

**INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS**

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Thesis Title: Development of a cost effective process for biodiesel production through model guided high cell density cultivation of *Chlorella* sp. FC2 IITG
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SHORT ABSTRACT

The initial characterization of *Chlorella* sp. FC2 IITG strain showcased the robustness as it was able to grow in a wide range of pH from 4 to 10 with a temperature ranging between 20-44°C. Glucose and sodium acetate when used as sole carbon source and lipid elicitor respectively yielded enhanced biomass and lipid content. Optimization of media for specific growth rate resulted in a highest growth rate of 2.115 day⁻¹ whereas 7.81 g L⁻¹ cell density was achieved in case of maximization of biomass titer. Optimized supplementation of 24 g L⁻¹ sodium acetate resulted in maximum of ~66% w/w, DCW neutral lipid accumulation. The multi-nutrient mechanistic model developed on the basis of segregation of intracellular nutrients into structural form of nutrient (SFN), readily utilizable intracellular stored nutrients (RUN), non-readily utilizable intracellular nutrients (Non-RUN) and their sequential consumption during starvation. Hypothesis developed on the preferential and sequential utilization of extra cellular nutrients (ECN) under nutrient sufficient condition followed by RUN and Non-RUN under starvation was established by an *in-silico* model and experimentally validated. Further, a biphasic fed batch approach was developed wherein, firstly, a high cell density cultivation was obtained through model guided optimized feeding strategy and nutrient driven pH control; secondly, intracellular lipid enrichment was attained via addition of sodium acetate. This strategy resulted in biomass titer of 90.15 g L⁻¹ with biomass and lipid productivity 19.75 g L⁻¹ day⁻¹ and 7.7 g L⁻¹ day⁻¹ respectively. Subsequently, a two-step chemostat cultivation methodology was designed by connecting two bioreactors in series where first reactor was operated at model optimized condition for biomass generation whereas the second reactor was solely dedicated to lipid induction. The strategy yielded the biomass and lipid productivity of 92.7 g L⁻¹ day⁻¹ and 9.76 g L⁻¹ day⁻¹ respectively, which was significantly high amongst similarly reported literatures. Finally, a metabolic model was developed which showed an enhanced lipid accumulation in biomass with concomitant 6 fold increase in non-growth associated (NGA) maintenance energy to combat stress during nutrient starvation whereas higher lipid induction in biomass with no significant increase in NGA maintenance energy was observed in case of sodium acetate supplementation. Hence sodium acetate was found to be a better option for lipid induction towards cost effective bioprocess development for biodiesel production. The biodiesel obtained from microalga FC2 grown under heterotrophic condition can be used for commercial applications as it satisfied American and European standards.