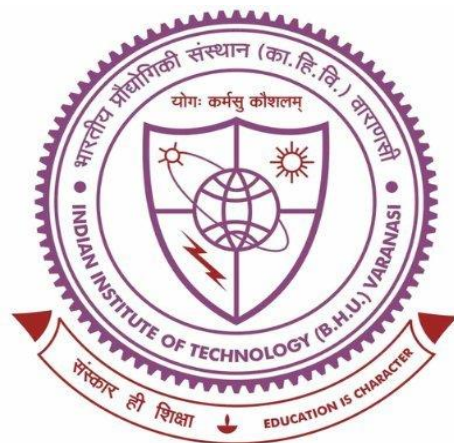


Production of Farnesene by Engineered Cyanobacteria

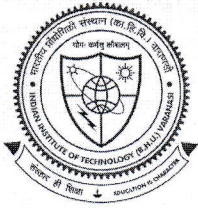


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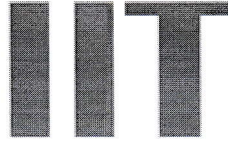
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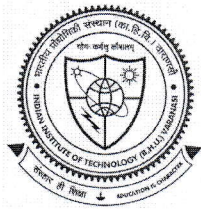
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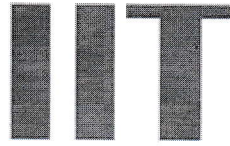
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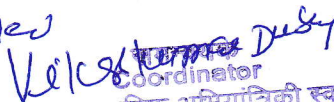
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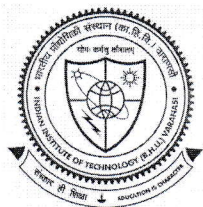
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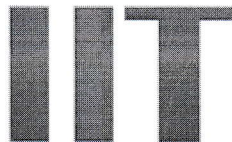
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*Dedicated to my loving
parents and brother*

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Knowledge without humility is worthless.

– **Mahamana Pt. Madan Mohan Malviya**
(Creator of the pious seat of learning, Banaras Hindu University)

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List of Abbreviations

1G	First generation
2G	Second generation
3G	Third generation
4G	Fourth generation
AFS/FS	Farnesene synthase
ASTM	American Society of Testing and Materials
AtoB	Acetoacetyl-CoA thiolase
bom	Basis of mobility
CapEx	Capital expenditure
CBB	Coffee berry borer
Chl ^R	Chloramphenicol resistance gene
CpcB	β -subunit of phycocyanin
CRISPR	Clustered regularly interspaced short palindromic repeats
DCW	Dry cell weight
DMAPP	Dimethylallyl diphosphate
DPOAEs	Distortion product otoacoustic emissions
DPPH	2,2-diphenyl-1-picryl-hydrazyl
DXP	1-deoxy-D-Xylulose-5-phosphate
DXR	DXP reductoisomerase
dxs	1-deoxy-D-Xylulose-5-phosphate synthase
EBF	(E)- β -farnesene
ENDS	Electronic nicotine delivery systems
FID	Flame ionization detector
FOC	Fixed operating cost
FP	Forward primer
FPP	Farnesyl diphosphate
FPPS/ispA	Farnesyl diphosphate synthase
FSLFPPS	Fusion of FS and FPPS genes by linker
GAP	Glyceraldehyde 3-phosphate
GC	Gas chromatography
GGPP	Geranyl geranyl diphosphate
GHG	Greenhouse gas
GPP	Geranyl diphosphate
GPPS	GPP synthase
GLS	Geranylgeranyl synthase
HMBPP	4-hydroxy-3-methylbut-2-enyldiphosphate
HMG-CoA	4-hydroxy-3-methyl-glutaryl-CoA
HMGs	HMG-CoA synthase
HMGR	HMG-CoA reductase
IC ₅₀	Half-maximal inhibitory concentration
idi	Isopentenyl diphosphate isomerase

idispA	Fusion of <i>idi</i> and <i>ispA</i> genes by linker
IM	Isopropyl myristate
IspG	HMBPP synthase
IspH	HMBPP reductase
IPP	Isopentenyl diphosphate
IPTG	Isopropyl- β -D-thiogalactopyranoside
IspS	Isoprene synthase
kan ^R	Kanamycin resistance
K _{cat}	Turnover number
kDa	Kilo Dalton
K _m	Michaelis constant
LB	Luria-Bertani broth
LCA	Life cycle assessment
LDH	Lactate dehydrogenase
MEP	Methyl-D-erythritol-4-phosphate
MeSa	Methyl salicylate
MFSP	Minimum farnesene selling price
MK	Mevalonate kinase
MVA	Mevalonate
NADH	Nicotinamide adenine dinucleotide
NASA	National Aeronautics and Space Administration
NO _x	Nitrogen oxides
NREL	National Renewable Energy Laboratory
NSI	Neutral site I
NSI'	Upstream NSI region
NSI''	Downstream NSI region
NSII	Neutral Site II
NSII'	Upstream NSII region
NSII''	Downstream NSII region
NSIII	Neutral site III
NSIII'	Upstream NSIII region
NSIII''	Downstream NSIII region
OBPs	Odorant binding proteins
OpEx	Operating expenditures
ORs	Odorant receptors
OUPF	Orientation-specific upstream forward primer
PBR	Photobioreactor
PCR	Polymerase chain reaction
PCX	Paclitaxel
PHLS	β -phellandrene synthase
PT	Prenyl transferase
PMD	Mevalonate 5-pyrophosphate decarboxylase
PMK	Mevalonate 5-phosphate kinase

ppm	Parts per million
RBS	Ribosome binding site
RDRP	Reversible deactivation radical polymerization
RFP	Red fluorescent protein
RP	Reverse primer
SARS	Severe acute respiratory syndrome
SCN	Soybean cyst nematode
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SIP	Iso paraffin
Sm ^R	Streptomycin and spectinomycin resistance
TEA	Techno-economic analysis
TPEs	Thermoplastic elastomers
tHMGR	Truncated HMGR
T _g	Glass transition temperature
TPS	Terpene synthase
VOC	Variable operating cost

Notations

α	Alpha
β	Beta
γ	Gamma
$^{\circ}\text{C}$	Degree celsius
$^{\circ}\text{C}/\text{min}$	Degree celsius per minute
h	Hour
Gt	1 billion tonnes
%	Percentage
\$	Dollar
\$/kg	Dollar per kilogram
mg	Milligram
mg/L	Milligram per liter
mg/g DCW	Milligram per gram dry cell weight
mg/L/day	Milligram per liter per day
g/L	Gram per liter
g/m ³ /day	Gram per meter cube per day
$\mu\text{mol photon}/\text{m}^2/\text{s}$	Micromole photons per meter square per second
μl	Microliter
μg	Microgram
$\mu\text{g}/\text{L}$	Microgram per liter
$\mu\text{g}/\text{L}/\text{day}$	Microgram per liter per day
M	Molar
mM	Millimolar
rpm	Revolution per minute
v/v	Volume by volume

Preface

In the current energy paradigms, most of the requirements, be it fuel for transportation, electricity generation, or thermoplastic materials production, are fulfilled by utilizing fossil-based fuels such as coal, petroleum, and natural gases. This leads to the generation of substantial amounts of greenhouse gases (GHG) into the atmosphere causing global warming. Carbon dioxide is the highest and accounts for 76% of total GHG emissions. Apart from fossil fuel combustion, deforestation and industrial processes also add up to the atmospheric CO₂. This represents a significant global challenge, impacting climate, ecosystem, and human health by climate fluctuations, irregular drought and flood patterns, and decreased crop productivity. Considering these repercussions, substantial research efforts and advancements have been dedicated over the past decade to support the objectives outlined in the “Agenda 2030” for sustainable development. The aim is to address 17 key goals encompassing social, economic, and environmental dimensions on a global scale. The primary target include limiting the average temperature increase to below 2 °C and halving greenhouse gas emissions by 2030. There are several strategies to sequester CO₂, including physical, chemical and biological methods. Out of these methods biological fixation has emerged to be the most feasible one to achieve carbon neutrality. Cyanobacteria transform atmospheric CO₂ into oxygen and can generate a variety of valuable products. Cyanobacteria are known for their biofixation efficiency which is 10-15 times higher than the terrestrial plants. Following the biofixation of CO₂ the cyanobacteria can produce carbohydrates, lipids, proteins which can be utilized in food and feed additives and also can be used as biofuel directly or indirectly.

The thesis reports the sustainable production of farnesene through CO₂ sequestration by cyanobacteria. The study is designed in three different sections. Wherein the first section integration vectors were constructed. Three integration vectors were

constructed to genetically modify fast growing cyanobacteria *Synechococcus elongatus* UTEX 2973. Conventional cloning, which includes restriction digestion and ligation, was used for the vector construction. The vectors namely were constructed such that the gene(s) of interest is inserted at the neutral site of the genome. The vectors were checked at each step to confirm the correct gene/fragment ligation, which was followed by the sequencing.

In the next section, the integration vectors constructed were transformed into *Synechococcus elongatus* UTEX 2973, emanating four genetically modified strains, UTEX *AFS*, UTEX *AFS::dxs*, UTEX *AFS::idispA*, and UTEX *AFS::dxs::idispA*. The UTEX *AFS::dxs::idispA* produced the highest farnesene level of 12.87 ± 0.7 mg/L in 5 days, equivalent to 12.48 mg/g DCW. With a productivity of 2.57 mg/L/day, UTEX *AFS::dxs::idispA* emerges as the superior photosynthetic farnesene producer compared to the existing cyanobacterial literature for farnesene production.

Further, a conceptual farnesene production plant is designed using engineered cyanobacteria which can utilize CO₂ from the flue gas. Techno-economic analysis and life cycle assessment of this conceptual plant are done. The capital expenses (CapEx), operating expenses (OpEx) and minimum farnesene selling price (MFSP) was calculated. The estimated CapEx for the plant amounts to \$74.36 MM. The total annual OpEx is projected to be \$19.42 MM. The key cost drivers of the MFSP were determined by single-point sensitivity analysis. The study identified the farnesene productivity and cost of organic solvent and inducer, majorly influencing the MFSP. An MFSP of \$5.77/kg was observed with a 40-fold increase in productivity, which was further reduced following the reduction in the cost of organic solvent. Moreover, a life cycle assessment of the conceptual process is assessed, indicating that the process is carbon neutral.

Thus, the present work successfully provides the framework for sustainable production of farnesene from cyanobacteria. The platform can also be used to produce other

value-added products by varying genes of interest in the vectors constructed and, hence, the modified cyanobacteria strains. The modified UTEX strain was able to produce farnesene with the highest productivity reported to date in cyanobacteria, which can be further improvised by adaptive evolutionary engineering, process parameters optimization and different modes of reactor operation. The TEA and LCA reveal that the process can be successfully upscaled and can give competition to the existing technologies marking a significant shift to a carbon-neutral economy.