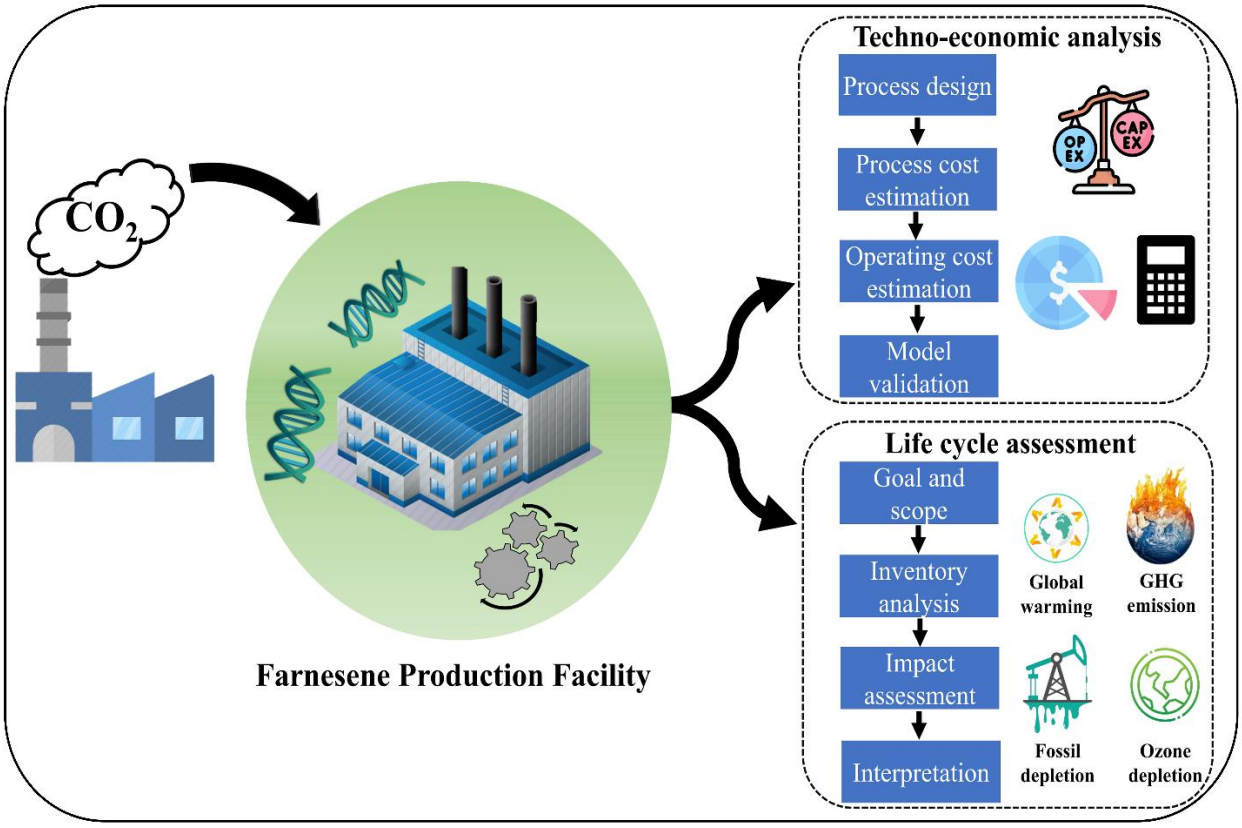
CHAPTER 5

Design and simulation of conceptual farnesene production plant using engineered cyanobacteria*



* **Akhil Rautela** et al. (2024) Techno-economic analysis and life cycle assessment of sustainable farnesene production by genetically engineered cyanobacteria utilizing carbon dioxide: A step towards commercial viability. (Communicated)

This chapter focuses on the techno-economic analysis (TEA) and life cycle assessment (LCA) of the conceptual farnesene production plant. This plant utilizes engineered cyanobacteria and carbon dioxide (CO_2) from the flue gas as a carbon source. Currently, Amyris Biotechnologies Inc., USA, with a production plant in Brazil, leads the global production of farnesene and sells under the brand name Biofene® (Mawhood et al., 2016). The company utilizes *Saccharomyces cerevisiae* equipped with an MVA pathway, which is engineered with a farnesene synthase (*AFS*) gene derived from *Artemisia annua* (Chandran et al., 2011). Total Energies, the world's seventh-largest oil and gas company, in partnership with Amyris, encouraged the commercialization of farnesene by fermentation and proposed that by 2025, farnesene production costs will match ethanol production costs. Presently Amyris is vending farnesene at a price of $2.15/Kg (Rautela et al., 2024a). Process scaling is one of the approaches for reducing the cost of production. Amyris initiated scaling up of farnesene production by high-throughput screening which is screening of best performing strain. A large number of microbial strain variants were screened at a nominal 300 µl scale. They constructed a regression model with a simplified equation with fermentation yield, recovery yield and rate. Gradually, the high-throughput screening scale (300 µl) was increased to a laboratory scale (500 ml), and all fermentation parameters were monitored likewise (Davison and Lievense, 2016).

Since yeast is a heterotrophic organism, sugar is required as a carbon source. Amyris employ sugarcane as a feedstock to facilitate yeast growth and farnesene production. The increasing cost of sugar production demands a shift towards sustainable sources for farnesene production (Cheng et al., 2019). CO_2 is one such source that cyanobacteria can assimilate to produce value-added products (Rautela and Kumar, 2022). National Oceanic and Atmospheric Administration has reported January 2024 CO_2 concentration to be 423 ppm, which was 365 ppm in 2002 (NASA). Uncontrolled utilization of fossil-based fuels

and other man-made activities are the pivotal causes of GHG emissions. These emissions are mostly in the form of flue gas with 3-6 % CO_2 (Choi et al., 2020). CO_2 is a heat-trapping gas that constitutes the major component of greenhouse gases. Since CO_2 concentration is increasing, there is an increase in global temperature making Earth 1.17 °C hotter in 2023. One of the major concerns is restricting this rise in temperature to 2 °C (Rawat et al., 2023). Elevated CO_2 levels significantly impact human health by causing sweat, high blood pressure and heart rate (Saravanan et al., 2022). Naturally, the plants and various microorganisms act as efficient CO_2 fixers (Nisar et al., 2021). Cyanobacteria are one such propitious host organism which sequesters CO_2 for its growth.

Cyanobacteria possess an MEP pathway to generate the precursor molecules for terpenoids. Since they lack the *AFS* gene, it has to be engineered to produce farnesene along with the bottleneck gene(s) of the MEP pathway to increase the production (Blanc-Garin et al., 2022; Chenebault et al., 2023; Lee et al., 2021; Pattharaprachayakul et al., 2019; Sun et al., 2023). The cyanobacterial strains studied have a higher doubling time corresponding to low product formation with low farnesene productivity. In the latest work from our lab, the engineered *Synechococcus elongatus* UTEX 2973 showed the highest farnesene productivity of 2.57 g/m^3/day (Rautela et al., 2024b). The strain was engineered with *AFS*, and bottleneck gene(s) of the MEP pathway, 1-deoxy-D-xylulose-5-phosphate synthase (*dxs*) and fusion of isopentenyl diphosphate isomerase and farnesyl diphosphate synthase (*idispA*) into the genomic neutral site to generate UTEX *AFS::dxs::idispA* strain. The engineered strains were grown using 5% CO_2.

The lab-scale studies show promising results for farnesene production in UTEX *AFS::dxs::idispA* with high productivity. Transitioning any new technology from the lab scale to an industrial scale necessitates thorough technical, economic, and environmental implications (Gangwar et al., 2024). TEA analyzes the economic feasibility or performance

of a process based on the already published literature. Whereas LCA analyzes the environmental impacts like carbon footprint, acidification potential, eutrophication potential, ozone layer depletion potential, photochemical smog potential, and human toxicity potential. To the best of our knowledge, no TEA and LCA are available for farnesene production from engineered cyanobacteria utilizing CO_2 from the flue gas. Therefore, the present study evaluates the economic potential and environmental sustainability of this process by computing the capital costs, operation costs, minimum farnesene selling price (MFSP) and environmental impact. Since the farnesene manufacturing plant is hypothetical, the process configurations and parameters are assumed to drive future research in the right direction.

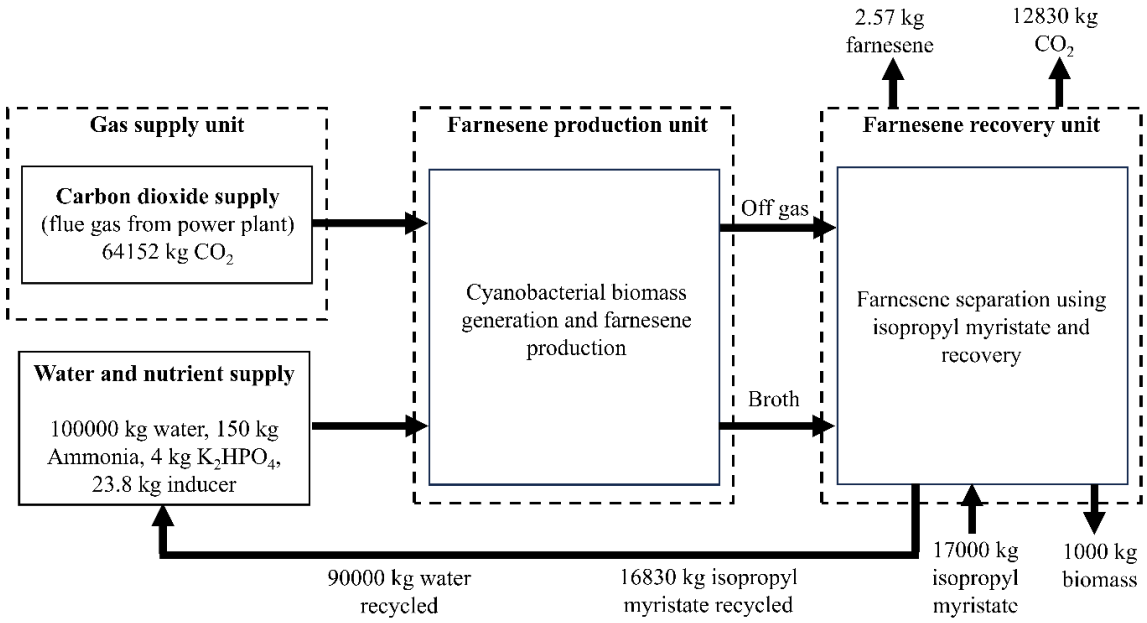


Fig. 5.1. Simplified block diagram for farnesene production by cyanobacteria. The values shown are for one PBR (100 m^3).

5.1. Materials and methods

5.1.1. Process overview

In the present work, a conceptual design of 100 m^3 batch fermentation is proposed for farnesene production by engineered cyanobacteria (UTEX *AFS::dxs::idispA*) using CO_2 as a carbon source (Rautela et al., 2024b). As shown in Fig. 5.1, a simplified process

flow diagram consists of gas supply, farnesene production and farnesene extraction unit. Aspen Plus software (AspenTech, Cambridge, MA, USA) was used to generate a simulation model for material and energy balance with a targeted annual capacity of 100 tonnes of farnesene based on the literature and preliminary data. Based on the model, an in-house Excel spreadsheet was generated, which was used for the estimation of process economics, which includes capital expenses (CapEx), operating expenses (OpEx), MFSP and farnesene revenue (FR). Table 5.1 summarizes the details of the assumptions used for farnesene production, which were acquired from the extensive literature review.

Table 5.1 Financial and productivity baseline assumptions for farnesene production.

Financial assumption	**Value**
Initial rate of return	10%
Plant financing by equity	50%
Plant life (years)	20
Income tax	20%
Interest rate of debt financing	8%
Term of debt financing (years)	10
Depreciation schedule (years)	7
Startup time (years)	0.5
Productivity baseline assumptions	
Production scale (tonnes/year)	100
Volume of PBR (m^3)	100
Number of PBRs	1177
Facility size (acre, PBRs only)	1455
Total facility size (acre)	1732
Farnesene productivity ($g/m^3/day$)	2.574[a]
Batch time (day)	50
Number of batches per year	6
Downtime (day)	5
Operating days per year	330

[a](Rautela et al., 2024b)

5.1.2. CO_2 supply component

Earlier studies have suggested the use of flue gas for terpenoids, such as squalene production by cyanobacteria (Choi et al., 2020; Choi et al., 2017b). Flue gas contains 3-6 % CO_2, which can be used as a carbon source by cyanobacteria, enabling them to thrive. Apart from CO_2, flue gas also contains 11.99% O_2, 21.72 ppm NOx, 1.43 ppm CO, water

vapour and dust (Choi et al., 2020). It is evident from recent studies that 5 % CO_2 can be used as the sole carbon source for farnesene production by engineered cyanobacteria (Rautela et al., 2024b). The utilization of flue gas for farnesene production will reduce the overall process cost by establishing CO_2 allowance incentives (Geissler and Maravelias, 2021). Therefore, the present TEA study envisioned utilizing CO_2 as a sole carbon source, which was delivered from a power plant near the facility to PBRs. To avoid pressure fluctuations in the PBRs, flue gas is compressed to 5 bar pressure before being fed to PBRs at a flow rate of 0.1 vvm (Liang et al., 2022). It is important to desulfurize the flue gas prior to being fed to the PBRs by passing it through the desulfurization unit. It is assumed that out of 100% CO_2 injected, 20% is released into the environment, and the remaining is distributed between farnesene production and biomass generation (Markham et al., 2016; Rueda et al., 2023).

5.1.3. Farnesene production

Farnesene production has been reported by many researchers in different cyanobacterial strains (Blanc-Garin et al., 2022; Chenebault et al., 2023; Lee et al., 2021). We recently engineered UTEX 2973 by expressing the *AFS* gene and overexpressing *dxs* and *idispA* (Rautela et al., 2024b). The highest productivity of 2.57 g/m^3/day was achieved, which was the highest among engineered cyanobacterial strains studied so far. In the proposed TEA, PBRs with 100 m^3 working volume are fed with CO_2, water and nutrients to generate biomass and farnesene, as described by equations 1 and 2, respectively. It is considered that 90% of the water is recycled to the PBRs (Lopes et al., 2019).

$$CO_2 + 0.622\ H_2O + 0.23\ NH_3 \rightarrow CH_{1.939}O_{0.473}N_{0.23} + 1.0745\ O_2 \quad (1)$$

$$15\ CO_2 + 12\ H_2O \rightarrow C_{15}H_{24} + 21\ O_2 \quad (2)$$

In the above stoichiometric equation, $CH_{1.934}O_{0.473}N_{0.23}$ represent cyanobacteria biomass. The equations are acquired from the already published literature (Liang et al., 2022). There

are several systems available for the cultivation of cyanobacteria, but given that farnesene, upon continuous bubbling, can escape the system, the use of closed tubular PBRs is proposed (Liang et al., 2022; Markham et al., 2016). PBRs have an advantage over open pond systems as they are less susceptible to contamination and can maintain monocultures effectively (Fasahati et al., 2019). Moreover, PBRs also prevent evaporative loss of media and the product (Markham et al., 2016). According to Davies et al. (Davis et al., 2011) and Markham et al. (Markham et al., 2016), it is estimated that a tubular PBR of 100 m^3 capacity covers an area of 1.23 acre. The PBRs will run for 50 days with 6 number of batches annually. After 50 days of run time, there will be a downtime of 5 days giving 330 operating days per year (Table 5.1).

5.1.4. Farnesene Extraction

In the lab scale, to capture farnesene, an overlay of immiscible organic solvents like dodecane, decane, hexadecane and isopropyl myristate (20% v/v) is applied (Lee et al., 2021, 2017). The problem associated with applying overlay is that it reduces the working volume of PBRs. In addition to this, the overlay proves to be toxic to the cyanobacteria and is not feasible to be used on a large scale (Rautela et al., 2024b). . Therefore, in the present TEA, a separate extraction vessel with isopropyl myristate (IM) is proposed in which the farnesene extraction will take place. The method relies on the difference in solubility of farnesene in the aqueous phase and organic phase (Sun et al., 2020). To avoid loss of farnesene in the off-gas, the off-gas is also directed inside the extraction vessel, where it will pass through the IM, and farnesene will solubilize into it. The solution from the extraction vessel is passed through a decanter centrifuge to separate the organic phase from the aqueous phase. Further, the organic phase is passed through a distillation column where the farnesene recovery occurs. 99% recovery of the organic phase is assumed, which is recycled back to the extraction vessel (Sun et al., 2020).

5.1.5. Biomass Recovery

The biomass is separated from the aqueous phase through a decanter centrifuge. The recovered biomass can be used for the production of value-added products and is important in food, energy, cosmetics, agriculture and medicine (Pathak et al., 2018). Therefore, cyanobacteria biomass can be sold at a price of $0.74/kg, contributing to the revenue generation for the process (Markham et al., 2016). This will aid in reducing the selling price of the farnesene.

5.1.6. Process economics evaluation

The economic feasibility of farnesene production from CO_2 using engineered cyanobacteria was analyzed by Aspen Plus economic evaluation software. After the simulation of the process, an Excel spreadsheet was generated, which was used to assess CapEx, OpEx, and MFSP ($/kg). The CapEx calculation was based on the total cost of equipment which were assumed from the previous reported studies, vendor quotations, and Aspen Plus analyzer. For instance, the cost of PBRs was deduced by using the following formulae from Markham et al. (Markham et al., 2016).

$$New\ cost = Base\ cost \times \left(\frac{New\ size}{Base\ size}\right)^{n} \quad (3)$$

Where ‘n’ is the scaling factor which varies with the equipment. The cost of other equipment to be used, such as pumps, decanter, distillation column, extraction vessel and preheater, were taken from Aspen Plus Economic Analyzer. The cost of land was assumed to be $3000 per acre, according to previous NREL reports and other TEA studies (Davis et al., 2016; Zhu et al., 2018).

The OpEx calculation was based on the cost of raw materials, which are summarized in Table 5.2. The raw materials and utility like ammonia, K_2HPO_4, water, inducer, organic solvent, and electricity make up the variable operating cost (VOC) which can vary with the production, while the salaries and facility maintenance make up the fixed

operating cost (FOC). The costs of these are majorly derived from the already published literature, vendor quotations and Aspen Plus simulation software. All the costs have been adjusted to 2023-dollar values according to the inflation rate.

Table 5.2 Cost of utilities and raw materials used in techno-economic analysis.

Raw materials	Cost ($/Kg)	References
Input		
Ammonia	0.15	(Kim et al., 2023)
K_2HPO_4	0.91	(Markham et al., 2016)
Water	0.0002	(Liang et al., 2022)
Organic Solvent	1.5	Vendor quotation
Inducer	7.0	Vendor quotation
Electricity	0.1 $/KW	(Liang et al., 2022)
Output		
Carbon credit	0.056	(Geissler and Maravelias, 2021)
Biomass credit	0.74	(Markham et al., 2016)

The prices have been adjusted to 2023-dollar values according to the inflation rate.

The MFSP is based on investment expense and is calculated using the following formulas (Sen et al., 2012):

$$DC = \frac{Acquisition\ cost\ (CapEx) - Residual\ (40\%\ of\ CapEx)}{20} \quad (4)$$

$$ROI = \frac{DR \times [1+DR]^{ELS}}{[1+DR]^{ELS}-1} \times TPI \quad (5)$$

$$IT = TR \times (FR + ER - OC - DC) \quad (6)$$

$$FR + ER = OC + ROI + IT \quad (7)$$

$$MFSP = \frac{FR}{Farnesene\ production} \quad (8)$$

$$Payback\ period = \frac{FC+WC}{Total\ revenue - FOC - VOC} \quad (9)$$

$$Net\ cash\ flow = Total\ revenue - OpEx \quad (10)$$

$$Net\ present\ value = \sum_{n=1}^{n=t} \frac{Cash\ flow}{(1+DR)^n} \quad (11)$$

Where ‘DC’ is depreciation cost, ‘ROI’ is the return on investment, ‘DR’ is the discount rate, ‘ELS’ is the economic life of the project (year), ‘TPI’ is total project investment, ‘TR’ is the tax rate, ‘ER’ is revenue without farnesene, ‘OC’ is operating cost, ‘IT’ is income tax

and 'FR' is farnesene revenue, 'FC' is fixed capital, 'WC' is working capital, 'FOC' is fixed operating cost, 'VOC' is variable operating cost, 'n' is project length in year, and 'DR' is discount rate. The MFSP was determined from the FR. The FR is calculated based on the sales of these process components, which is decided at the breakeven point where total revenues and total costs are equal. All the values used in the formulas to derive MFSP can be seen in supplementary data. Finally, sensitivity analysis was performed to quantify the resulting cost impact on the overall MFSP.

Apart from this evaluation of Payback period (PBP), Net present value (NPV) and internal rate of return was done while considering the following assumptions:

1. The revenue from the production is assumed to be 50% for the first year followed by 100% for the following years.
2. 100% CapEx and 50% is utilized in the first year.
3. 6.74% is the discount rate used for NPV calculation.

Table 5.3 Life cycle inventory for per Kg farnesene production.

Materials	Value
Ammonia	11.7 Kg
K_2HPO_4	0.31 Kg
CO_2	4430 Kg
Water	7770 Kg
Organic solvent	66 Kg
Electricity	3138.94 KWh

5.1.7. Life cycle assessment

A cradle-to-gate LCA was performed to assess the potential environmental impact of farnesene production from genetically engineered cyanobacteria. The cradle-to-gate approach involves analysis starting from the raw materials to the production of farnesene. In the study, CCaLC2 LCA software (University of Manchester, UK) was used to quantify the impact of all inputs and outputs associated with the production process (Adsal et al.,

2020; Bor and Üçtuğ, 2022). The detailed instructions for the LCA are indicated in ISO 14040 and ISO 14044 (ISO 14040, 2006; ISO 14044, 2006). According to the guidelines, goal of the study was defined with the consideration of using 1 kg of farnesene as functional unit to investigate global warming potential. Further, based on the assumptions and process conditions defined in section 2.1-2.5 a life cycle inventory for farnesene production was made (Table 5.3). The amount of raw materials (ammonia, K_2HPO_4, inducer) required for the process were based on the lab scale experiments (Rautela et al., 2024b). As mentioned in TEA the biomass will be sold and will be utilized in a manner to be sequestered. Some of the applications in which biomass can be sequestered are biochar formation, soil amendments, and bio composites (Park et al., 2024; Kheirfam, 2020; In-na et al., 2020; Goodchild-Michelman et al., 2023)

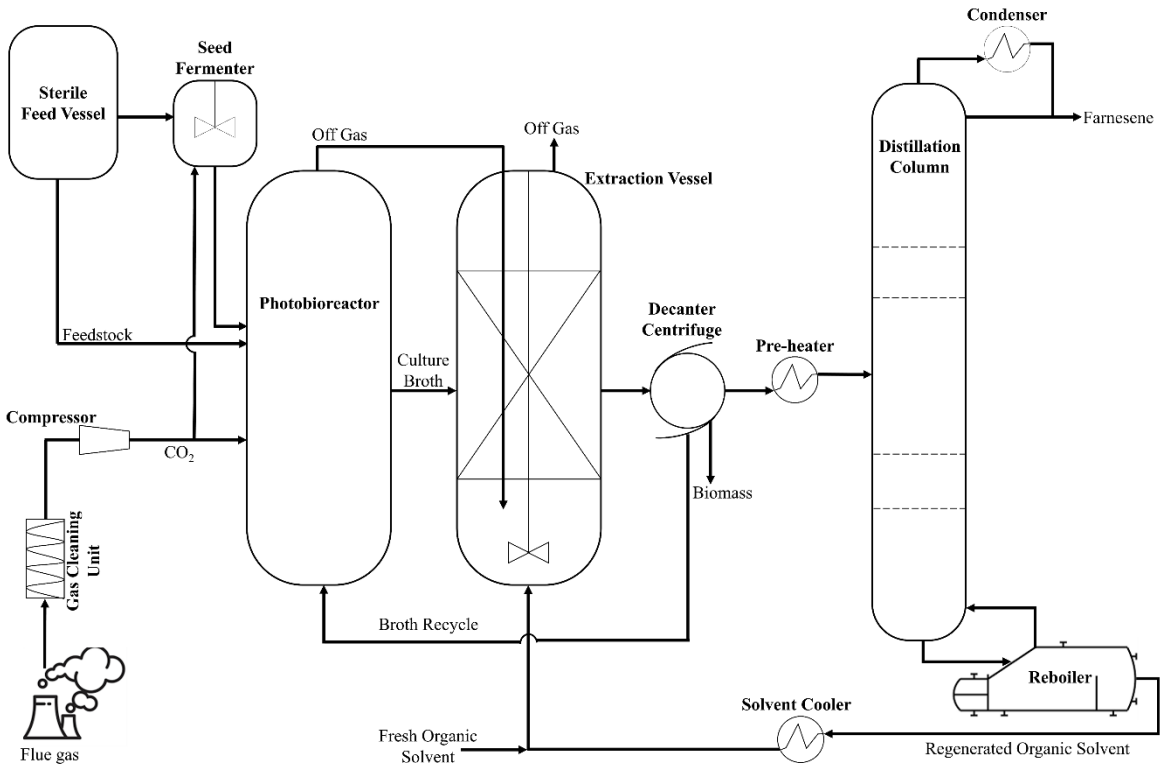


Fig. 5.2. Schematic process flow diagram for farnesene production through genetically engineered cyanobacteria utilizing carbon dioxide.

5.2. Results and discussion

5.2.1. Base case economics

In the previous study from our lab, we engineered UTEX 2973 to produce farnesene (Rautela et al., 2024b). The *AFS*, *dxs* and *idispA* genes were integrated into the genomic DNA of UTEX 2973 at NSI, NSII and NSIII, respectively, resulting in the strain UTEX *AFS::dxs::idispA*. The highest productivity of 2.574 g/m^3/day was obtained from the UTEX *AFS::dxs::idispA* strain. In the present study, this productivity was assumed to be the base productivity (2.574 g/m^3/day). This productivity assumed here is not a theoretical limit and can be further increased by improving strains at the genetic level and optimization of process parameters. It is important to highlight here that the analysis provided is based on pre-commercial technology. Therefore, the analysis is conceptual and reflects reasonable projections for scaling up the technology. The schematic representation of process flow is shown in Fig. 5.2. The results of TEA for farnesene production from engineered cyanobacteria are discussed below, with MFSP determined at the productivity of 2.574 g/m^3/day or higher.

5.2.2. Capital expenses distribution

The CapEx was determined by taking into account the purchase and installed cost of equipment and other costs derived from previous TEAs, commissioning charges, vendor quotations and Aspen plus economic evaluation software (Davis et al., 2016; Fei et al., 2020; Kumar et al., 2020; Liang et al., 2022; Markham et al., 2016; Pandey et al., 2020). Table 5.4 lists the cost of equipment and other costs such as land, piping, paint, and IM. The total cost for installed equipment was reckoned to be $10.90 MM, whereas the total CapEx was $28.16 MM. The other costs (having commissioning charges) account for the largest portion of CapEx i.e., $17.25 MM followed by costs of PBRs and land. In the previous study by Markham et al. (2016) the largest portion of CapEx was accounted by cost of PBRs due to the fact that commissioning charges was not considered. Reduction in

the number of PBRs will decrease the land requirement and hence land cost. This can be achieved by increasing farnesene productivity from the base case or reducing the targeted annual capacity. Apart from PBRs, there are other equipment required for the proper functioning of the facility, such as gas compressor, pumps, extraction vessel, decanter centrifuge, distillation column and preheater, which contribute 14% of the capital investment (Fig. 5.3(a)). About 8% of the total CapEx is contributed by overhead contracts, contingencies, and construction of the facility, which includes piping and paint.

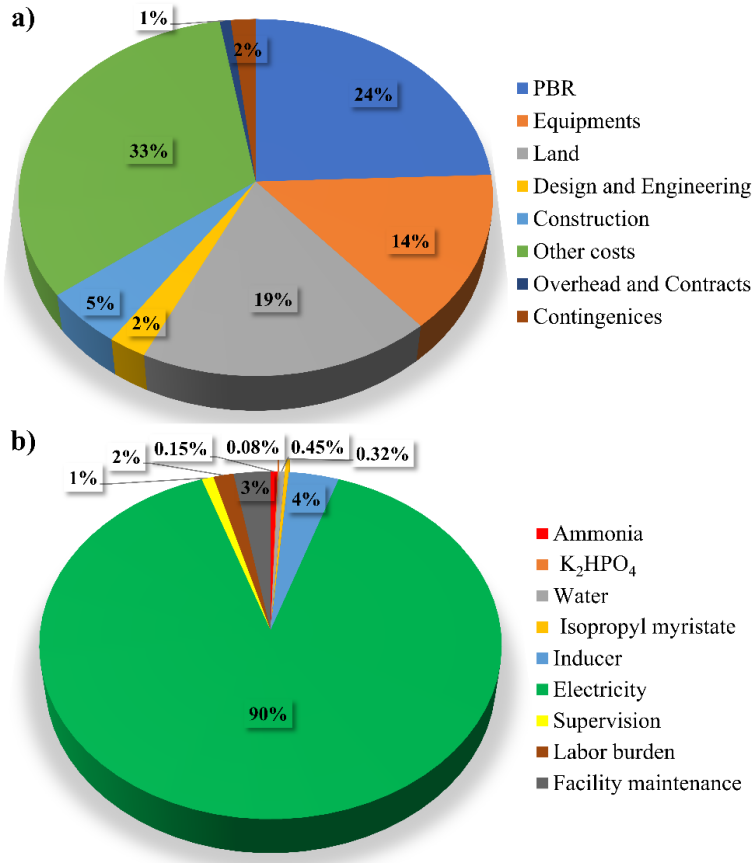


Fig. 5.3. Cost distribution of the proposed farnesene production plant. **a)** annual capital expenditure (CapEx) and **b)** annual operating expenditure (OpEx).

5.2.3. Operating expenses distribution

The operating expenses are divided into VOC and FOC and is shown in Table 5.5. The total operation cost amounts to $30.75 MM, out of which VOC and FOC account for 95% and 5%, respectively (Fig. 5.3(b)). The VOC includes the cost of raw materials (water, K_2HPO_4, ammonia, inducer, isopropyl myristate, and electricity), while the FOC includes

salaries, labour burden and facility maintenance. The largest operating expense is shared by the electricity consumption, followed by inducer cost. The cost of electricity consumption is deemed to be $27.62 MM annually, accounting for 90% of total operating costs. The annual cost of inducer accounts for 4% of the OpEx. To annihilate the inducer cost, strong constitutive promoters or light-inducible promoters can be used to express the gene(s) of interest (Kobayashi et al., 2022; Till et al., 2020). Since it is assumed that the flue gas is being used as a sole carbon source and is getting supplied from the powerplant near the facility, the cost of CO_2 is not added to the operating cost. Moreover, utilizing CO_2 from the flue gas generates revenue in the form of carbon incentives at $0.056 per kg of CO_2 consumed (Geissler and Maravelias, 2021).

5.2.4. Minimum farnesene selling price

The MFSP for the design basis of 100 tonnes annual capacity was calculated to be $148.44/kg. The MFSP was calculated based on the capital costs, production costs and financial assumptions with base farnesene productivity of 2.574 g/m^3/day. Upper and lower bound of base case farnesene productivity was taken into account for the MFSP calculation. A negative correlation between farnesene productivity and MFSP can be seen in Fig. 5.4. This was in accordance with the previous studies where an increase in productivity decreases the minimum selling price of a product (Sun et al., 2020). The highest cost driver of the MFSP was the electricity consumption. The next highest contribution belongs to the capital cost of PBRs. The revenue generated by carbon credits and biomass reduces the total impact on the plant by generating revenue of $22.55 MM/year and $0.52 MM/year, respectively. It is worth noting that farnesene's market price, as per Amyris's report, ranges between $3.07 to $6.15 per kg (Amyris' Sweet-'N-High). This cost is for the farnesene derived from a heterotrophic source, i.e., yeast (*Saccharomyces cerevisiae*). The highest farnesene productivity by *S. cerevisiae* was reported to be 4710 g/m^3/day (4.71 g/L/day)

(Wang et al., 2023). This is approximately more than 1800 times the productivity assumed in this study (2.574 g/m^3/day). In order to meet the market price, the farnesene productivity needs to be at least 180.18 g/m^3/day (where MFSP is $5.57 per kg). This implies that to make the process economically feasible, a 70-fold enhancement in productivity is imperative.

Table 5.4 Capital investments (CapEx) of the process.

Equipment	**Installed cost ($ MM)**
Flue gas desulfurization unit	0.006
Gas compressor	1.073
Pumps	0.072
Closed tubular PBRs	6.87
Decanter	1.52
Distillation Unit	1.2
Extraction vessel	0.161
Preheater	0.00337
Total equipment cost	**10.90537**
Other costs	
Commissioning Charges	7.68
Equipment Setting	0.002185
Land	5.19
Piping	0.103721
Civil	0.013835
Steel	0.011238
Instrumentation	0.570811
Electrical	0.691579
Insulation	0.025664
Paint	0.005988
Other	1.1878
G & A Overhead	0.060022
Contract Fee	0.168106
Total Design, Eng, Procurement Cost	0.6783
Contingencies	0.523291
Isopropyl myristate	0.334150
Total other costs	17.25629
Total CapEx (equipment cost + other costs)	**28.16166**

This improvement can be brought up by strain improvement strategies, which include promoter and ribosome binding site engineering, metabolic engineering, genome mining, and selection of high-yielding mutants (Yadav et al., 2021). Specifically, the strain can be improved by increasing the number of copies of the *AFS* gene. This can be done by

integrating the *AFS* gene into the genome coupled with its plasmid-borne expression (Blanc-Garin et al., 2022; Chenebault et al., 2023). Apart from this the optimization of culture conditions can also significantly improve the farnesene productivity (Bao et al., 2024). For instance, using dairy wastewater with whey can significantly increase the growth of cyanobacteria and, hence, the production by acting as a heterotrophic carbon source (Ding et al., 2021). It also gives high production in comparison to IPTG induced cultures.

Table 5.5 Annual Operating cost (OpEx) distribution.

Variable operating cost (VOC)	**Annual cost ($ MM)**
Ammonia	0.158
K_2HPO_4	0.025
Water	0.141
Isopropyl myristate	0.020
Inducer	1.176
Electricity	27.62
Total VOC	**29.142**
Fixed operating cost (FOC)	
Salaries	0.277
Labor burden	0.475
Facility maintenance	0.864
Total FOC	**1.616**
Total OpEx (VOC+FOC)	**30.758**

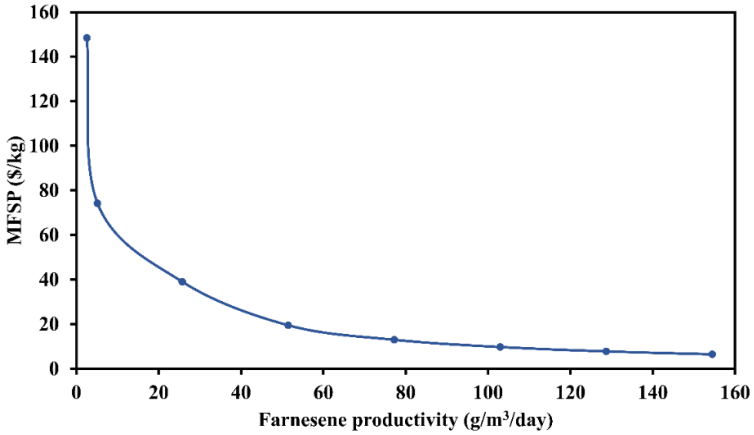


Fig. 5.4 Variation of MFSP over a range of farnesene productivity ($g/m^3/day$).

5.2.5. Sensitivity analysis: Impact of uncertainty in production factors on MFSP

Since the production process outlined in the present study is at the conceptual stage, there exist inherent risks associated with the process itself and the economic parameters. In pursuit to address these risks and to gain thorough insight into how various factors influence the economic parameters a single point or one-to-one sensitivity analysis was performed. The sensitivity analysis was performed for the design variables. The baseline for the variables were same as assumed earlier. The analysis involves adjusting one variable at a time within its lower and upper bound while keeping the other variables constant (Liang et al., 2022; Sun et al., 2020).

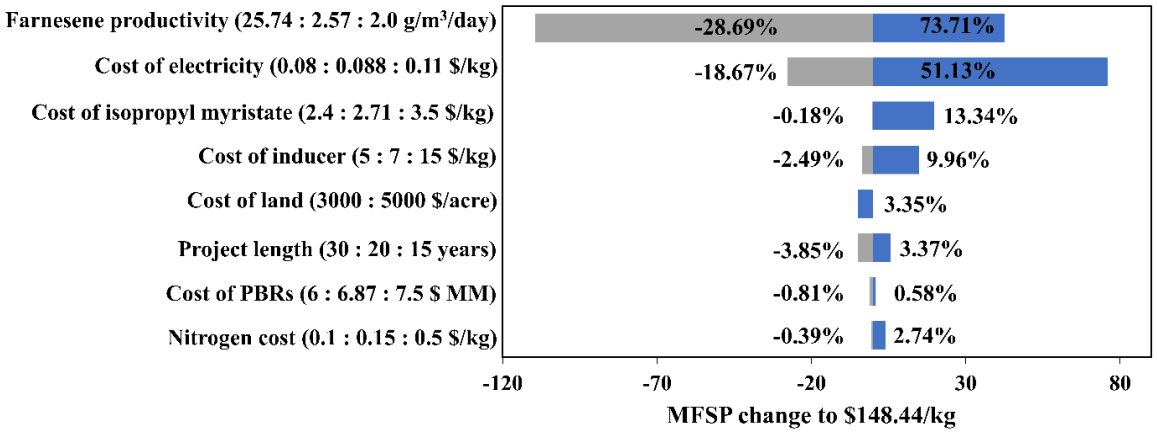


Fig. 5.5 Single-point sensitivity analysis showing impacts of different variables on MFSP (base case price: $87.83/Kg).

As shown in Fig. 5.5, farnesene productivity, cost of electricity, isopropyl myristate, and inducer impact the overall cost of farnesene, acting as the key cost drivers. The farnesene productivity and project length are negatively correlated, whereas the cost of electricity, isopropyl myristate, and inducer are positively correlated. How the variations in assumptions affects the overall cost can be spreadsheet provided as supplementary data. Since the farnesene productivity predominates as a cost-driving force, it significantly impacts MFSP. This is due to the fact that as the productivity increases, so does the yield, thereby reducing the necessity for large numbers of PBRs (whose cost accounts for 24% of

CapEx) and reduction in the total land area (and hence the cost of land, which is 19% of CapEx) (Fei et al., 2020; Markham et al., 2016). As mentioned in section 3.4, the farnesene productivity can be increased by strain improvement strategies and media optimization. Up to a 9% rise in the MSFP was observed on increasing the cost of inducer. In the present study, the inducer is crucial as all the genes engineered in the genetically modified strains are under the control of trc promoter (IPTG inducible promoter) (Rautela et al., 2024b). Inducible promoters provide a regulation of the product formation if the product is toxic to the host organism (Tegel et al., 2011). To reduce the cost of the inducer, whey can be used as a substitute which induces trc promoter as efficiently as IPTG (Viitanen et al., 2003). Whey not only acts as an inducer but can also be used as a carbon source by the microorganisms, increasing the biomass and hence the product formation (Ding et al., 2021). To further reduce the price of using whey, cheese whey wastewater can be used in the production plant. This not only eliminates the requirement for freshwater but also aids in the bioremediation of wastewater. In addition to this, naturally, inducible promoters like light-inducible can be used (Liu et al., 2023).

Following the farnesene productivity, electricity cost is the driving factor for MFSP. The reduced electricity cost reduces the MFSP. This cost can be reduced by utilizing electricity generated through renewable sources such as solar energy (Musi et al., 2017). An increase in the cost of isopropyl myristate also increases MFSP. Previously, Supelpak 2SV resins filled in a column attached to the exit port were used to trap farnesene (Halfmann et al., 2014). However, due to their higher cost and the replacement of resins every 3 days, organic solvents are now being sought to extract farnesene (Rautela et al., 2024b; Wang et al., 2021; Xu et al., 2023). Utilization of organic solvents can be a reliable method at the lab scale, but as observed, larger quantities are required at the industrial scale, which adds up to the selling cost of the product. This calls for the exploration of better

alternative methods for trapping farnesene rather than extracting it in the organic solvent (Yang et al., 2021). Utilization of the strategies discussed can lead to a substantial decrease in MFSP and a successful scale-up of the technology.

5.2.6. Financial performance indicators evaluation

Three financial performance indicators PBP, NPV and IRR were analyzed. PBP is a useful metric and aids in determining the time taken to recover the initial investment costs (Chandel et al., 2014). In the present study, a PBP of 7.9 years was calculated and compared to the economic life of the year (20 years). Shorter PBP are preferred, indicating quicker recovery and a more appealing investment opportunity (Luthra et al., 2015; Han et al., 2022). PBP of ~7.9 years can be further reduced upon utilizing the strategies defined in section 3.5. Meanwhile, the PBP does not consider factors such as the time value of money, cash flows and overall profitability (Luthra et al., 2015). In this regard, NPV and IRR provide a better understanding. NPV analysis utilizes projected cash flows, discount rate and the project length. NPV can be positive or negative, indicating that the project is expected to generate more value than its cost or might lead to a loss, respectively (Abdelhady, 2021). The NPV of $12.87 MM was estimated for the farnesene production plant, which considers CapEx, OpEx, total revenue generated, cash flow, discounted cash flow and length of the project. This indicates that the impact on the CapEx, OpEx and total revenue generated will have a direct impact on NPV. IRR is the discount rate at which the investment's NPV becomes zero, signifying the breakeven point for cash flow (Patrick and French, 2016). A positive IRR corresponds to a profitable investment. An IRR of 12% was calculated indicating a 12% discount rate to reach zero NPV.

Table 5.6 Potential environmental impacts associated with the production of 1 Kg of farnesene.

Environmental impacts	Total	Unit
Acidification potential	12.32	Kg SO_2 eq.
Eutrophication potential	0.88	Kg PO_4 eq.
Ozone layer depletion potential	0.00	Kg R11 eq.
Photochemical smog potential	0.70	Kg C_2H_4 eq.
Human toxicity potential	159.23	Kg DCB eq.

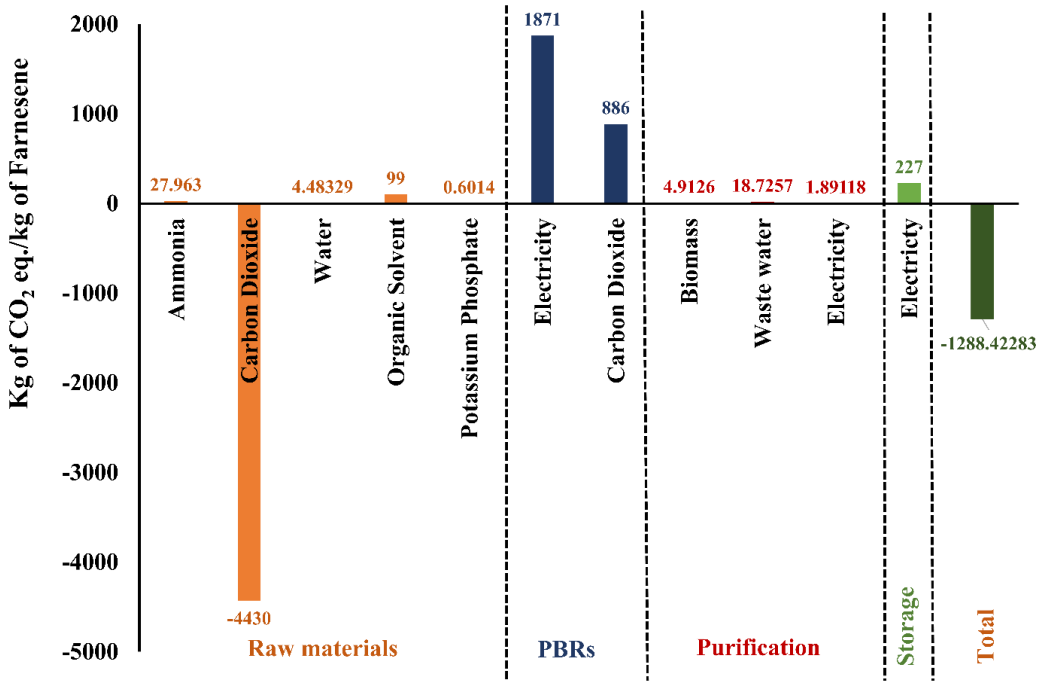


Fig. 5.6 Global warming potential analysis of cradle-to-gate life cycle assessment for per Kg of farnesene produced by engineered cyanobacteria.

5.2.7. Life cycle assessment for potential environmental impacts

Aside from the economics of the process, evaluation of the environmental impacts of the process plays a crucial role in scaling up the process to the industrial level. Hence, LCA was conducted to determine potential environmental impacts linked to producing 1 kg of farnesene (Table 5.6). The global warming potential (GWP) values associated with the different stages of farnesene production can be seen in Fig. 5.6. The most significant GWP value is attributed to the production stage in which the highest CO_2 emission is from the energy consumed by PBRs required for the fermentation process. This was followed by

the raw materials used in the process, such as carbon source (CO_2), nitrogen (NH_3) and potassium and phosphorus source (K_2HPO_4). Since the process utilizes the CO_2 (as a carbon source) from flue gas, unlike the farnesene production from the sugarcane feedstock, there is no CO_2 equivalent for the cultivation of sugarcane and pretreatment (Michailos, 2018; Moreira et al., 2014). Therefore, the total carbon emission of the current process is negative, and the process is carbon neutral.

5.3. Conclusion

The present study explored the economic and environmental aspects of farnesene production from engineered cyanobacteria, building upon previous research where *Synechococcus elongatus* UTEX 2973 was genetically engineered to produce farnesene with the highest productivity. The analysis serves as a conceptual framework for scaling up farnesene production with 100 tonnes annual capacity, highlighting key factors influencing MFSP and potential avenues for increasing productivity and reducing the cost of the process. The majority of investments in CapEx are attributed to plant commissioning charges, followed by PBRs and land cost. Whereas in OpEx, it is predominated by the cost of electricity. Sensitivity analysis highlighted farnesene productivity, electricity cost as the factors affecting MFSP. The NPV of $12.87 MM indicates that the plant will generate profit. Further, LCA reinforced the sustainability potential of cyanobacteria-based farnesene production compared to the conventional methods utilizing sugarcane as feedstock.