

PREFACE

Driven by growing concerns about diminishing fossil fuel resources and their environmental impact, sustainable biofuels production has emerged as a crucial challenge for the modern world. Isoprene, a versatile terpenoid, has immense potential as a platform chemical for liquid transportation fuels and a precursor for diverse products like rubber and adhesives. It is commercially produced from crude oil refining processes. As a result of fossil fuel depletion, efforts to develop technologies to produce renewable isoprene in a sustainable and carbon-neutral way from CO₂ using photosynthetic microorganisms such as cyanobacteria and microalgae are gaining significant attention for its industrial and biofuel applications.

This study commences to explore a sustainable alternative: engineering the fast-growing cyanobacterium *Synechococcus elongatus* UTEX 2973 for efficient isoprene production. Our strategy is based on engineering the cyanobacterium with the isoprene synthase gene (*IspS*) to unlock its ability to synthesize isoprene. Furthermore, enhancement of isoprene production is accomplished by overexpressing a bottleneck enzyme (*IDI*) and employing an inhibitor (alendronate) which are involved in the methyl erythritol 4-phosphate (MEP) metabolic pathway. The engineered *S. elongatus* UTEX 2973 strains are analyzed for growth and isoprene production under controlled process conditions. The preliminary production study shows promising potential of recombinant *S. elongatus* UTEX 2973 strains for sustainable isoprene production. Moreover, alendronate concentration is optimized to enhance isoprene production using the engineered recombinant *S. elongatus* UTEX 2973 strain. The productivity of isoprene is influenced by process operating conditions such as growth temperature, light intensity and concentration of carbon source. To further enhance the isoprene yield, Box-Behnken Design (BBD) tool is used to generate experimental datasets for optimization of process parameters such as light intensity,

NaHCO₃ concentration and growth temperatures using statistical response surface methodology (RSM) and artificial neural network-genetic algorithm (ANN-GA) models.

Fossil fuels have long served as the backbone of isoprene production, but their existence is approaching its end. This study steps into a new era, unveiling a conceptual plant design for sustainable isoprene production by harnessing the power of recombinant cyanobacteria and utilizing industrial waste flue gas as the sole CO₂ source. With a target of 1000 tonnes of annual production, the isoprene production plant was designed and simulated using the Aspen Plus software. We also conduct a preliminary techno-economic analysis, ensuring this approach not only governs on environmental principles but also succeeds in the actual application.

This study breaks new ground by establishing a potential combination of alendronate inhibition and machine learning optimization as a superior method for enhancing isoprene production in fast-growing recombinant cyanobacteria. This novel approach surpasses conventional statistical optimization and provides a crucial foundation for production scaleup and further improving yields. Furthermore, the insights gained from recombinant strains and metabolic pathway inhibition studies suggest immense potential for the photosynthetic production of hemiterpenes, opening doors for exciting future developments in this domain. This study also provides a platform for designing cost-optimization strategies for sustainable production of isoprene and other commodity chemicals utilizing flue gas.