.org/10.1016/j.biotechadv.2015.02.013

Ciric, A., Krajnc, B., Heath, D., & Ogrinc, N. (2020). Response surface methodology and arti fi cial neural network approach for the optimization of ultrasound-assisted extraction of polyphenols from garlic. *Food and Chemical Toxicology*, *135*(July 2019), 110976. https://doi.org/10.1016/j.fct.2019.110976

Coates, J. (2006). Interpretation of Infrared Spectra, A Practical Approach. In R. A. Meyers (Ed.), *Encyclopedia of Analytical Chemistry* (pp. 1–23). https://doi.org/10.1002/9780470027318.a5606

Culture Collection of Algae and Protozoa. (2015). 3N-BBM+V (Bold Basal Medium with 3-fold Nitrogen and Vitamins; modified). Retrieved September 1, 2015, from https://www.ccap.ac.uk/

Danny, T. (2018, December 11). PM launches B10 biodiesel programme – to reduce CO2 by 10%, boost demand for Malaysian palm oil. *Paultan.Org*, p. 1.

Das, A. K., & Dewanjee, S. (2018). Optimization of Extraction Using Mathematical Models and Computation. In S. D. Sarker & L. Nahar (Eds.), *Computational Phytochemistry* (pp. 75–106). https://doi.org/https://doi.org/10.1016/B978-0-12-812364-5.00003-1

de-Bashan, L. E., & Bashan, Y. (2010). Immobilized microalgae for removing pollutants: Review of practical aspects. *Bioresource Technology*, *101*(6), 1611–1627. https://doi.org/10.1016/j.biortech.2009.09.043

de-Bashan, L. E., Bashan, Y., Moreno, M., Lebsky, V. K., & Bustillos, J. J. (2002). Increased pigment and lipid content, lipid variety, and cell and population size of the microalgae *Chlorella* spp. when co-immobilized in alginate beads with the microalgae-growth-promoting bacterium *Azospirillum brasilense*. *Canadian Journal of Microbiology*, *48*(6), 514–521. https://doi.org/10.1139/w02-051

Deconinck, N., Muylaert, K., Ivens, W., & Vandamme, D. (2018). Innovative harvesting processes for microalgae biomass production: A perspective from patent literature. *Algal Research*, *31*(September 2017), 469–477. https://doi.org/10.1016/j.algal.2018.01.016

Delrieu, P. E., & Ding, L. (2001). *Patent No. #6,319,507.* United States Patent: United States Patent.

Deshmukh, S., Kumar, R., & Bala, K. (2019). Microalgae biodiesel: A review on oil extraction, fatty acid composition, properties and effect on engine performance and emissions. *Fuel Processing Technology*, *191*(May), 232–247. https://doi.org/10.1016/j.fuproc.2019.03.013

Doan, Q. C., Moheimani, N. R., Mastrangelo, A. J., & Lewis, D. M. (2012). Microalgal biomass for bioethanol fermentation: Implications for hypersaline systems with an

industrial focus. *Biomass and Bioenergy*, *46*, 79–88.

Draget, K. I., Skjåk Bræk, G., & Smidsrød, O. (1994). Alginic acid gels: the effect of alginate chemical composition and molecular weight. *Carbohydrate Polymers*, *25*(1), 31–38. https://doi.org/10.1016/0144-8617(94)90159-7

El-Helaly, S. N., Habib, B. A., & El-Rahman, M. K. A. (2018). Resolution V fractional factorial design for screening of factors affecting weakly basic drugs liposomal systems. *European Journal of Pharmaceutical Sciences*, *119*, 249–258. https://doi.org/https://doi.org/10.1016/j.ejps.2018.04.028

El-Sheekh, M., Abomohra, A. E. F., Eladel, H., Battah, M., & Mohammed, S. (2018). Screening of different species of *Scenedesmus* isolated from Egyptian freshwater habitats for biodiesel production. *Renewable Energy*, *129*, 114–120. https://doi.org/10.1016/j.renene.2018.05.099

El Arroussi, H., Benhima, R., El Mernissi, N., Bouhfid, R., Tilsaghani, C., Bennis, I., & Wahby, I. (2017). Screening of marine microalgae strains from Moroccan coasts for biodiesel production. *Renewable Energy*, *113*, 1515–1522. https://doi.org/10.1016/j.renene.2017.07.035

Enamala, M. K., Enamala, S., Chavali, M., Donepudi, J., Yadavalli, R., Kolapalli, B., Kuppam, C. (2018). Production of biofuels from microalgae - A review on cultivation, harvesting, lipid extraction, and numerous applications of microalgae. *Renewable and Sustainable Energy Reviews*, *94*(June), 49–68. https://doi.org/10.1016/j.rser.2018.05.012

Energy Data and Research Unit, S. P. and C. D. (2019). *Malaysia energy statistics handbook 2019*. Putrajaya, Malaysia: Suruhanjaya Tenaga.

Eroglu, E., Smith, S. M., & Raston, C. L. (2015). Application of Various Immobilization Techniques for Algal Bioprocesses. In N.R. Moheimani et al. (Ed.), *Biomass and Biofuels from Microalgae* (2nd ed., Vol. 2, pp. 19–44). https://doi.org/10.1007/978-3-319-16640-7_2

Fan, Y., Wu, Y., Fang, P., & Ming, Z. (2017). Facile and effective synthesis of adsorbent - Utilization of yeast cells immobilized in sodium alginate beads for the adsorption of phosphorus in aqueous solution. *Water Science and Technology*, *75*(1), 75–83. https://doi.org/10.2166/wst.2016.491

Fasaei, F., Bitter, J. H., Slegers, P. M., & van Boxtel, A. J. B. (2018). Techno-economic

evaluation of microalgae harvesting and dewatering systems. *Algal Research*, *31*(April), 347–362. https://doi.org/10.1016/j.algal.2017.11.038

Gani, P., Mohamed Sunar, N., Matias-Peralta, H., Abdul Latiff, A. A., & Mohamad Fuzi, S. F. Z. (2017). Growth of microalgae *Botryococcus* sp. in domestic wastewater and application of statistical analysis for the optimisation of flocculation using alum and chitosan. *Preparative Biochemistry and Biotechnology*, *14*(4), 333–341.

Gani, P., Sunar, N. M., Matias-peralta, H., & Aziz, A. (2017). Effect of pH and alum dosage on the efficiency of microalgae harvesting via flocculation technique. *International Journal of Green Energy*, *14*(4), 395–399. https://doi.org/10.1080/15435075.2016.1261707

Ghosh, S., Banerjee, S., & Das, D. (2017). Process intensification of biodiesel production from *Chlorella* sp. MJ 11/11 by single step transesterification. *Algal Research*, *27*(March), 12–20. https://doi.org/10.1016/j.algal.2017.08.021

Glicksman, M. (1986). *Food Hydrocolloids*. Boca Raton, FL: CRC Press.

Gonzalez-Fernandez, C., Sialve, B., Bernet, N., & Stayer, J.-P. (2012). Impact of microalgae characteristics on their conversion to biofuel. Part I: Focus on cultivation and biofuel production. *Biofuel Bioproduct Biorefinery*, *6*, 105-13.

Gopinath, A., Puhan, S., & Govindan, N. (2010). Effect of unsaturated fatty acid esters of biodiesel fuels on combustion, performance and emission characteristics of a DI diesel engine. *International Journal of Energy and Environment.*, *1*(3), 411–430.

Gui, M. M., Lee, K. T., & Bhatia, S. (2008). Feasibility of edible oil vs. non-edible oil vs. waste edible oil as biodiesel feedstock. *Energy*, *33*(11), 1646–1653. https://doi.org/10.1016/j.energy.2008.06.002

Guiseley, K. B. (1989). Chemical and physical properties of algal polysaccharides used for cell immobilization. *Enzyme and Microbial Technology*, *11*(11), 706–716. https://doi.org/10.1016/0141-0229(89)90119-1

Gupta, S. Sen, Shastri, Y., & Bhartiya, S. (2016). Model-based optimization of biodiesel production from microalgae. *Computers & Chemical Engineering*, *89*, 222–249. https://doi.org/10.1016/j.compchemeng.2016.01.014

Habib, M. A. ., Yusoff, F. M., Phang, S. M., & Mohamed, S. (2003). Growth and Nutritional Values of Moina micrura Fed on *Chlorella vulgaris* Grown in Digested Palm Oil Mill Effluent. *Asian Fisheries Science*, *16*, 107–119.

Haigh-Flórez, D., de la Hera, C., Costas, E., & Orellana, G. (2014). Microalgae dual-head biosensors for selective detection of herbicides with fiber-optic luminescent O2 transduction. *Biosensors and Bioelectronics*, *54*, 484–491. https://doi.org/https://doi.org/10.1016/j.bios.2013.10.062

Hamedi, S., Mahdavi, M. A., & Gheshlaghi, R. (2016). Improved lipid and biomass productivities in *Chlorella vulgaris* by differing the inoculation medium from the production medium. *Biofuel Research Journal*, *3*(2), 410–416. https://doi.org/10.18331/BRJ2016.3.2.6

Hamzaoui, A. H., Jamoussi, B., & M'nif, A. (2008). Lithium recovery from highly concentrated solutions: Response surface methodology (RSM) process parameters optimization. *Hydrometallurgy*, *90*(1), 1–7. https://doi.org/10.1016/j.hydromet.2007.09.005

Han, Y.-H., Lee, J.-S., Kwak, J.-K., Lee, E.-H., & Cho, M.-G. (1999). High-density cultivation of microalgae using microencapsulation. *Journal of the Korean Fisheries Society*, Vol. 32, pp. 186–191.

Hayder, J., Chaouch, M. A., Amira, N., Ben, M., Majdoub, H., Chaubet, F., & Maaroufi, R. M. (2018). Co-immobilization of chitosan and dermatan sulfate from Raja montagui skin on polyethylene terephthalate surfaces : Characterization and antibiofilm activity. *International Journal of Polymeric Materials and Polymeric Biomaterials*, *67*(5), 277–287. https://doi.org/10.1080/00914037.2017.1320664

Ho, S.-H., Chen, C.-Y., Lee, D.-J., & Chang, J.-S. (2011). Perspectives on microalgal CO_2 - emission mitigation systems – a review. *Biotechnology Advances*, *29*, 189–198.

International Energy Agency. (2019). *Global Energy & CO2 Status Report*.

Joelianingsih, Maeda, H., Hagiwara, S., Nabetani, H., Sagara, Y., Soerawidjaya, T. H., Abdullah, K. (2008). Biodiesel fuels from palm oil via the non-catalytic transesterification in a bubble column reactor at atmospheric pressure: A kinetic study. *Renewable Energy*, *33*(7), 1629–1636. https://doi.org/10.1016/J.RENENE.2007.08.011

Johnston, D., Kumar, P., Choonara, Y. E., du Toit, L. C., & Pillay, V. (2013). Modulation of the nano-tensile mechanical properties of co-blended amphiphilic alginate fibers as oradurable biomaterials for specialized biomedical application. *Journal of the Mechanical Behavior of Biomedical Materials*, *23*, 80–102. https://doi.org/10.1016/j.jmbbm.2013.03.026

Joo, D. S., Cho, M. G., Lee, J. S., Park, J. H., Kwak, J. K., Han, Y. H., & Bucholz, R. (2001). New strategy for the cultivation of microalgae using microencapsulation. *Journal of Microencapsulation*, *18*(5), 567–576. https://doi.org/10.1080/02652040010018065

Kansedo, J., & Lee, K. T. (2012). Transesterification of palm oil and crude sea mango (*Cerbera odollam*) oil: The active role of simplified sulfated zirconia catalyst. *Biomass and Bioenergy*, *40*, 96–104.

Karazhiyan, H., Razavi, S. M., & Phillips, G. O. (2011). Extraction optimization of a hydrocolloid extract from cress seed (*Lepidium sativum*) using response surface methodology. *Food Hydrocolloids*, *25*, 915–920.

Karpagam, R., Raj, K. J., Ashokkumar, B., & Varalakshmi, P. (2015). Characterization and fatty acid profiling in two fresh water microalgae for biodiesel production: Lipid enhancement methods and media optimization using response surface methodology. *Bioresource Technology*, *188*, 177–184. https://doi.org/10.1016/j.biortech.2015.01.053

Katam, K., & Bhattacharyya, D. (2019). Simultaneous treatment of domestic wastewater and bio-lipid synthesis using immobilized and suspended cultures of microalgae and activated sludge. *Journal of Industrial and Engineering Chemistry*, *69*, 295–303. https://doi.org/10.1016/j.jiec.2018.09.031

Kendirlioglu, G., Agirman, N., & Cetin, A. K. (2015). The effects of photoperiod on the growth, protein amount and pigment content of *Chlorella vulgaris*. *Turkish Journal of Science & Technology*, *10*(2), 7–10.

Kin, W. C., Yusup, S., Lai, F. C., & Bokhari, A. (2016). Physio-chemical Studies of Locally Sourced Non-Edible Oil: Prospective Feedstock for Renewable Diesel Production in Malaysia. *Procedia Engineering*, *148*, 451–458. https://doi.org/10.1016/j.proeng.2016.06.460

Kitcha, S., & Cheirsilp, B. (2014). Enhanced lipid production by co-cultivation and co-encapsulation of oleaginous yeast *Trichosporonoides spathulata* with microalgae in alginate gel beads. *Applied Biochemistry and Biotechnology*, *173*(2), 522–534.

https://doi.org/10.1007/s12010-014-0859-5

Knothe, G. (2005). Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. *Fuel Processing Technology*, *86*(10), 1059–1070. https://doi.org/http://dx.doi.org/10.1016/j.fuproc.2004.11.002

Kong, W. B., Hua, S. F., Cao, H., Mu, Y. W., Yang, H., Song, H., & Xia, C. G. (2012). Optimization of mixotrophic medium components for biomass production and biochemical composition biosynthesis by *Chlorella vulgaris* using response surface methodology. *Journal of the Taiwan Institute of Chemical Engineers*, *43*(3), 360–367. https://doi.org/10.1016/j.jtice.2011.11.007

Kosourov, S. N., & Seibert, M. (2009). Hydrogen Photoproduction by Nutrient-Deprived *Chlamydomonas reinhardtii* Cells Immobilized Within Thin Alginate Films Under Aerobic and Anaerobic Conditions. *Biotechnology and Bioengineering*, *102*(1), 50–58. https://doi.org/10.1002/bit.22050

Kousha, M., Farhadian, O., Dorafshan, S., Soofiani, N. M., & Bhatnagar, A. (2013). Optimization of malachite green biosorption by green microalgae-Scenedesmus quadricauda and *Chlorella vulgaris*: Application of response surface methodology. *Journal of the Taiwan Institute of Chemical Engineers*, *44*(2), 291–294. https://doi.org/10.1016/j.jtice.2012.10.009

Kumar, M., & Sharma, M. P. (2016). Kinetics of *Chlorella protothecoides* microalgal oil using base catalyst. *Egyptian Journal of Petroleum*, *25*(3), 375–382. https://doi.org/10.1016/j.ejpe.2015.08.005

Lahin, F. A., Sarbatly, R., & Suali, E. (2016). Polishing of POME by *Chlorella* sp. in suspended and immobilized system. *IOP Conference Series: Earth and Environmental Science*, *36*(1), 1–10. https://doi.org/10.1088/1755-1315/36/1/012030

Lam, M. K., & Lee, K. T. (2012). Immobilization as a feasible method to simplify the separation of microalgae from water for biodiesel production. *Chemical Engineering Journal*, *191*, 263–268. https://doi.org/10.1016/j.cej.2012.03.013

Lam, M. K., Yusoff, M. I., Uemura, Y., Lim, J. W., Khoo, C. G., Lee, K. T., & Ong, H. C. (2017). Cultivation of *Chlorella vulgaris* using nutrients source from domestic wastewater for biodiesel production : Growth condition and kinetic studies. *Renewable Energy*, *103*, 197–207. https://doi.org/10.1016/j.renene.2016.11.032

Lau, P. S., Tam, N. F. Y., & Wong, Y. S. (1998). Effect of carrageenan immobilization on the physiological activities of *Chlorella vulgaris*. *Bioresource Technology*, *63*(2), 115–121. https://doi.org/10.1016/S0960-8524(97)00111-9

Lee, B. H., & Park, J. K. (1996). Encapsulation of Whole Cell β-Galactosidase of *Escherichia coli*. *Korean Journal of Biotechnology and Bioengineering*, *11*, 398–404.

Lee, K. Y., & Mooney, D. J. (2012). Alginate: properties and biomedical applications. *Progress in Polymer Science*, *37*(1), 106–126. https://doi.org/10.1016/j.progpolymsci.2011.06.003

Leite, L. D. S., Hoffmann, M. T., & Daniel, L. A. (2019). Coagulation and dissolved air flotation as a harvesting method for microalgae cultivated in wastewater. *Journal of Water Process Engineering*, *32*(August), 100947. https://doi.org/10.1016/j.jwpe.2019.100947

Lim, S., & Lee, K. T. (2010). Recent trends, opportunities and challenges of biodiesel in Malaysia: An overview. *Renewable and Sustainable Energy Reviews*, *14*(3), 938–954. https://doi.org/10.1016/j.rser.2009.10.027

Ling, G. P., & Winifred, A. (2018, November 26). Malaysia plans to start using B-10 biodiesel from Dec. 1. *Nikkei Asian Review*, p. 1.

Liu, J., Lin, R., Nan, Y., & Tavlarides, L. L. (2015). Production of biodiesel from microalgae oil (*Chlorella protothecoides*) by non-catalytic transesterification: Evaluation of reaction kinetic models and phase behavior. *Journal of Supercritical Fluids*, *99*, 38–50. https://doi.org/10.1016/j.supflu.2015.01.010

Liu, S., Xu, J., Yu, H., Zhao, C., Chen, W., & Ma, F. (2018). Comparing the Effect of C, N, and P Factors on Photosynthesis, Biomass, and Lipid Production in *Chlorella* sp. *Journal of Environmental Engineering (United States)*, *144*(11), 1–7. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001459

Liu, X. D., Yu, W. Y., Zhang, Y., Xue, W. M., Yu, W. T., Xiong, Y., Yuan, Q. (2002). Characterization of structure and diffusion behaviour of Ca-alginate beads prepared with external or internal calcium sources. *Journal of Microencapsulation*, *19*(6), 775–782. https://doi.org/10.1080/0265204021000022743

Malaysian Palm Oil Council. (2019). The implications of EU Resolution to the Malaysian Palm Oil Industry. *MPOC*, 1.

Mat Yasin, M. H., Mamat, R., Najafi, G., Ali, O. M., Yusop, A. F., & Ali, M. H. (2017). Potentials of palm oil as new feedstock oil for a global alternative fuel: A review. *Renewable and Sustainable Energy Reviews*, *79*(May), 1034–1049. https://doi.org/10.1016/j.rser.2017.05.186

Mata, T. M., Martins, A. A., & Caetano, N. S. (2010). Microalgae for biodiesel production and other applications: A review. *Renewable and Sustainable Energy Reviews*, *14*(1), 217–232. https://doi.org/10.1016/j.rser.2009.07.020

Mathimani, T., Beena Nair, B., & Ranjith kumar, R. (2016). Evaluation of microalga for biodiesel using lipid and fatty acid as a marker – A central composite design approach. *Journal of the Energy Institute*, *89*(3), 436–446. https://doi.org/10.1016/j.joei.2015.02.010

Mathimani, Thangavel, & Mallick, N. (2018). A comprehensive review on harvesting of microalgae for biodiesel - Key challenges and future directions. *Renewable and Sustainable Energy Reviews*, *91*(April), 1103–1120. https://doi.org/10.1016/j.rser.2018.04.083

Mathimani, Thangavel, Uma, L., & Prabaharan, D. (2018). Formulation of low-cost seawater medium for high cell density and high lipid content of *Chlorella vulgaris* BDUG 91771 using central composite design in biodiesel perspective. *Journal of Cleaner Production*, *198*, 575–586. https://doi.org/10.1016/j.jclepro.2018.06.303

Matinja, A. I., Mohd Zain, N. A., Suhaimi, M. S., & Alhassan, A. J. (2019). Optimization of biodiesel production from palm oil mill effluent using lipase immobilized in PVA-alginate-sulfate beads. *Renewable Energy*, *135*, 1178–1185. https://doi.org/10.1016/j.renene.2018.12.079

Md Sai'aan, N. H., Soon, C. F., Tee, K. S., Ahmad, M. K., Youseffi, M., & Khagani, S. A. (2017). Characterisation of encapsulated cells in calcium alginate microcapsules. *IECBES 2016 - IEEE-EMBS Conference on Biomedical Engineering and Sciences*, 611–616. https://doi.org/10.1109/IECBES.2016.7843522

Menegazzo, M. L., & Fonseca, G. G. (2019). Biomass recovery and lipid extraction processes for microalgae biofuels production: A review. *Renewable and Sustainable Energy Reviews*, *107*(July 2018), 87–107. https://doi.org/10.1016/j.rser.2019.01.064

Miao, X., & Wu, Q. (2006). Biodiesel production from heterotrophic microalgal oil. *Bioresource Biotechnology*, *110*, 85–93.

Milano, J., Ong, H. C., Masjuki, H. H., Chong, W. T., Lam, M. K., Loh, P. K., & Vellayan, V. (2016). Microalgae biofuels as an alternative to fossil fuel for power generation. *Renewable and Sustainable Energy Reviews*, *58*, 180–197. https://doi.org/http://dx.doi.org/10.1016/j.rser.2015.12.150

Mitropoulou, G., Nedovic, V., Goyal, A., & Kourkoutas, Y. (2013). Immobilization Technologies in Probiotic Food Production. *Journal of Nutrition and Metabolism*, *2013*, 1–15. https://doi.org/10.1155/2013/716861

Mofijur, M., Masjuki, H. H., Kalam, M. A., Hazrat, M. A., Liaquat, A. M., Shahabuddin, M., & Varman, M. (2012). Prospects of biodiesel from Jatropha in Malaysia. *Renewable and Sustainable Energy Reviews*, *16*(7), 5007–5020. https://doi.org/10.1016/j.rser.2012.05.010

Mohammad Mirzaie, M. A., Kalbasi, M., Mousavi, S. M., & Ghobadian, B. (2016). Investigation of mixotrophic, heterotrophic, and autotrophic growth of *Chlorella vulgaris* under agricultural waste medium. *Preparative Biochemistry and Biotechnology*, *46*(2), 150–156. https://doi.org/10.1080/10826068.2014.995812

Mohanadoss, P., Fadhil, M., & Md Din, M. F. (2013). Effect of Light/Dark cycle on Biomass and Lipid Productivity by *Chlorella pyrenoidosa* using Palm Oil Mill Effluent (POME). *Journal of Scientific and Industrial Research*, *72*, 703–706.

Mohiuddin, M., Kumar, B., & Haque, S. (2017). Biopolymer Composites in Photovoltaics and Photodetectors. In K. K. Sadasivuni, D. Ponnamma, J. Kim, J.-J. Cabibihan, & M. A. AlMaadeed (Eds.), *Biopolymer Composites in Electronics* (pp. 459–486). https://doi.org/https://doi.org/10.1016/B978-0-12-809261-3.00017-6

Moreno-Garrido, I. (2008). Microalgae immobilization: Current techniques and uses. *Bioresource Technology*, *99*(10), 3949–3964. https://doi.org/10.1016/j.biortech.2007.05.040

Moreno García, L., Adjallé, K., Barnabé, S., & Raghavan, V. (2017). Microalgae biomass production for a biorefinery system: Recent advances and the way towards sustainability. *Renewable and Sustainable Energy Reviews*, *76*, 493–506. https://doi.org/10.1016/j.rser.2017.03.024

Mubarak, M., Shaija, A., & Suchithra, T. V. (2015). A review on the extraction of lipid from microalgae for biodiesel production. *Algal Research*, *7*, 117–123. https://doi.org/10.1016/j.algal.2014.10.008

Muda, N., & Pin, T. J. (2012). On Prediction of Depreciation Time of Fossil Fuel in Malaysia. *Journal of Mathematics and Statistics*, *8*(1), 136–143. https://doi.org/10.3844/jmssp.2012.136.143

Muhd Nasrah, N. S., Mohd Zahari, M. A. K., Masngut, N., & Ariffin, H. (2017). Factorial experimental design for biobutanol production from oil palm frond (OPF) juice by *Clostridium acetobutylicum* ATCC 824. *Chemical Engineering Research Bulletin*, *19*, 36. https://doi.org/10.3329/cerb.v19i0.33774

Mujtaba, G., Rizwan, M., Kim, G., & Lee, K. (2018). Removal of nutrients and COD through co-culturing activated sludge and immobilized *Chlorella vulgaris*. *Chemical Engineering Journal*, *343*, 155–162. https://doi.org/https://doi.org/10.1016/j.cej.2018.03.007

Mujtaba, G., Rizwan, M., & Lee, K. (2017). Removal of nutrients and COD from wastewater using symbiotic co-culture of bacterium Pseudomonas putida and immobilized microalga *Chlorella vulgaris*. *Journal of Industrial and Engineering Chemistry*, *49*, 145–151. https://doi.org/http://dx.doi.org/10.1016/j.jiec.2017.01.021

Muto, M., Nojima, D., Yue, L., Kanehara, H., Naruse, H., Ujiro, A., Tanaka, T. (2017). Potential of water surface-floating microalgae for biodiesel production: Floating-biomass and lipid productivities. *Journal of Bioscience and Bioengineering*, *123*(3), 314–318. https://doi.org/10.1016/j.jbiosc.2016.09.015

Nadiah, W., Kadir, A., Kee, M., Uemura, Y., Wei, J., & Teong, K. (2018). Harvesting and pre-treatment of microalgae cultivated in wastewater for biodiesel production : A review. *Energy Conversion and Management*, *171*(March), 1416–1429. https://doi.org/10.1016/j.enconman.2018.06.074

Nandi, S., & Bhattacharyya, R. (2015). Production of biodiesel from *Jatropha Curcas* oil with recycling of enzyme. *International Journal on Applications in Civil and Enviornmental Engineering*, *1*(1), 1–5. https://doi.org/10.1016/j.compchemeng.2008.09.012

Nautiyal, P., Subramanian, K. A., & Dastidar, M. G. (2014). Kinetic and thermodynamic studies on biodiesel production from *Spirulina* platensis algae biomass using single stage extraction-transesterification process. *Fuel*, *135*, 228–234. https://doi.org/10.1016/j.fuel.2014.06.063

Neoh, C., Yahya, A., Adnan, R., Abdul Majid, Z., & Ibrahim, Z. (2012). Optimization of decolorization of palm oil mill effluent (POME) by growing cultures of

Aspergillus fumigatus using response surface methodology. *Environmental Science and Pollution Research International*, *20*(5), 2912–2923. https://doi.org/10.1007/s11356-012-1193-5

Nirmala, N., & S, D. S. (2020). Phylogenetic analysis for identification of lipid enriched microalgae and optimization of extraction conditions for biodiesel production using response surface methodology tool. *Biocatalysis and Agricultural Biotechnology*, *25*(April), 101603. https://doi.org/10.1016/j.bcab.2020.101603

Nussinovitch, A. (2010). Bead Formation, Strengthening, and Modification. In *Polymer Macro- and Micro-Gel Beads: Fundamentals and Applications*. https://doi.org/10.1007/978-1-4419-6618-6

Nwuche, C. O., Ekpo, D. C., Eze, C. N., Aoyagi, H., & Ogbonna, J. C. (2014). Use of Palm Oil Mill Effluent as Medium for Cultivation of *Chlorella sorokiniana*. *British Biotechnology Journal*, *4*(3), 305–316. https://doi.org/10.9734/bbj/2014/6921

Oh, T. H., Hasanuzzaman, M., Selvaraj, J., Teo, S. C., & Chua, S. C. (2018). Energy policy and alternative energy in Malaysia: Issues and challenges for sustainable growth – An update. *Renewable and Sustainable Energy Reviews*, *81*(June), 3021–3031. https://doi.org/10.1016/j.rser.2017.06.112

Onumaegbu, C., Alaswad, A., Rodriguez, C., & Olabi, A. (2019). Modelling and optimization of wet microalgae *Scenedesmus quadricauda* lipid extraction using microwave pre-treatment method and response surface methodology. *Renewable Energy*, *132*, 1323–1331. https://doi.org/10.1016/j.renene.2018.09.008

Othman, F. (2016). *Jurnal Teknologi*. *8*(August), 47–51. https://doi.org/10.11113/jt.v78.9285

Pahazri, N. F., Mohamed, R., Al-Gheethi, A., & Kassim, A. H. M. (2016). Production and harvesting of microalgae biomass from wastewater: a critical review. *Environmental Technology Reviews*, *5*(1), 39–56. https://doi.org/10.1080/21622515.2016.1207713

Parichehreh, R., Gheshlaghi, R., Akhavan, M., & Elkamel, A. (2019). Optimization of lipid production in *Chlorella vulgaris* for biodiesel production using fl ux balance analysis. *Biochemical Engineering Journal*, *141*(May 2018), 131–145. https://doi.org/10.1016/j.bej.2018.10.011

Perullini, M., Ferro, Y., Durrieu, C., Jobbágy, M., & Bilmes, S. A. (2014). Sol–gel silica platforms for microalgae-based optical biosensors. *Journal of Biotechnology*, *179*, 65–70. https://doi.org/https://doi.org/10.1016/j.jbiotec.2014.02.007

Pezzolesi, L., Samorì, C., & Pistocchi, R. (2015). Flocculation induced by homogeneous and heterogeneous acid treatments in *Desmodesmus* communis. *ALGAL*, *10*, 145–151. https://doi.org/10.1016/j.algal.2015.04.024

Phalakornkule, C., Mangmeemak, J., Intrachod, K., & Nuntakumjorn, B. (2010). Pretreatment of palm oil mill effluent by electrocoagulation and coagulation. *ScienceAsia*, *36*(2), 142–149. https://doi.org/10.2306/scienceasia1513-1874.2010.36.142

Phytotechlab. (2019). Phytotechlab. Retrieved from Phytotechlab website: https://phytotechlab.com/

Quah, E. (2019, March 4). QSR to turn oil waste into biofuel. *The Star Online*, p. 1.

Raheem, A., Prinsen, P., Vuppaladadiyam, A. K., Zhao, M., & Luque, R. (2018). A review on sustainable microalgae based biofuel and bioenergy production: Recent developments. *Journal of Cleaner Production*, *181*, 42–59. https://doi.org/10.1016/j.jclepro.2018.01.125

Rajanren, J. R., & Ismail, H. M. (2017). Investigation of *Chlorella vulgaris* microalgae as a source for renewable fuel. *Biofuels*, *8*(1), 37–47. https://doi.org/10.1080/17597269.2016.1200861

Rajkumar, R., & Sobri Takriff, M. (2016). Prospects of Algae and their Environmental Applications in Malaysia: A Case Study. *Journal of Bioremediation & Biodegradation*, *07*(01), 1–12. https://doi.org/10.4172/2155-6199.1000321

Rashid, N., Ur Rehman, M. S., Sadiq, M., Mahmood, T., & Han, J. I. (2014). Current status, issues and developments in microalgae derived biodiesel production. *Renewable and Sustainable Energy Reviews*, *40*, 760–778. https://doi.org/10.1016/j.rser.2014.07.104

Rashid, U., Anwar, F., Moser, B. R., & Knothe, G. (2008). Moringa oleifera oil: A possible source of biodiesel. *Bioresource Technology*, *99*(17), 8175–8179. https://doi.org/http://dx.doi.org/10.1016/j.biortech.2008.03.066

Rawat, I., Ranjith Kumar, R., Mutanda, T., & Bux, F. (2013). Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. *Applied Energy*, *103*, 444–467. https://doi.org/http://dx.doi.org/10.1016/j.apenergy.2012.10.004

Rees, D. A. (1981). Polysaccharide shapes and their interactions - some recent advances. *Pure and Applied Chemistry*, *53*(1), 1–14. https://doi.org/10.1351/pac198153010001

Robbins, M. P., Evans, G., Valentine, J., Donnison, I. S., & Allison, G. G. (2012). New opportunities for the exploitation of energy crops by thermochemical conversion in Northern Europe and the UK. *Progress in Energy and Combustion Science*, *38*(2), 138–155. https://doi.org/http://dx.doi.org/10.1016/j.pecs.2011.08.001

Robinson, P. K., Goulding, K. H., Mak, A. L., & Trevan, M. D. (1986). Factors affecting the growth characteristics of alginate–entrapped Chlorella. *Enzyme and Microbial Technology*, *8*, 729–733.

Sakarika, M., & Kornaros, M. (2019). *Chlorella vulgaris* as a green biofuel factory : Comparison between biodiesel , biogas and combustible biomass production. *Bioresource Technology*, *273*(November 2018), 237–243. https://doi.org/10.1016/j.biortech.2018.11.017

Sarmento, B., Ferreira, D., Veiga, F., & Ribeiro, A. (2006). Characterization of insulin-loaded alginate nanoparticles produced by ionotropic pre-gelation through DSC and FTIR studies. *Carbohydrate Polymer*, *66*(1), 1–7. https://doi.org/10.1016/j.carbpol.2006.02.008

Sartori, C. (1997). *The characterisation of alginate systems for biomedical applications* (Brunel University). https://doi.org/http://bura.brunel.ac.uk/handle/2438/7399

Seki, H., & Suzuki, A. (2002). Adsorption of heavy metal ions to floc-type biosorbents. *Journal of Colloid and Interface Science*, *249*(2), 295–300. https://doi.org/10.1006/jcis.2002.8297

Selesu, N. F. H., V. de Oliveira, T., Corrêa, D. O., Miyawaki, B., Mariano, A. B., Vargas, J. V. C., & Vieira, R. B. (2016). Maximum microalgae biomass harvesting via flocculation in large scale photobioreactor cultivation. *The Canadian Journal of Chemical Engineering*, *94*(2), 304–309. https://doi.org/10.1002/cjce.22391

Seyed Hosseini, N., Shang, H., & Scott, J. A. (2018). Optimization of microalgae-

sourced lipids production for biodiesel in a top-lit gas-lift bioreactor using response surface methodology. *Energy*, *146*, 47–56. https://doi.org/10.1016/j.energy.2017.08.085

Shamsuddin, N. H. A., Abdullah, R., Hamzah, Z., & Yusof, S. J. H. M. (2015). The improvement of screening the significant factors of oil blends as biolubricant base stock. *Malaysian Journal of Analytical Sciences*, *19*(1), 88–96.

Shen, Y., Yang, T., Zhu, W., & Zhao, Y. (2017). Wastewater treatment and biofuel production through attached culture of *Chlorella vulgaris* in a porous substratum biofilm reactor. *Journal of Applied Phycology*, *29*(2), 833–841. https://doi.org/10.1007/s10811-016-0981-6

Shen, Yu, Gao, J., & Li, L. (2017). Municipal wastewater treatment via co-immobilized microalgal-bacterial symbiosis: Microorganism growth and nutrients removal. *Bioresource Technology*, *243*, 905–913. https://doi.org/https://doi.org/10.1016/j.biortech.2017.07.041

Singh, G., & Patidar, S. K. (2018). Microalgae harvesting techniques: A review. *Journal of Environmental Management*, *217*, 499–508. https://doi.org/10.1016/j.jenvman.2018.04.010

Singh, Y. (2003). Photosynthetic activity, and lipid and hydrocarbon production by alginate-immobilized cells of *Botryococcus* in relation to growth phase. *Journal of Microbiology and Biotechnology*, *13*(5), 687–691.

Skorupskaite, V., Makareviciene, V., & Levisauskas, D. (2015). Optimization of mixotrophic cultivation of microalgae *Chlorella* sp. for biofuel production using response surface methodology. *Algal Research*, *7*, 45–50. https://doi.org/10.1016/j.algal.2014.12.001

Smidsrød, O., & Skjåk-Bræk, G. (1990). Alginate as immobilization matrix for cells. *Tibtech*, *8*(4), 71–78. https://doi.org/10.1016/0167-7799(90)90139-O

Song, W., Rashid, N., Choi, W., & Lee, K. (2011). Biohydrogen production by immobilized *Chlorella* sp . using cycles of oxygenic photosynthesis and anaerobiosis. *Bioresource Technology*, *102*(18), 8676–8681. https://doi.org/10.1016/j.biortech.2011.02.082

Srinuanpan, S., Cheirsilp, B., Boonsawang, P., & Prasertsan, P. (2019). Immobilized oleaginous microalgae as effective two-phase purify unit for biogas and anaerobic

digester effluent coupling with lipid production. *Bioresource Technology*, *281*(February), 149–157. https://doi.org/10.1016/j.biortech.2019.02.085

Srinuanpan, S., Cheirsilp, B., Prasertsan, P., & Kato, Y. (2018). Strategies to increase the potential use of oleaginous microalgae as biodiesel feedstocks : Nutrient starvations and cost-effective harvesting process. *Renewable Energy*, *122*, 507–516. https://doi.org/10.1016/j.renene.2018.01.121

Suastes-Rivas, J. K., Hernández-Altamirano, R., Mena-Cervantes, V. Y., Barrios Gómez, E. J., & Chairez, I. (2020). Biodiesel production, through intensification and profitable distribution of fatty acid methyl esters by a microalgae-yeast co-culture, isolated from wastewater as a function of the nutrients' composition of the culture media. *Fuel*, *280*(June), 118633. https://doi.org/10.1016/j.fuel.2020.118633

Suganya, T., & Renganathan, S. (2012). Optimization and kinetic studies on algal oil extraction from marine macroalgae Ulva lactuca. *Bioresource Technology*, *107*, 319–326. https://doi.org/10.1016/j.biortech.2011.12.045

Sugawara, S., Imai, T., & Otagiri, M. (1994). The controlled release of prednisolone using alginate gel. *Pharmaceutical Research*, *11*(2), 272–277. https://doi.org/10.1023/A:1018963626248

Sukumaran, P., Nulit, R., Zulkifly, S., Halimoon, N., Omar, H., & Ismail, A. (2014). Potential of fresh POME as a growth medium in mass production of Arthrospira platensis. *International Journal of Current Microbiology and Applied Sciences*, *3*, 235–250.

Talukder, M. M. R., Das, P., & Wu, J. C. (2014). Immobilization of microalgae on exogenous fungal mycelium: A promising separation method to harvest both marine and freshwater microalgae. *Biochemical Engineering Journal*, *91*, 53–57. https://doi.org/10.1016/j.bej.2014.07.001

Tan, C., Nik Meriam, N. S., Loh, S. K., & Phang, S. (2016). *Chlorella* Biomass Production in Annular Photobioreactor Using Palm Oil Mill Effluent (Pome): Effect of Hydrodynamics and Mass Transfer , Irradiance , Aeration Rate and Pome. *Journal of Oil Palm Research*, *28*(4), 496–509.

Taparia, T., Mvss, M., Mehrotra, R., Shukla, P., & Mehrotra, S. (2015). Developments and challenges in biodiesel production from microalgae : A review. *Biotechnology and Applied Biochemistry*, *63*(5), 715–726. https://doi.org/10.1002/bab.1412

Thani, M. I., Hussin, R., Wan Ibrahim, W. R., & Sulaiman, M. S. (1999). *Industrial Processes and the Environment (Handbook No.3): Crude Palm Oil Industry.* Kuala Lumpur: Department of Environment.

Thanos, C. G., Bintz, B. E., & Emerich, D. F. (2007). Stability of alginate–polyornithine microcapsules is profoundly dependent on the site of transplantation. *Journal of Biomedical Materials Research Part A*, *81*(1), 1–11.

Tijana, R., Irena, K.-V., Marko, J., Biljana, J.-S., & Darko, I. (2014). Comparison of full factorial design, central composite design, and box-behnken design in chromatographic method development for the determination of fluconazole and its impurities. *Analytical Letters*, *47*, 1334–1347. https://doi.org/10.1080/00032719.2013.867503

Topallar, H., & Geçgel, Ü. (2000). Kinetics and thermodynamics of oil extraction from sunflower seeds in the presence of aqueous acidic hexane solutions. *Turkish Journal of Chemistry*, *24*(3), 247–253.

Tran, N. H., Bartlett, J. R., Kannangara, G. S. K., Miller, S. A., Volk, H., & Wilson, M. A. (2010). Catalytic upgrading of biorefinery oil from micro-algae. *Fuel*, *89*, 265–274.

Travieso, L., Benitez, F., Weiland, P., Sánchez, E., Dupeyrón, R., & Dominguez, A. R. (1996). Experiments on immobilization of microalgae for nutrient removal in wastewater treatments. *Bioresource Technology*, *55*(3), 181–186. https://doi.org/https://doi.org/10.1016/0960-8524(95)00196-4

Vasilieva, S. G., Lobakova, E. S., Lukyanov, A. A., & Solovchenko, A. E. (2016). Immobilized microalgae in biotechnology. *Moscow University Biological Sciences Bulletin*, *71*(3), 170–176. https://doi.org/10.3103/S0096392516030135

Vishnu Priya, M., Ramesh, K., Sivakumar, P., Balasubramanian, R., & Anirbid, S. (2019). Kinetic and thermodynamic studies on the extraction of bio oil from *Chlorella vulgaris* and the subsequent biodiesel production. *Chemical Engineering Communications*, *206*(3), 409–418. https://doi.org/10.1080/00986445.2018.1494582

Wan, C., Alam, M. A., Zhao, X. Q., Zhang, X. Y., Guo, S. L., Ho, S. H., Bai, F. W. (2015). Current progress and future prospect of microalgal biomass harvest using various flocculation technologies. *Bioresource Technology*, *184*, 251–257. https://doi.org/10.1016/j.biortech.2014.11.081

Wang, S., Zhu, J., Dai, L., Zhao, X., Liu, D., & Du, W. (2016). A novel process on lipid extraction from microalgae for biodiesel production. *Energy*, *115*, 963–968. https://doi.org/10.1016/j.energy.2016.09.078

Wang, W., Liu, X., Xie, Y., Zhang, H., Yu, W., Xiong, Y., Ma, X. (2006). Microencapsulation using natural polysaccharides for drug delivery and cell implantation. *Journal of Materials Chemistry*, *16*(32), 3252. https://doi.org/10.1039/b603595g

Weschler, M. K., Barr, W. J., Harper, W. F., & Landis, A. E. (2014). Process energy comparison for the production and harvesting of algal biomass as a biofuel feedstock. *Bioresource Technology*, *153*, 108–115. https://doi.org/10.1016/j.biortech.2013.11.008

Wu, J., Alam, M. A., Pan, Y., Huang, D., Wang, Z., & Wang, T. (2017). Enhanced extraction of lipids from microalgae with eco-friendly mixture of methanol and ethyl acetate for biodiesel production. *Journal of the Taiwan Institute of Chemical Engineers*, *71*, 323–329. https://doi.org/10.1016/j.jtice.2016.12.039

Wu, T. Y., Mohammad, A. W., Md. Jahim, J., & Anuar, N. (2010). Pollution control technologies for the treatment of palm oil mill effluent (POME) through end-of-pipe processes. *Journal of Environmental Management*, *91*(7), 1467–1490.

Yan, W., Loke, P., Ching, J., Chang, J., & Chuan, T. (2018). Microalgae cultivation in palm oil mill effluent (POME) for lipid production and pollutants removal. *Energy Conversion and Management*, *174*(June), 430–438. https://doi.org/10.1016/j.enconman.2018.08.057

Yang, Y. H., Klinthong, W., & Tan, C. S. (2015). Optimization of continuous lipid extraction from *Chlorella vulgaris* by CO_2-expanded methanol for biodiesel production. *Bioresource Technology*, *198*, 550–556. https://doi.org/10.1016/j.biortech.2015.09.076

Ye, Y., Huang, Y., Xia, A., Fu, Q., Liao, Q., Zeng, W., & Zheng, Y. (2018). Optimizing culture conditions for heterotrophic-assisted photoautotrophic biofilm growth of *Chlorella vulgaris* to simultaneously improve microalgae biomass and lipid productivity. *Bioresource Technology*, *270*(August), 80–87. https://doi.org/10.1016/j.biortech.2018.08.116

Ye, Y., Huang, Y., Xia, A., Fu, Q., Liao, Q., Zeng, W., Zhu, X. (2018). Optimizing culture conditions for heterotrophic-assisted photoautotrophic biofilm growth of *Chlorella vulgaris* to simultaneously improve microalgae biomass and lipid

productivity. *Bioresource Technology*, *270*, 80–87. https://doi.org/10.1016/J.BIORTECH.2018.08.116

Zeng, X., Danquah, M. K., Halim, R., Yang, S., Chen, X. D., & Lu, Y. (2013). Comparative physicochemical analysis of suspended and immobilized cultivation of *Chlorella* sp. *Journal of Chemical Technology and Biotechnology*, *88*(2), 247–254. https://doi.org/10.1002/jctb.3821

Zhuang, L. L., Yu, D., Zhang, J., Liu, F. fei, Wu, Y. H., Zhang, T. Y., Hu, H. Y. (2018). The characteristics and influencing factors of the attached microalgae cultivation: A review. *Renewable and Sustainable Energy Reviews*, *94*(June), 1110–1119. https://doi.org/10.1016/j.rser.2018.06.006