

# METABOLICALLY ENGINEERED *E. coli* FOR THE PRODUCTION OF BIOFUEL:AN *IN SILICO* APPROACH

[Project sponsored by Karnataka State Council for Science and Technology, Bangalore under 37<sup>th</sup> Student Project Programme]

*Project Report Submitted by*

**Akshaya Yathish Poonja**  
(4NM10BT002)

**JyothsnaPais**  
(4NM10BT014)

**Karthik D Hegde**  
(4NM10BT015)

UNDER THE GUIDANCE OF

**Mr. Bharath B.R**  
Lecturer

*in partial fulfillment of the requirements for the award of the Degree of*

***Bachelor of Engineering (Biotechnology)***

*from*

***Visvesvaraya Technological University, Belgaum***



**DEPARTMENT OF CIVIL ENGINEERING**  
**N.M.A.M. INSTITUTE OF TECHNOLOGY**

(An Autonomous Institution under VTU, Belgaum)  
(AICTE approved, NBA Accredited, ISO 9001:2008 Certified)  
**NITTE –574 110, Udupi District, KARNATAKA**

**2013-2014**

## ABSTRACT

India, whose per capita energy consumption is among the world's lowest, has little room to reduce fuel use as it needs to power industry to bolster its fast-sliding economy and imports over 75% of its crude oil. Hence there is a need for researchers to find an alternative for the improved production of fuel using edible and non-edible sources like sugarcane, corn, vegetable oils, liquid animal fats, green diesel, and algae. Recent studies have shown that even microorganisms can be used for biofuel production and also suggested that metabolic engineering of microorganisms can also enhance the yield of biofuel. Metabolic engineering is the science that combines systematic analysis of metabolic and other pathways with molecular biological techniques to improve cellular properties by designing and implementing rational genetic modifications. In that concern the current study provides an *in silico* approach to metabolically engineer *Escherichia coli* K12 to improve its potency to yield bio-ethanol in the glucose supplemented culture media. In the present approach systems biology methods like mass conservation and time scale analysis were adopted to identify the key metabolite in *E. coli* K12 system consequently NADH dehydrogenase was predicted as a key enzyme to improve the yield of bio ethanol. The approach established during the study was subjected for validation for its sensitivity and accuracy by employing *in vitro* experiments. The *in vitro* studies have positively supported the predictions made by an *in silico* approach by showing improved yield in ethanol production on inhibiting NADH dehydrogenase using MgCl<sub>2</sub>. The reaction mixture treated with 6.7mM MgCl<sub>2</sub> has shown improved yield of six fold during 48hr of fermentation.

**Keywords:** *Bioethanol, Metabolic engineering, Escherichia coli K12, in silico, in vitro*