INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: Sustainable way of producing biodiesel using oleaginous *Tetradesmus obliquus* KMC24

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Thesis Submitted to the Department/ Center	:	School of Energy Science and Engineering
Date of completion of Thesis Viva-Voce Exam	:	08-04-22
Key words for description of Thesis Work	:	Microalgae; Biodiesel; Biorefinery; Circular Bioeconomy; Catalyst

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SHORT ABSTRACT

Oleaginous microalgae are deemed as cell bio-factories for biodiesel production. However, the lower biomass and lipid productivity, along with the high cost of downstream processing such as harvesting, lipid extraction and conversion to biodiesel makes microalgae-based biodiesel production system economically unviable. To address this challenge, the present study aims to develop a sustainable biodiesel production system by employing various strategies such as selection of potential microalgal strain, modification of cultivation mode, optimization of culture conditions, development of low-cost harvesting and lipid to biodiesel conversion techniques. In the present study, a novel microalgae, *Tetradesmus obliquus* KMC24 was isolated and exposed to nutrient stress (nitrogen and/or phosphorus) for a short period via two-stage cultivation to obtain maximum biomass and lipid. The effect of nutrient starvation on the morphology, biomass concentration, photosynthetic activity, and biochemical composition of *Tetradesmus obliquus* KMC24 was investigated. Two days nitrogen-starved cells (-N2) were able to accumulate the maximum amount of lipid (39.93 ± 0.44%) without affecting the biomass

concentration (2.15 ± 0.04 g L^{-1}). During nitrogen (-N) and phosphorus (-P) starvation, photosynthetically fixed carbon pool was diverted to lipid biosynthesis. Pearson's correlation analysis suggested that stress-induced lipid accumulation is associated with an increased intracellular reactive oxygen species (ROS) level. The ROS fluorescence intensity was highest in –N2 cultures (17051.49 ± 93.15 a.u.), suggesting highly oxidative stress-tolerant cells. A high degree of fatty acid saturation was obtained under nitrogen starvation as compared to other culture conditions. Biodiesel properties such as cetane number, saponification number, and iodine value were improved under nitrogen starvation.

High-energy requirement for harvesting microalgal biomass poses a major challenge during downstream processing. Addressing this challenge, the present study developed a sustainable and efficient harvesting technique by valorizing waste eggshell. Herein, waste eggshell-derived bioflocculant was used for harvesting *T. obliquus* in a circular bioeconomy approach. It was found that 120 mg L^{-1} bioflocculant can flocculate 98.62 ± 0.43% of *T. obliquus* cells within 25 min at optimal pH 4.0 and temperature 35 °C. The influence of bioflocculant concentration, pH and temperature on zeta potential was evaluated to understand the flocculation mechanism. Microscopic and FESEM-EDX images were analyzed to evaluate the microalgal structural changes. Adsorption mechanism of bioflocculant over the microalgal cells was determined by performing adsorption kinetic studies. Pseudo-second order kinetic model was a suitable fit for the data obtained from the experiments, which indicated chemisorption as the probable mechanism. The spent medium recovered after harvesting process was successfully recycled for subsequent cultivation of *T. obliquus* KMC24, thus reducing the dependency on fresh medium. The FAME composition of the biomass treated with bioflocculant was not altered.

The last study was focused on developing and characterizing low-cost and eco-friendly catalyst for microalgal lipid transesterification to biodiesel. Herein, a novel carbon-based solid acid catalyst was synthesized by carbonization of de-oiled microalgal biomass followed by sulfonation. The effect of catalyst synthesis conditions such as carbonization temperature, sulfonation time, and H_2SO_4 concentration on the surface acidity of the catalyst and free fatty acid conversion was determined. The de-oiled microalgal biomass-based (DMB) solid acid catalyst was predominantly composed of carboxylic, phenolic, and sulfonic groups as indicated

by the FTIR analysis and supported by the XPS analysis. The catalyst was further characterized by various methods to determine its physiochemical properties. A maximum fatty acid methyl ester (FAME) yield of 94.23% for microalgal oil (AO) and 96.25% for waste cooking oil (WCO) was obtained under optimized transesterification conditions. The catalyst exhibited high catalytic activity (FAME yield >90%) until the fourth cycle. Most of the biodiesel properties were within the permissible limit of EN 14,212 and ASTM D6751 standards.