

## Chapter – 6

### Conclusion and Future Prospects

Cyanobacteria are possible photosynthetic microbial cell factories for isoprene and other value-added products in biorefinery systems for developing circular bio-economy. The study emphasizes renewable isoprene production from CO<sub>2</sub> using recombinant cyanobacteria, addressing concerns about energy security while mitigating climate change through carbon sequestration and highlights the potential of isoprene as a promising alternative to fossil-based fuels. The MEP pathway was modified by integrating plant origin *IspS* and bacterial *IDI* genes into the genome of the *S. elongatus* UTEX 2973 and implementing a novel CrtE enzyme inhibition strategy with alendronate. Docking studies confirmed that alendronate preferentially binds to CrtE without affecting other crucial enzymes in the isoprene biosynthesis pathway and provided valuable insights into the interaction between CrtE and alendronate, paving the way for further optimization strategies. Furthermore, expressing heterologous genes and employing enzyme inhibition strategy significantly increased isoprene yield, offering a viable pathway for large-scale production. Compared to using only the inducer IPTG, alendronate supplementation increased isoprene production by 4.7-fold, reaching 1.92 mg/g DCW in 6 days of production study. This demonstrates the effectiveness of CrtE inhibition in enhancing isoprene yield. Thus, recombinant *S. elongatus* UTEX 2973 *IspS*.*IDI* strain, being a fast-growing cyanobacteria, could be a potential photosynthetic cell factory for sustainable and improved isoprene production using alendronate inhibitor. Furthermore, isoprene production by optimizing both process parameters and alendronate concentration we achieved a remarkable improvement in isoprene yield. This targeted optimization, particularly with the assistance of the ANN-GA model, led to a significant 29.52-fold increase in isoprene yield, which demonstrates the immense potential of engineered cyanobacteria for sustainable chemical production. This innovation paves the way for

further research on scaling up this sustainable technology and unlocking its potential as a platform for producing not only isoprene but also other valuable commodity chemicals. While cyanobacteria biorefineries hold immense potential for sustainable production, several key challenges persist. Expensive downstream processes, high input costs, and limited product diversification threaten economic viability. Additionally, photobioreactor limitations like self-shading hinder efficient biomass accumulation. Overcoming these obstacles requires collaborative efforts across diverse fields, including bioengineering, process optimization, and cost analysis.

This techno-economic analysis demonstrates the exciting potential of sustainable isoprene production using engineered cyanobacteria. Our proposed design, based on continuous photo-fermentation in a 100 m<sup>3</sup> photobioreactor and cryogenic separation, reveals that photosynthetic isoprene can be economically competitive with existing microbial production systems. The estimated minimum selling price of 4.85 \$/kg matches current isoprene market, opening the door for a significant shift towards more eco-friendly production. Sensitivity analysis identifies key factors like productivity, capital cost, and project duration that can be optimized for further cost reduction. By collaboratively enhancing these factors, we can unlock the full potential of this sustainable technology and revolutionize the isoprene industry. This study provides a platform for engineering and cost optimization strategies for photosynthetic sustainable isoprene and other platform chemicals production utilising flue gases. Our findings offer a promising avenue for future research and development, pushing the boundaries of sustainable biofuel production and contributing to a greener future. The next steps could involve scaling up the process, improving efficiency, and exploring alternative inhibition strategies for even greater isoprene production. The findings of this work support the scheme of large-scale process development using recombinant cyanobacteria, a step towards scale-up the sustainable isoprene production.