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OPTIMIZATION OF MIXOTROPHIC MARINE MICROALGA Tetraselmis sp. FTC 208 CULTIVATION IN STIRRED TANK PHOTOBIOREACTOR

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By

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MAY 2014

Chairman : Professor Arbakariya Ariff, PhD

Faculty : Biotechnology and Biomolecular Sciences

Microalgal lipid is gaining a reputation as the third generation renewable biofuel. Harnessing of efficient strains that can produce considerable neutral lipid in open systems are the interest of many researchers. Major hindrance lies in low biomass yield of photoautotrophic farming. The possibility to increase biomass and lipid productivity of local microalga isolate, *Tetraselmis* sp. FTC208, was assessed under photoautotrophic, mixotrophic and heterotrophic cultivation conditions. Four non-linear growth models (logistic, logistic with lag, modified Gompertz and Baranyi-Roberts) were assessed for the predictive ability of microalgal culture performance under different trophic conditions, followed by medium optimization using statistical methods (response surface methodology (RSM) and artificial neural network (ANN)) to enrich the nutrients of W-30 medium. The effect of hydrodynamic due to agitation on *Tetraselmis* growth was initially investigated in a 2 L stirred tank photobioreactor (ST-PBR) fitted with dual Rushton turbines. The prospect of improving the existing agitation system in 10 L ST-PBR was then explored by conducting a non-biological, stirred tank - specific preliminary mixing performance modelling to design the triple-impeller system for handling shear sensitive cells. The potential impellers tested include Rushton turbine (RT), Bakker's turbine (BT-6), A315 hydrofoil (HYD), and Narcissus turbine (NS). Attempt on up-scaling the cultivation of *Tetraselmis* sp. to 10 L ST-PBR was then made by taking into consideration the preferred hydrodynamics for microalga growth and mixing performance in 2 L ST-PBR.

Through 16S rDNA and phylogenetic analysis, *Tetraselmis* sp. FTC208 has the closest homology to *Tetraselmis striata*. Baranyi-Roberts model was statistically accepted for the estimation of microalga growth kinetics under different culture conditions. Walne's medium was redesigned with 'biomass capacity' (ϕ) of at least 100% fixed for all micronutrient elements, while a sequential increased of 10% were imposed on deficient macronutrients. New medium (W-30) achieved X_{max} of 1.505 g dcw/L and P_{max} of 376 mg lipid/L (~ 25% *w/w*), representing 1.8

and 3.7-fold improvements relative to the original Walne's formulation. Cultivation in mixotrophic (with light) gave better biomass and lipid production over heterotrophic (without light) conditions. W-30 medium supplemented with 30 g/L glucose boosted microalga growth to 8.08 g dcw/L when compared to the strictly photoautotrophic run, which gave biomass and lipid productivity of 404 mg dcw/L·day and 90.9 mg lipid/L·day, respectively. A quadratic model of RSM and an ANN network with 10 hidden neurons produced comparable results, albeit ANN formulation was observed to give higher output response. Optimized formulation utilizing W-30 as basal medium was composed of added glucose (24.05 g/L), $NaNO_3$ (4.70 g/L) and yeast extract (0.93 g/L) eventually produced X_{max} of 12.38 g dcw/L, lipid yield per cell, P_{max}/X_{max} of 195.77 mg lipid/g dcw and productivity, Pr_{lipid} of 173.11 mg lipid/L·day.

For cultivation in 2 L ST-PBR, the steady state biomass concentration rose with increased agitation relative to the "just suspended" speed (N_{js}) of 150 rpm (tip speed 0.409 s^{-1}) at gas velocity (U_g) fixed at 0.5 VVM. The highest X_{max} (16.88 g dcw/L) and Pr_{cell} (928.95 mg dcw/L·day), in conjunction with lipid yield (227.10 mg lipid/g dcw) and productivity (213 mg lipid/L·day) were observed at 250 rpm (tip speed 0.681 s^{-1}), which corresponded to P_g/V and k_La of 190.93 W/m^3 and 0.0118 s^{-1}, respectively. Based on the cultivation data of 2 L ST-PBR, the impeller tip speed (πND) at 0.681 m/s and k_La at 0.0118 s^{-1} were adapted in 10 L ST-PBR equipped with three Rushton turbines (RT) as well as another photobioreactor unit retrofitted with NS-BT6-NS. This triple impeller configuration was believed to impose moderate shear effect without risking the oxygen transfer rate to the medium. Scale-up criterion employing constant k_La in NS-BT6-NS system was found to exert better influence on microalgal growth over strategy that was based on equal tip speed. Highest X_{max} was obtained at 18.07 g dcw/L with 22.6% *w/w* of lipid bodies.

The fatty acid profile of lipid derived from the 10 L ST-PBR mixotrophic culture exhibited C16:0 (18.5%), C18:1n9c (15.7%), C18:0 (9.3%) and C18:3n6 (6.3%) in addition to an uncommon monounsaturated C12:1 (15.1%). *Tetraselmis* bio-oil merits a further investigation as feedstock for producing biodiesel since its saponification value (SV = 148.48), iodine value (IV = 104.38) and cetane number (CN = 59.57) all adhered to the range set by the established biofuel standards.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PENGOPTIMUMAN PEMBIAKAN MIKROALGA *Tetraselmis* sp. FTC 208 SECARA MIKSOTROFIK DI DALAM FOTOBIOREAKTOR TANGKI TERADUK

Oleh

MOHD SHAMZI MOHAMED

MEI 2014

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Lipid dari mikroalga semakin mencapai reputasi sebagai "bahanapi boleh diperbaharui" generasi ketiga. Sehingga kini, pemanfaatan strain yang cekap dalam menghasilkan sejumlah besar lipid neutral di dalam sistem terbuka menjadi tumpuan ramai penyelidik. Halangan utama biasanya terletak pada pengeluaran biojisim yang rendah hasil dari pertanian secara fotoautotrofik. Kebarangkalian peningkatan pengeluaran biojisim dan lipid dari mikroalga tempatan yang dipencilkan, *Tetraselmis* sp. FTC208, dinilai di bawah keadaan fotoautotrofik, miksotrofik dan heterotrofik. Empat model pertumbuhan bukan linear (logistik, logistik dengan masa tertunda, Gompertz diubahsuai dan Baranyi-Roberts) juga dinilai dari segi kebolehramalan prestasi kultur mikroalga pada keadaan trofik berlainan. Ini disusuli dengan pengoptimuman media menggunakan kaedah statistik (kaedah sambutan permukaan (RSM) dan kaedah jaringan neural buatan (ANN)) untuk memperkayakan lagi nutrien dalam media W-30. Kesan hidrodinamik pergolakan ke atas pertumbuhan *Tetraselmis* pada awalnya disiasat menggunakan fotobioreaktor tangki teraduk (ST-PBR) 2 L yang dipasangkan dua turbin Rushton. Prospek penambahbaikan sistem pengaduk sedia ada pada ST-PBR berskala pepandu 10 L kemudian diterokai dengan menjalankan pemodelan prestasi percampuran awalan dalam tangki teraduk tanpa mengambilkira unsur biologi. Ini untuk tujuan merekabentuk sistem tiga - pengaduk terbaik untuk sel yang sensitif pada ricihan. Pengaduk berpotensi termasuk turbin Rushton (RT), turbin Bakker (BT-6), hidrofoil A315 (HYD), and turbin Narcissus (NS). Cubaan peningkatan skala pengeluaran *Tetraselmis* sp. kepada 10 L ST-PBR dilaksana setelah mengambilkira keadaan hidrodinamik yang menggalak pertumbuhan mikroalga di dalam sistem ST-PBR 2 L.

Melalui analisa 16S rDNA dan filogeni, *Tetraselmis* sp. FTC 208 dikenalpasti mempunyai pertalian homologi terhampir dengan *Tetraselmis striata.* Model

Baranyi-Roberts diterimapakai secara statistik untuk penganggaran kinetik pertumbuhan mikroalga di bawah keadaan trofik berlainan. Media Walne direka semula dengan 'kapasiti biojisim' (ϕ) ditetapkan sekurang-kurangnya 100% untuk semua unsur mikronutrien, manakala makronutrien yang dianggap kurang dinaikkan pada kadar 10% secara berperingkat. Media baru (W-30) mencapai biojisim maksimum (X_{max}) sebanyak 1.505 g dcw/L dan lipid maksimum (P_{max}) sebanyak 376 mg lipid/L (~ 25% *w/w*), ini mewakili peningkatan 1.8 dan 3.7 kali ganda berbanding formulasi asal Walne. Pembiakan secara miksotrofik (bercahaya) didapati menghasilkan pengeluaran biojisim dan lipid yang lebih baik berbanding heterotrofik (tanpa cahaya). Media W-30 yang ditambah 30 g/L glukos pula merangsang pertumbuhan mikroalga sehingga 8.08 g dcw/L berbanding pertumbuhan secara fototropik mutlak, yang mana pengeluaran biojisim dan lipid dicapai pada 404 mg dcw/L·hari and 90.9 mg lipid/L·hari.Satu persamaan kuadratik RSM dan juga jaringan ANN dengan 10 neuron tersembunyi didapati memberikan keputusan setanding. Walau bagaimana pun, formulasi ANN memberikan output sambutan yang lebih tinggi. Formula optimum di mana W-30 adalah media basal dengan tambahan glukos (24.05 g/L), $NaNO_3$ (4.70 g/L) dan ekstrak yis (0.93 g/L) akhirnya menghasilkan X_{max} pada 12.38 g dcw/L, penghasilan lipid per sel, P_{max}/X_{max} sebanyak 195.77 mg/g dcw dan produktiviti lipid, Pr_{lipid} sejumlah 173.11 mg/L·hari.

Untuk pembiakan di dalam ST-PBR berisipadu 2 L, kepekatan biojisim pada keadaan mantap meningkat dengan bertambahnya kadar adukan relatif kepada kelajuan "hanya terampai" (N_{js}) iaitu 150 rpm (laju hujung bilah 0.409 s^{-1}) dengan halaju gas (U_g) ditetapkan pada 0.5 VVM. X_{max} tertinggi (16.88 g dcw/L) dan Pr_{cell} (928.95 mg dcw/L·hari) seiringan dengan hasil lipid (227.10 mg lipid/g dcw) dan produktiviti lipid (213 mg lipid/L·hari) diperolehi pada 250 rpm (laju hujung bilah 0.681 s^{-1}). Kelajuan ini menyamai input kuasa, P_g/V dan k_La masing-masing pada 190.93 W/m^3 dan 0.0118 s^{-1}. Berdasarkan data pembiakan ST-PBR berisipadu 2 L, kelajuan hujung bilah (πND) pada 0.681 m/s dan k_La pada 0.0118 s^{-1} telah diadaptasi untuk ST-PBR berskala pepandu 10 L yang dilengkapi tiga pengaduk Rushton (RT) beserta satu lagi unit fotobioreaktor yang dipasang NS-BT6-NS. Konfigurasi ini dianggap menghasilkan kesan ricihan sederhana tanpa memberikan risiko kepada kadar pemindahan oksigen ke media. Kriteria peningkatan skala berdasarkan penetapan k_La bersama sistem NS-BT6-NS didapati lebih mempengaruhi pertumbuhan mikroalga berbanding strategi yang menyamakan kelajuan hujung bilah. X_{max} tertinggi diperolehi pada 18.07 g dcw/L dengan kandungan jasad lipid sebanyak 22.6% *w/w*.

Profil asid lemak dari lipid yang terhasil dari kultur ST-PBR berisipadu 10 L secara miksotrofi menunjukkan kehadiran C16:0 (18.5%), C18:1n9c (15.7%), C18:0 (9.3%) dan C18:3n6 (6.3%) di samping kewujudan asid lemak mono-tak-tepu yang jarang dikesan, C12:1 (15.1%). Minyak-bio *Tetraselmis* wajar diselidiki dengan lebih terperinci sebagai bahan suapan untuk biodiesel berdasarkan nilai saponifikasi (SV = 148.48), nilai iodin (IV = 104.38) dan nombor setana (CN = 59.57) kesemuanya mematuhi julat piawaian-piawaian bahanapi-bio sedia ada.

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"We don't grow when things are easy; we grow when we face Challenges"

I certify that a Thesis Examination Committee has met on 19th May 2014 to conduct the final examination of Mohd Shamzi bin Mohamed on his thesis entitled "Optimization of Mixotrophic Marine Microalga *Tetraselmis* sp. FTC 208 Cultivation in Stirred Tank Photobioreactor" in accordance with the Universities and University Colleges Act 1971 and the constitution of the Universiti Putra Malaysia [P.U. (A) 106] March 15, 1998. The committee recommends that the candidate be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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