INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: **Microalgal Bio-refinery: Process optimization and intensification for production of lipids and other value-added products**

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SHORT ABSTRACT

A potential and promising green liquid fuel is biodiesel, which can be blended with petroleum diesel. The new and economic alternate feedstocks for biodiesel include microalgal lipids. Simultaneously, microalgae are promising producers of various other biofuels, such as bioethanol, glycerol and biomaterials. This thesis has assessed the potential of a natural freshwater microalgal strain for bio-refinery that produces biodiesel, bioethanol, β-carotene and glycerol. A native freshwater microalgae was isolated and was named *Tetradesmus obliquus* SGM19. The preliminary optimization of the growth conditions was done. The strain exhibited high lipid (28%), carbohydrate (24%) and protein (42%) concentrations, and was also rich in pigments. The growth cycle of *T. obliquus* SGM19 was further optimized using statistical experimental design for simultaneous production of lipid and β-carotene. Application of 33 kHz and 1.4 bar ultrasound at 10% duty cycle was revealed to enhance the lipid and β-carotene yields by 34.5% and 31.5%, respectively. Kinetic analysis of substrate and product profiles in control and test experiments revealed both lipid and β-carotene to be growth-associated products. Consistently higher NAD(H) concentrations were observed for sonicated samples; indicating faster metabolism. The viability of ultrasound-exposed microalgal cells (assessed with flow cytometry) was >80%. Further, the lipid in *T. obliquus* SGM19 biomass was subjected to transesterification for producing biodiesel. The biodiesel synthesis process was optimized in two steps, viz. (1) optimization of the conditions/pretreatment of biomass, and (2) statistical optimization of transesterification process parameters. *In-situ* base-catalyzed transesterification of the lyophilized biomass had the highest biodiesel yield of 37.5% (w/w DCW). Next, statistical optimization of transesterification parameters (catalyst loading, methanol to biomass ratio,

temperature and reaction time) was done. Finally, intensification of transesterification was attempted by replacing mechanical agitation of reaction mixture with sonication. Sonication reduced the overall activation energy of transesterification process from 24.66 to 19.82 kJ/mol. Furthermore, the FAME profile for ultrasound-assisted transesterification process was characterized with GC-MS. Major fatty acids present in the lipids were palmitic, heptadecanoic, linoleic, linolenic and arachidic acids. The biodiesel properties were found to be as per ASTM D6751 standards. Finally, the *T. obliquus* SGM19 biomass was subjected to sequential treatment for extraction and synthesis of β-carotene, biodiesel, bioethanol and glycerol. It was observed that the *in-situ* transesterification resulted in higher yields of end-products in comparison to 2-step transesterification and were: 0.11 g β-carotene, 29 g biodiesel, 2.6 g glycerol and 12 g bioethanol. This thesis has thus presented a comprehensive study on lab-scale processes of cultivation and growth of a wild microalgal strain, extraction of lipids and nutraceutical (β-carotene), and further synthesis of green biofuels like biodiesel and bioethanol, and finally intensification of these processes with external stimulus of ultrasound.