INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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Thesis Title: Improved microbial lipid production from *Rhodococcus opacus* PD630: Molecular and systems biology approach

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SHORT ABSTRACT

Biofuels are a better source of alternative energy in the state of fossil fuel exhaustion and energy crisis. After long years of research, scientists have discovered several potential microbial hosts being able to synthesize biofuel intracellularly. *Rhodococcus opacus* PD630 (*R. opacus* PD630) is one of them, which has caught attention in the field of industrial biodiesel production. Moreover, this microbe is also well known to degrade complex substances into simpler forms that had been used into waste management.

The genes and their products used in biodiesel production are not well explored in *R. opacus* PD630. In the current study, *R. opacus* PD630 was used for simultaneous production of biodiesel and treatment of synthetic dairy wastewater (SDWW) with 80% of chemical oxygen demand (COD) removal as an outcome. Physiology of any organism is best known through its complete metabolism extended from its genome. Systems biology approach to see through its metabolic ability is a superior way to identify genomic problems when there is a lack of definite literature available. Taking into consideration, genomic scale metabolic model (GeM) is designed and constructed (iROPD630) to locate the functionality of genes involved in the complex blueprint, which eventually gives rise to designated phenotypes.

Biodiesel consists primarily of Fatty acid methyl esters (FAMEs) or Fatty acid ethyl Esters (FAEEs), which occurs by transesterification reaction between methanol/ethanol and free fatty-acids in the presence of sulphuric acid or sodium hydroxide acting as catalyst. Methanol is a toxic substance and the handling alone makes it a little challenging apart from producing it in large volumes. Thus, ethanol is largely considered over methanol for biodiesel production. Though, ethanol has been achieved to be conveniently produced from microbes in a large volume, it adds an extra economical, time and manual burden in the biodiesel production. To have an alternative to this issue, we constructed a superior strain of *R. opacus* PD630 with an ability to synthesize FAEE intracellularly without an external supply of ethanol. The cultivations led a FAEEs of 28.84% and 31.28% of the dry cell weight (DCW) in lactose and glucose respectively. The experimental data sets obtained from lactose were used to simulate the *i*ROPD630MD (obtained from *i*ROPD630) GeM for better understanding its physiology through metabolic flux analysis. With ethanol as the objective function, the model with carbon-constraint flux variability analysis correctly predicted the ethanol yield. This makes the GeM all the more viable and can be used to predict targets for enhancing ethanol or biodiesel yield.

All these collective approaches on exploring biofuels for the betterment of mankind shall further ease the dependence on fossil fuels and pave a new way for the rise of biofuels.