INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: Development of sustainable bioprocess for biodiesel production from novel freshwater microalga *Chlorella sorokiniana* FC6 IITG

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SHORT ABSTRACT

Exhaustive fuel reserves worldwide has renewed and invigorated interest towards alternate sources of fuel which would serve dual function of being renewable and sustainable to the environment. In purview of modern trends, microalgal cultivation has gained significant interest as a pioneer for the sustainable production of biodiesel attributed to its innate ability to accumulate substantially large amounts of neutral lipids as compared to oil plants. Current state of art makes it economically infeasible for commercialization which can be accounted to the several bottlenecks that persist during the upstream and downstream processes. Rationale for strain improvement as well as process strategy modification are being essential for development towards a more sustainable process as far as biodiesel production is concerned.

In the present study, indigenous microalgal strains were isolated and screened for maximum neutral lipid accumulation. The best strain with inherent ability to accumulate neutral lipid was further taken for detailed characterization and evaluation under different cultivation conditions such as photoautotrophic, heterotrophic and mixotrophic mode. Oil quality in terms of fatty acid compositions of the biomass obtained from different cultivation conditions were also evaluated by gas chromatography (GC). In order to achieve enhanced biomass and lipid productivity two process engineering strategies were developed: (i) one stage two phase high cell density mixotrophic fed-batch cultivation and (ii) synchronized growth and neutral lipid accumulation under mixotrophic growth in fed-batch and chemostat mode achieved via manipulation of substrates feeding mode and supplementation of lipid elicitors in the growth medium. Finally, electrochemical harvesting method was employed to pre-concentrate the microalgal culture broth as a sustainable alternatives to the existing methods and the biomass was converted to biodiesel via in situ transesterification.

The key findings were: A novel indigenous microalgal strain *Chlorella sorokiniana* FC6 IITG was isolated and identified (GENEBANK Accession no.: JX453208) to accumulate total lipid up to 16% (w/w, DCW) under un-

optimized growth conditions in shake flask condition. Characterization of the strain under different pH and temperature revealed the inherent robustness of the strain in terms of its growth in wide range of pH from 4 to 10 and at temperatures of range 20-44°C. The strain was also found to be capable of utilizing organic carbon sources under heterotrophic and mixotrophic growth conditions. Media optimization resulted in 19% improvement of biomass titer (0.69 g L-1) for the strain as compared with the un-optimized photoautotrophic growth conditions. Further evaluation of the strain in automated bioreactor under different trophic (photoautotrophic, heterotrophic and mixotrophic) modes showed significant variation in the biomass productivity (142.06 to 455.50 mg L-1 day-1) and total lipid productivity (47.20 to 111.85 mg L-1 day-1). Mixotrophic batch cultivation was found to be superior to photoautotrophic & heterotrophic mode in terms of biomass and lipid productivity of 455.5 mg L-1 day-1 and 111.85 mg L-1 day-1 respectively. A single-stage two phase fed-batch mixotrophic cultivation strategy resulted in higher biomass titer of 15.81 g L-1 with a lipid content of 54.95%, w/w DCW and significant improvement in biomass and lipid productivity (1.93 g L-1 day-1 and 550 mg L-1 day-1 respectively) from batch cultivation. Further, improvement in biomass and lipid productivity was achieved by process engineering strategy to achieve simultaneous growth and lipid accumulation by using lipid inducers (a combination of sodium chloride and sodium acetate). Synchronized growth and lipid accumulation under fed-batch & chemostat operation mode resulted in lipid productivity of 0.97 g L-1 day-1 and 1.27 g L-1 day-1 respectively. Maximum lipid productivity of 1.27 g L-1 day-1 which is comparable to the existing cultures reported in various literatures. Electrochemical harvesting showed 72% microalgae recovery efficiency at optimum operational parameter which could be a viable option for pre-concentration of microalgae. Fatty acid methyl ester (FAME) composition analysis reveals the majority of fatty acid was C16:0, C16:1, C18:0, C18:1 and C18:2 which depicts its suitability as biodiesel. Therefore, biodiesel obtained from *Chlorella sorokiniana* FC6 IITG grown under mixotrophic fed-batch or chemostat condition under the influence of inducer molecule can be used for commercial applications as it satisfies overall ASTM D6751-15a and EN 14,214 standards. The abovementioned qualitative and quantitative analysis further strengthens the claim of this novel indigenous strain as a potent cell factory for biodiesel production.