INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title:
Production of biodiesel and exopolysaccharides from *Scenedesmus abundans* cell factory in flat panel photobioreactor with autoflocculation harvesting strategy

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SHORT ABSTRACT

Fatty acid methyl ester (FAME, biodiesel) production from microalgae has been gaining momentum in order to mitigate current energy demand but still facing major challenges such as low biomass production in open pond cultivation systems, unsuccessful closed photobioreactor (PBR) design, inappropriate media balancing under natural sunlight, inefficient harvesting technology and unsuitable transesterification process. Moreover, in order to make biodiesel economically viable, there is a need to exploit other products concomitantly apart from biodiesel and reduce downstream processing costs. Exopolysaccharides (EPS) are one of the major valuable byproducts secreted by some microalgae directly into the media. Concomitant synthesis of intracellular and extracellular product fractions in microbial cell factories would be an excellent biorefinery outlook for the researchers to reduce overall cost of their production and yield profits. Major factors that influence oleaginous microalgae growth and lipid production under natural sunlight are photobioreactor design, adequate supply of macronutrients and micronutrients, availability of sunlight intensity, CO_2 supply, nitrogen starvation, mixing and mass transfer, process pH and culture temperature. Therefore, the algal biorefinery approach should focus on PBR design, media balancing for enrichment of multi-products, efficient harvesting technology for biomass recovery and desirable transesterification process for producing biodiesel. Arising from the above, the objectives of the study were formulated to develop process strategy for construction and characterization of medium scale flat panel PBR for producing biodiesel and exopolysaccharides from *S. abundans*. Accordingly, modulation of macronutrients and micronutrients were studied to enhance lipid content of *S. abundans* in single stage medium scale flat panel PBR under natural sunlight. Also, multi-objective optimization of nutrients was performed in this study to enhance biodiesel, autosedimentation of *Scenedesmus abundans* and exopolysaccharides production in single stage medium scale flat panel PBR under diurnal natural sunlight. Finally, free lipase and immobilized lipase mediated direct transesterification optimized strategy was developed to enhance biodiesel from microalgal biomass in presence of methanol. Parallel mini flat panel PBR and medium scale flat panel PBR were constructed for the cultivation of *S. abundans*. Customized unidirectional LED lighting system was developed to supply light energy to microalgae inside the laboratory. A high mass transfer efficient membrane sparger was designed and equipped at the bottom of flat panel PBR. The medium scale flat panel PBR produced overall mass transfer coefficient of CO_2 (K_{La,CO_2}) and mixing time (t_m) of 0.0125 s^{-1} and 8 sec respectively at 0.43 cm/s superficial gas velocity. Maximum biomass titer of 6.9 g/l was achieved at end of growth phase using optimized growth media in medium scale flat panel PBR at 2162 μE/m^2/s, 1 VVM indoor condition. The productions were 1.53 g/l (22% of DCW) FAME with productivity of 67 mg/l/ day and 236 mg/l EPS with yield of 37 mg/g biomass under nitrogen starvation. This microalgal strain have potential to produce multi-products (biodiesel, EPS) and also has the capability of natural autoflocculation.

Further to improve lipid production of *S. abundans*, the effect of macro and micronutrients were investigated. The optimized lipid media was formulated for enhancing lipid content in microalgae. Comparison of single stage and two stage strategies were also carried out in this study. The single stage produced 2.5 fold higher %FAME in DCW than two stage and economical as harvesting steps could be minimized. Biomass titer of 2.79 g/l with maximum FAME content of 46% was achieved using developed media in medium scale flat panel PBR under diurnal natural sunlight. FAME concentration, FAME productivity and maximum EPS concentration of 1.28 g/l, 27 mg/l/day and 155 mg/l were obtained under sunlight study. Therefore, the exploitation of *S. abundans* as a co-producer of biodiesel and EPS under outdoor sunlight could be a feasible approach. In addition to that, multi-objective optimization approach has been developed to increase the production of biodiesel, secretion of EPS and enhance autoflocculation (ASF) using *S. abundans* under diurnal light. For this study, Plackett-Burman design (PBD) was used for screening significant process variables. Diurnal simulated light intensity was generated from pulse width modulation (PWM) and OPTO22 control system that controls LED (light emitting diode) light intensity ranging from 0 to 2200 μE/m^2/s (capable of mimicking sunlight intensities in laboratory). PBD determines urea, $CoCl_2.6H_2O$, $CuSO_4.5H_2O$ and $Na_2MoO_4.2H_2O$ as significant process variables in modified Chu-13 media that influences FAME content in dry biomass, FAME concentration, ASF and EPS concentration by *S. abundans*. The quadratic model equations of responses from RSM-CCD (response surface methodology-central composite design) enhance single objective at a time. Therefore, these model equations from RSM-CCD are used with composite desirability approach for formulating optimized multi-objective RSM media. The developed media increased the FAME production by 1.72 fold (70% in DCW), FAME titer by 2.3 fold (3.83 g/l), ASF by 1.08 fold (1.93) and EPS by 1.60 fold (462.50 mg/l) as compared to unoptimized media under diurnal LED light. The formulated media produced 55% of FAME content in dry biomass, 1.79 g/l FAME, ASF of 1.83 within 1 hr, 290 mg/l EPS under natural sunlight. For this study, immobilized lipase mediated direct transesterification process was developed. Methanol was used as lipid extraction solvent, acyl acceptor and reaction medium. The optimized conditions for free lipase were 16:1 methanol to biomass mass ratio, lipase amount with respect to biomass (80%) with 50 mg/ml lipase concentration, 30 ºC reaction temperature (room temperature) and works optimum without initial pH adjustments. Lipase loading of 10 mg/alginate bead (62.5 mg/ml enzyme concentration) produced highest FAME content in DCW (44%) among different enzyme loadings using calcium alginate entrapment method. Kinetic study reveals that transesterification efficiency increases with increase in reaction time for both optimized free lipase and immobilized lipase. Water behaves as a better storage medium as compared to buffers for immobilized lipase in reusability experiments. Lipase loading of 20 mg/alginate bead (125 mg/ml enzyme concentration) showed better reusability up to 5th cycle. As far as our knowledge is concerned, this is the first study of multi-objective optimization of nutrients to enhance FAME, autoflocculation and EPS concomitantly in *Scenedesmus abundans*. Also, very few studies have reported immobilized lipase mediated direct transesterification of algal biomass in presence of methanol to produce biodiesel. Hence, enhancement of biodiesel and EPS under sunlight without photoinhibition by natural autoflocculation in medium scale flat panel PBR paved way for biorefinery. Therefore, generation of multi-products by *S. abundans*, natural autoflocculation based biomass harvesting and lipase immobilization by entrapment method can make the process sustainable.