**PRODUCING NEXT-GENERATION BIOFUELS FROM FILAMENTOUS
CYANOBACTERIA: AN ECONOMIC FEASIBILITY ANALYSIS**

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Abbreviations

AC: Activated carbon
AISIM: Algae Income Simulation Model
ARID: Algae Raceway Integrated Design cultivation system
BOD5: Five-day biochemical oxygen demand
CIP: Clean in place units
DAF: Dissolved air flotation
EC: Electrocoagulation
FARM: Farm-level Algae Risk Model
HRAP: High rate algal wastewater pond
HTL-CHG: Hydrothermal Liquefaction-Catalytic Hydrothermal Gasification
KOV: Key output variable
MWWT: Municipal wastewater treatment
NAABB: National Alliance for Biofuels and Bioproducts
NPV: Net present value
NREL: National Renewable Energy Laboratory
PBR: Photobioreactor
VOC: volatile organic compound
VS: volatile solids

Abstract

The need for renewable, sustainable sources of biofuels continues to increase as the world's population continues to grow. Using microorganisms as biofuel producers is one area that is being researched extensively for this purpose. *Anabaena* sp. PCC 7120 is a filamentous strain of cyanobacteria capable of fixing atmospheric nitrogen, and has been genetically engineered to produce limonene, a cyclic hydrocarbon which has potential as a next-generation biofuel. This study analyzed the economic feasibility of a theoretical next-generation production facility that uses genetically engineered *Anabaena* 7120 to produce limonene. The economic feasibility of a limonene production facility was analyzed using the Farm-level Algae Risk Model (FARM). This model is an integrated systems compilation of numerous technoeconomic models that has been used previously in several algal production scenarios. FARM simulated 10 years of operation for the production facility for two scenarios. The 1st scenario used actual

limonene productivity data (0.018 mg/L/d) from a genetically engineered strain of filamentous cyanobacteria, while the 2nd scenario used a ‘best case’ assumption that limonene productivity can be increased 100-fold (1.8 mg/L/d). It was determined that the average probability of economic success of the 1st scenario at year 5 was 0%, while the average probability of success of the 2nd scenario was 100%. Assuming no fractional reductions in OPEX and CAPEX, the average net present value (NPV) at year 5 of the 1st scenario was -\$588 million, compared to \$392 million for the 2nd scenario. Further analysis determined that a limonene productivity of 1.02 mg/L/d is needed to yield an NPV of 0 dollars at year 5. This study shows strong evidence that a next-generation biofuel production facility utilizing genetically engineered strains of filamentous cyanobacteria could become economically feasible in the future if strains are developed with increased biofuel productivities.

Keywords

Anabaena sp. PCC 7120; Biofuel; Economic feasibility analysis; Farm-level algae risk model; Filamentous cyanobacteria; Limonene

1. Introduction

Environmental factors and market forces continue to drive the demand for next-generation biofuels. Adverse environmental impacts of greenhouse gas emissions from fossil fuel use include ozone depletion, global warming, and smog formation [1]. The widespread use of fossil fuels has led pollution, global climate change, and detrimental effects on the health of many organisms [2]. Production of next-generation biofuels using microorganisms, such as photosynthetic cyanobacteria, is one area being researched extensively to decrease dependency on fossil fuels. Cyanobacteria are prime candidates for this application due to their natural metabolic diversity [3]. Cyanobacteria are present in diverse habitats, ranging from polar regions to the tropics, [4-6], and have existed on Earth for at least 2.7 billion years [7]. They have morphologies ranging from unicellular to filamentous, and utilize the same photosynthetic process as higher plants [8]. Cyanobacteria also have the ability to grow on wastewater, which make them promising candidates for wastewater remediation [9].

Many filamentous strains of cyanobacteria capable of fixing atmospheric nitrogen have emerged as promising platforms in which to engineer production of fuels and chemicals [10]. Thus far, researchers have engineered filamentous cyanobacteria to produce high-value chemicals and potential next-generation biofuels such as limonene [11], farnesene [12], and linalool [13].

Process-model based technoeconomic analyses are commonly used to compare alternative processes and products [21-24]. Several programs capable of simulating processes for technoeconomic analyses are available. These include: Aspen Plus[®] [25], HOMER[®] [26], and Cadsim Plus[®] [27]. The Farm-level Algae Risk Model (FARM), formerly known as the Algae Income Simulation Model (AISIM), is a Monte Carlo firm level economic simulation model that

goes beyond a technoeconomic analysis. The FARM is designed to simulate the annual cultivation, harvesting, extraction, and financial/economic activities of an algae farm [28]. The model is an integrated systems compilation of several technoeconomic models for different phases of a commercial algae farm, and also includes the financial, marketing, and income tax aspects of a business [21]. The simulation results provide estimates of the probability of economic success for different pathways, and the sensitivity of production costs to changes in capital and operating expenses [21]. It is a preferred model for this study as it was designed specifically for processes involving photoautotrophic microorganisms. The similarities between algae and cyanobacteria allows for economic feasibility analyses with filamentous cyanobacteria using FARM.

Multiple algal farm scenarios have been modeled using FARM for the National Alliance for Biofuels and Bioproducts (NAABB) [15]. Of these, a scenario using a genetically modified algae grown in an Algae Raceway Integrated Design (ARID) cultivation system, harvested with an electrocoagulation (EC) harvesting system, and converted into biofuel using the Hydrothermal Liquefaction-Catalytic Hydrothermal Gasification (HTL-CHG) extraction method was the most promising [15]. We hypothesized that a system producing 4th generation biofuels directly from engineered filamentous cyanobacteria would have a higher probability of success than 3rd generation biofuels produced from algae, since downstream processes to convert oil into biofuels would not be needed. The highest-volume application for the engineered metabolism of microorganisms is production of transportation fuels [29], and at this time it appears that cyanobacteria are the sole renewable resource capable of meeting the global demand of transportation fuels [30-32]. This strongly suggests that cyanobacteria hold great potential for future next-generation biofuel production.

This study analyzed two scenarios with different limonene productivities. The 1st scenario used actual limonene production data from an engineered strain of filamentous cyanobacteria (0.018 mg/L/h) [11]. The 2nd scenario used a ‘best-case’ scenario which assumed a 100-fold increase in productivity to 1.8 mg/L/h. The latter productivity was calculated based on a claim from Joule Unlimited, Inc. that cyanobacteria can be engineered to produce 10 mg/L/h ethanol [33]. Ethanol’s molecular weight is 46 g/mole, while ethylene is 28 g/mole. Thus, the equivalent hydrocarbon productivity of 10 mg/L/h ethanol would be 6 mg/L/h, and we chose a more conservative productivity of 1.8 mg/L/h.

Limonene was chosen as the product for this system due to its high-value and the fact that production data from genetically engineered cyanobacteria is available [11]. However, this production system could also be used for other high-value volatile organic compounds (VOCs). Limonene is a cyclic hydrocarbon, which has potential as a biofuel [11, 34, 35] and as a precursor to jet fuel [36, 37]. Limonene’s immiscibility in water and low freezing point makes it a promising candidate as a biofuel [11]. While, limonene is not currently utilized as a source of fuel, it is used as a flavor, fragrance, and a green solvent [38]. However, as mentioned previously, the highest-volume application of genetically engineered microorganisms is the production of biofuels. Thus, in this study limonene was evaluated as a biofuel.

Fourth generation biofuels, such as limonene produced by filamentous cyanobacteria are fuels produced by genetically engineered algae and cyanobacteria that produce drop-in fuels [39]. The benefit of using drop-in fuels derived from cyanobacteria and algae is that they can be mixed with crude derivatives without the need to develop new fuel infrastructures [40]. The advantages of using photoautotrophic microorganisms to produce biofuels include their ability to grow rapidly due to their simple structure and they can convert CO₂ into organic compounds

[41]. Advantages that cyanobacteria have compared to algae regarding biofuel production are: cyanobacteria are generally easier to genetically manipulate, and algal biomass is typically used for biodiesel, while cyanobacteria can be engineered to produce target desired biofuels. Another advantage that cyanobacteria have is that cyanobacteria generally excrete their target products into the cultivation medium, thus the expensive operations of cell harvesting, disruption, and product separation can be avoided [42]. As mentioned above, limonene was selected for this study based on the fact that production data is available.

While next-generation biofuel production from filamentous N₂-fixing cyanobacteria is technically feasible, a thorough literature review has yielded no projections regarding economic viability. However, many studies on algal production of high-value chemicals and next-generation biofuels are available, and could serve as a model for comparison [14-20]. An economic feasibility analysis of a next-generation biofuel production process from filamentous cyanobacteria would provide a valuable comparison to well-established algal models, while at the same time identifying bottlenecks in the process. This will elucidate targets for future research to move towards the economically feasible production of next-generation biofuels from filamentous cyanobacteria.

2. The Farm-level Algae Risk Model (FARM)

FARM requires the Simetar[®] add-in for Microsoft[®] Excel to incorporate risk. Simetar[®] has been used extensively for risk analysis in business models and prospective businesses [43]. FARM can be considered as an integrated systems compilation of numerous technoeconomic and financial models for different phases on an algae farm [20]. FARM has been used in various scenarios [14-16, 18, 19] to determine economic feasibility of specific systems or technologies

related to algal farming. Richardson et al., [15] provides an overview of the functionality of FARM.

3. Process description and assumptions

Figure 1 illustrates the proposed limonene production process which is comprised of photobioreactors (PBRs), a gas cleaning unit, a gas stripping and limonene recovery unit, a solid fraction recovery unit, an anaerobic digestion unit, and a wastewater treatment unit [16, 44-46]. Table 1 presents the stream components of the process for limonene production in the 2nd scenario. In this economic feasibility analysis, data regarding cyanobacteria cultivation and growth were obtained from a recent study in which a strain of filamentous cyanobacteria had been genetically engineered to produce limonene [11]. For the cultivation phase, model data from the NAABB and National Renewable Energy Laboratory (NREL) were updated, modified, and used in FARM [15, 47, 48]. The number of PBRs and harvesting units were selected based on the desired throughput and required annual limonene production of the proposed system. The information on operating and capital expenses for several technologies used in this model was provided by the NAABB specific economic model (FARM) of algae crude oil production at a commercial size scale. For harvesting, dewatering and drying, data was obtained from a study by Berhane et al., [49].

In this study, a mass balance was determined on the different units in the process (Fig. 1). The mass balance accounts for the limonene production capacity of the facility and also the operational conditions for individual subunits. Also, a mass and energy balance was determined for the entire integrated system (including all 6 units shown in Figure 1). The notable assumptions for this study are summarized in Table 2. The operational data was adapted from

different technical reports and peer-reviewed research journals (Table 2), and the input-output data was calculated accordingly for both limonene production scenarios described below. Based on the limonene production capacity, cyanobacterial limonene productivity, and the cyanobacterial cultivation cycles, the total required volume of growth medium for the PBRs was calculated. The required land for the limonene production facility was considered to be non-cultivable and the PBR geometries were based on a study by Davis et al. [25]. In the solid fraction recovery unit, the growth medium needed per batch was calculated. Then, assuming a final biomass concentration of 10 g/L, the final biomass production after each cultivation batch was calculated. The annual amount of CO₂ required for the production system was calculated based on the amount of carbon fixed in the cultivated strain of cyanobacteria, amount of carbon dissipated in the gas stream, and the amount of carbon fixed as limonene in the system (Table 1). The required amount of activated carbon for the adsorption unit was calculated based on the required hourly adsorbing capacity, which was calculated based on the assumed limonene production capacity. For a complete limonene recovery cycle, 8 hours of adsorption and 8 hours of desorption was assumed.

Figure 1: Process flow diagram for limonene production.

Numbers in Figure 1 are used to indicate different streams in the process flow diagram. Solid lines indicate a liquid stream and dashed lines indicate a gas stream. Stream #1 is water which will be fed to the PBRs. Stream #2 is the nutritional stream where the chemicals needed for BG11 (Table 2) will be mixed with the water and used in the PBRs for cultivation. Stream #3 is a gaseous exhaust containing limonene that is sent to the adsorption unit. Stream #4 consists of the microwave regenerated activated carbon (AC) along with a 10% (w/w) of fresh activated carbon make up. Stream #5 consists of the spent AC that will undergo regeneration, limonene, and water. Stream #6 is limonene rich gaseous stream, resulting from microwave regeneration of spent AC. Streams #7-9 are the biomass + cultivation medium that concentrate the biomass. From the DAF unit, water is sent to algal ponds where it undergoes wastewater treatment and is recycled back into the system. Stream #10 is the biomass + liquid (160 g L^{-1}) that undergoes anaerobic digestion. Stream #11 is the produced biogas and biomass residues from anaerobic digestion that undergo combustion. Stream #12 is the gas produced from combustion that goes to the gas turbine. The thermal energy generated during combustion is used to create electricity in the gas turbine. Stream #13 is the gas that goes to the gas cleaning unit. Stream #14 is offsite flue gas that is used in the process. Stream #15 is the biomass residues that goes from the bottom of the clarifier to the anaerobic digestion unit.

223 **Table 1: Stream components in the process presented in Figure 1 for the 2nd scenario.** In the 1st scenario, all values are identical
 224 except for limonene, which is decreased 100-fold.

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Input and Outputs	1	2	3 ^b	4	5	6	7	8	9	10	11 ^{b,g}	12,13 ^{b,h}	14 ^b	15 ^b
	(ML batch ⁻¹)	(ton batch ⁻¹)	(m ³ s ⁻¹)	(kg batch ⁻¹)	(ton batch ⁻¹)	(kg h ⁻¹)	(ton day ⁻¹)	(ton day ⁻¹)	(ton day ⁻¹)	(ton day ⁻¹)	(m ³ batch ⁻¹)	(m ³ batch ⁻¹)	(m ³ s ⁻¹)	(L d ⁻¹)
water	2.348	43.52	0.181 ^c	0	11.469	45.5	159.44	94.663 ^f	9.566	5.979	358.8	5237.8	0	5979
CO2	0	0	0.033	0	0	0	0	0	0	0	1865.5	6744.5	0.39	0
Limonene	0	0	0.007	0	38.230	106	0	0	0	0	0	0	0	0
Solid biomass	0	0	0	0	0	0	0.956	0.956	0.956	0.956	0	0	0	0
Nitrogen	0	0	3.263	0	0	0	0	0	0	0	71.8	39032	2.67	0
Activated carbon	0	0	0	8080 ^d	0.404 ^e	0	0	0	0	0	0	0	0	0
Methane	0	0	0	0	0	0	0	0	0	0	4879	0	0	0
Other	0	4.35 ^a	0	0	0	0	0	0	0	0	0	0	0.21	0
Total	2.35	47.88	3.48	8080	50.103	151.5	160.39	96.619	10.523	6.936	7175	51014	3.26	5979

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228 *a: Nutrients used are shown in Table 2. b: represents gas streams. c: gas considered to be saturated contains 27.7 g_w/kg_{air}. d: Activated carbon in the adsorber- life time of carbon*
 229 *is considered to be 5 years. e: make up activated carbon. f: evaporation of water was considered to be 1 ton.day⁻¹. g: composition of the produced biogas after AD was considered*
 230 *as 68% CH₄, 26 % CO₂, 1% N₂, 0% O₂, 5 % H₂O h: composition of the flue gas was considered as N₂ 82%, CO₂ 12%, O₂ 5.5%.*

232 **Table 2: Notable assumptions used for this study.**

Process	Assumptions	Reference
Adsorption unit	Annual limonene production: 32,433 L/yr (Scenario 1) Condenser efficiency: 90% Adsorption unit efficiency: 85%. Adsorption/Desorption period: 8:8 h/h. Carbon make up: 10%. Carbon adsorption capacity: 30 %. Carbon bed density: 480.554 kg m ³	[11, 50] [51-43] [54, 55] [55]
Cultivation	Actual productivity data: 0.018 mg/L/d The 'best-case' scenario productivity: 1.8 mg/L/d Nutrient demands for cyanobacterial cultivation: BG ₁₁ medium for the required land estimation. Cost: \$ 500/acre Productivity of strain: 1.25 kg. m ⁻³ day ⁻¹ Sparged gas: 5% CO ₂ and 95% air - flow rate: 0.25 L/L/min Cyanobacteria growth cycle: 15 days. Log phase 12 days. Wastewater taken after secondary treatment	[11] [33] [16, 17, 25, 56] [25] [57]
Harvesting	Biomass concentration send for harvesting: 10 g L ⁻¹ Settling tank: Autoflocculation: concentrates the cyanobacteria to 1% Dissolved air flotation (DAF): concentrates the cyanobacteria to 10%. Electrocoagulation unit concentrates the slurry to 16%.	[25] [25] [49, 58] [45, 59, 60]
Energy recovery	Anaerobic digester: 0.5 m ³ methane per kg of residual biomass The composition of the flue gas from the gas turbine: 82% N ₂ , 12% CO ₂ , and 5.5% O ₂ based on 68% methane biogas produced in the AD	[61-63] [62, 64]
Wastewater treatment	Number of clarifiers in WWT system: 1 The retention time of the clarifier: 2 hours Overflow rate of the clarifier: 30 – 50 m ³ /m ² /d Wastewater hydraulic retention time in HRAPs: 4 days g of BOD ₅ removed per g of algae: 1.4	[65, 66] [67] [66, 67] [45, 65] [68]

3.1 Cyanobacteria cultivation

The strain utilized in the production system is a genetically engineered *Anabaena* sp. PCC 7120 (herein referred to as *Anabaena* 7120) capable of limonene production [11]. The required nutrients, growth rates, productivity and limonene production yield data for the cultivation phase were obtained from recent studies on this engineered strain [11, 50]. *Anabaena* 7120 is capable of fixing atmospheric nitrogen, which could be an advantage in biofuel production since combined nitrogen is a major cost in large scale biofuel strategies [11, 69, 70]. Currently the engineered strain contains the limonene synthase gene on a plasmid, thus requiring addition of an antibiotic (100 µg/ml neomycin) to the cultivation medium to ensure plasmid retention. The use of antibiotics is undesirable in industrial applications due to the additional costs of the antibiotics and the metabolic burden associated with retaining plasmids [71, 72]. For the purpose of this study, it is thus assumed that the gene can be integrated into the chromosome without any change in limonene production, thereby eliminating the need for antibiotics.

The PBRs used in this study are assumed to be tubular plastic cylinders with a 38 cm diameter and a height of ~2 m. There are 10,544 PBRs in the facility, and the volume of each reactor is 0.227 m³. The cycle time of each PBR is 15 d. Based on energy costs and current productivity, Schenk et al. [31] state that PBR costs should not exceed \$15 m⁻², thus this number was used in this analysis. The PBRs will be cooled using a sprinkler system that sprays water on the PBR surface [19, 25]. Nutrient demands for cyanobacteria cultivation will be met using BG11 medium [73].

Cyanobacteria production systems require a CO₂ supply for a carbon source, as well as to control pH, since pH is linked to the CO₂ concentration [74]. The CO₂ supply must be precisely controlled so that carbon does not become limiting, yet does not reach a concentration that

becomes inhibitory due to high CO₂ partial pressures [74, 75] or low pH. A mixture of 5% CO₂ and 95% air will be sparged into the PBRs at a rate of 0.25 L/L/min [57]. The CO₂-enriched air flow will also provide gas exchange by stripping out excess dissolved oxygen, as well as the produced limonene. The sparged gas will also create turbulence sufficient to keep cells in suspension. In this study, it is assumed that the PBRs can utilize light as their primary source and that no artificial light is needed for cultivation. It is assumed that cultivation takes place in a greenhouse as described by Halfmann et al., [11].

3.2 Limonene recovery

Limonene is a cyclic monoterpene that is a promising biodiesel and next-generation biofuel candidate due to its immiscibility with water and low freezing point [11, 76]. It is also a biodegradable solvent that can replace products such as acetone in organic solvents [77]. Limonene was chosen as the product for this system due to its high-value and the fact that production data from genetically engineered cyanobacteria is available [11]. This production system could also be used for other high-value volatile organic compounds (VOCs).

Product recovery must be inexpensive for any bioprocess to become economically feasible. An economically favorable means of limonene recovery from dilute fermentation streams may be gas-stripping [78]. Gas-stripping is a process by which a gas is constantly bubbled through the culture fluid to volatilize the chemical and some water vapor, which is then condensed [79]. One issue with using gas-stripping with cyanobacteria cultures is that the gas stream exiting the PBR would be very dilute. Cold condensation temperatures (-15°C) would be required to condense limonene from the vapor stream. This is because the temperature required to condense limonene from a vapor stream is directly correlated with the limonene concentration.

The additional costs involved with cooling, would offset the benefit of gas-stripping, thus the gas-stripping vapor can be passed through an absorber unit initially to concentrate the chemical. This would take place instead of using direct condensation (Figure 1). Then, a small volume of gas can be used to displace limonene from the absorber unit, which would result in a higher concentration of limonene in the secondary vapor. This process would allow cold water to be used to condense limonene [51].

Activated carbon (AC) has been used previously to adsorb volatile organic compounds, such as formaldehyde, n-hexane, benzene, toluene, ethanol, and limonene from gas streams from various industries [80]. Positive attributes of AC include: high porosity, large surface area, rapid adsorption capabilities, and a variety of pore sizes and structures. These attributes strongly suggest that AC would provide a relatively inexpensive option for recovering and concentrating limonene in gas-stripping vapor. To collect limonene produced from genetically engineered cyanobacteria, a column packed with AC will be connected to the outlet of the PBRs. The gas-stripped limonene vapor will enter the column and be adsorbed by the porous AC. After the AC column is saturated, hot air will be passed through the column to desorb limonene, and this limonene-enriched air will be passed through a cold water condenser to condense limonene and water vapor, which will then phase separate. [52]. The regenerated AC column can then be placed back into operation.

3.3 Solid Fraction Recovery

A successful low-energy and low-cost method of harvesting cell biomass is essential for the proposed system. The harvesting method must have low capital, operating, and maintenance costs. The design of the harvesting unit should provide ease of operation and integration with

cultivation facilities to limit pumping and power requirements. In addition, it should have low environmental impacts and low carbon and water footprints [81]. The harvesting strategy used in this study was based on the most promising of several NAABB developed technologies modeled by Richardson and Johnson [15].

Biomass will be harvested using a retention tank that concentrates the cyanobacteria to 10 g/L via autoflocculation [25]. Autoflocculation occurs due to high pH in cyanobacteria cultures caused by photosynthesis, in which precipitation of Ca, Mg, and phosphate ions form a chemical flocculating agent that results in cell settling [64]. This will be followed by dissolved air flotation (DAF) which thickens the material to 100 g/L, and electrocoagulation (EC) which concentrates the slurry to 160 g/L [44, 49]. DAF involves dissolving air at a high pressure in a saturator via a pump and releasing at a high pressure in the flotation cell. The sudden reduction in pressure of the water stream causes the formation of bubbles which creates the driving force on the biomass to move upward [58]. EC works by generating coagulating ions via electro-oxidation of an anode *in situ* when direct current is employed [59] In this study, the capacity of the EC unit was determined based on Kovalcik et al. [39]. This combination of solid fraction recovery steps was chosen as presumably it will minimize downstream processing costs [60].

3.4 Anaerobic Digestion

In anaerobic digestion (AD) units, organic biomass is biologically converted to biogas and residual sludge, which can be used as fertilizer. The organic matter is partially degraded by different anaerobic microorganisms by a succession of biological reactions: hydrolysis, acidogenesis, acetogenesis, and methanogenesis [64, 82]. Advantages of using AD in an industrial process include: considerable reduction in the organic and pollution load, improved

economic profile, improved energy recovery, and odor suppression [83]. The chemical composition and the physical characteristics of the produced biogas are highly dependent on the substrate and the organic matter load. Typically, it is a mixture of methane and CO₂ and may contain other gases including: N₂, O₂, H₂O, H₂S and NH₃ [64, 83].

Anabaena spp. contain approximately 5% lipids [84], 45% proteins [85] and 31% carbohydrates [86] which makes it an attractive substrate for AD [87]. The theoretical methane yield is defined as liters of methane per gram of volatile solids (VS) and is expressed as L CH₄ per g VS. It can be calculated based on the stoichiometric conversion of organic matter to methane and CO₂ [63]. Lipids have a higher theoretical methane yield (1.0 L CH₄ per g VS) compared to proteins and carbohydrates (0.85 L CH₄ per g VS and 0.42 CH₄ per g VS, respectively [61]. However, methane yields for algal biomass are lower (0.09 – 0.34 CH₄ per g VS) due to the resistance of algal cell membrane components to digestibility, and it is assumed cyanobacteria will have similar characteristics. Thus, the retention times required for digestibility in the AD would be high (~20-30 days). Typically, the AD units will produce a sulfur-free high energy content biogas (up to 68%), which is non-corrosive and suitable for engine generators [61, 62]. In the theoretical production facility evaluated in this study, the pre-concentrated biomass from the solid fraction recovery unit is fed to a high-rate AD [61] to produce biogas, which can be further converted to electrical and thermal energy. It is also assumed that during the combustion process, the steam produced in the combustor will be fed into an energy recovery system, which will generate electricity via a steam turbine. The high protein content of *Anabaena* spp. suggests that the biomass could also be used as a single cell protein, which has been done previously with microalgae strains [88]. This is an area that could be explored and potentially used in future modeling studies.

3.5 Wastewater treatment and gas cleaning units

A wastewater treatment unit, based on conventional municipal wastewater treatment (MWWT) facilities was considered in this study with some minor modifications. In our system, the flow rate is much lower than that of conventional MWWTs [66]. Also, in the primary treatment stage of a municipal wastewater facility, a primary clarifier is employed as a sedimentation basin for continuous removal of solid fractions. In this study, the primary clarification unit was omitted, assuming that primary solids will be fractionated in high rate algal wastewater ponds (HRAPs) [65]. HRAPs are wastewater treatment lagoons that use heterotrophic bacteria for the aerobic degradation of organic compounds and microalgae for oxygen production [70, 89, 90]. In this study, wastewater was assumed to be directly sent to the HRAPs with an average hydraulic retention time of 4 days [91].

One way that the concentration of algae in ponds can be calculated based on the required oxygen to satisfy five-day biochemical oxygen demand (BOD₅) in the pond. It was assumed that 1.55 g O₂ was produced per gram of algae biomass, and 1.1 grams O₂ is required per gram of BOD₅ removed. Therefore, 1 g of algae would remove 1.4 g of BOD₅ [68]. The retention time of the clarifier was assumed to be 2 hours and the overflow rate was assumed to be 30 – 50 m³/m²/d [67]. These solids will be sent to an AD unit for biological conversion, where energy is required for the solids scraper as well as for sump pumps to transfer the solids to the AD unit [91].

In this study, it was assumed that the flue gases from the gas turbine are used as the primary source of CO₂ required for cyanobacterial cultivation. We also assume that the limonene production facility will be constructed next to a coal or natural gas power plant and that the production facility can utilize the flue gases from the plant. The AD units produce a sulfur-free biogas which is burned in the turbine and results in a sulfur-free flue gas stream [62]. The

composition of the flue gas from the gas turbine is 82% N₂, 12% CO₂, and 5.5% O₂ based on 68% methane biogas produced in the AD [62]. Conventional flue gases, such as what will enter the production facility from the neighboring power plant, contain mainly carbon monoxide (CO), nitrogen oxides (NO_x), sulfur dioxide (SO₂), nitrous oxide (N₂O), hydrogen chloride (HCl), and hydrogen fluoride (HF). Thus, a gas cleaning unit is required.

There are different technologies available for gas cleaning including dry scrubbing processes and wet processes. The wet processes, which are usually proposed for hazardous waste and household waste combustion facilities, are costly and energy extensive [92]. In this production facility, a conventional dry gas cleaning unit was used. In this technology, dust and hazardous materials are removed in a fabric filter based on physical sorption or chemistry sorption. The blowing agent, NaHCO₃, is initially blown into the flue gas stream where pollutants are chemically bonded to the agent [92]. Then, the agent is deposited in a bag filter and the clean gas is sent to the PBRs.

3.6 Other notable assumptions

For this hypothetical production process, location has minimal impact on the productivity of the system as cultivation takes place in a greenhouse where light will not be a limiting, as described by Halfmann et al. [11]. The land needed for this hypothetical production facility is dependent on the size and involved technology of the production system. Environmental, geographic, and technological characteristics of the land are important determinants for the suitability of the land. The land used for this production system is considered to be located in the Southwestern USA. [16]. In addition, the land is considered to be non-arable to improve the economics of the system. Based on the selected region, the cost for land is assumed to be very low at \$ 500/acre [17, 56], and located next to a coal or natural gas power plant. In the event that

land this cheap is not available next to a power plant, the change in CAPEX will be minimal due to the small amount of land needed to cultivate cyanobacteria in PBRs. For example, if land for the production facility was purchased at \$5000/acre, the change in CAPEX would only be \$23,310, and the change in CAPEX would only be 1.6%. Considering the estimated production cost of \$107.9 million of both scenarios at year 5, this number is inconsequential.

The cyanobacterial biomass consists of 40 to 50% carbon [93], which suggests that 1.5 to 2.0 kg of CO₂ is required for each 1 kg of cyanobacteria produced in the cultivation phase. CO₂ is directly injected into PBRs via internal sparging through 3 mm diameter nozzles located at the bottom of the illuminated reactors. CO₂ expenses from the production facility are assumed to be \$0 due to the neighboring power plant. The production facility is assumed to be operational continuously for ~8000 h/yr (330 d/yr, 24 h/d). Cultivation and harvesting are considered at a continual basis for 24 h/d for plant operation of 330 d/yr. Cyanobacterial productivity is assumed to be 1.25 kg/m³/d based on Davis et al. [25]. Thus, harvesting volume is limited to 37.5 kg/m³/month based on the required annual production. For cyanobacterial cultivation, a lag phase of 3 days was considered in which the microbes adapt to the growth conditions. Afterwards, 10 days of log phase was considered with a limonene production of 0.018 mg/L/d (1st scenario) or 1.8 mg L/d (2nd scenario). Day 14 is assumed to be the beginning of the stationary phase where chemical production typically decreases and cultivation medium is sent for harvesting.

The condenser for recovering limonene is considered to be a refrigerated type due to the high expected VOC contents in the gas stream. For these condensers, the efficiency is expected to be > 90%, thus 90% is assumed for this system. The condenser efficiency and associated costs are taken from a study by Shareef et al., [53]. Fixed-bed units are employed for limonene adsorption from the gas stream. During adsorption, limonene outlet will increase from zero (at

the start of the cycle) to the breakthrough concentration (at the end of the cycle). The efficiency is considered as a function of time, and can be calculated via integration over the length of the absorption cycle. An overall efficiency of 85% is considered in this study for a shorter adsorption cycle. Overall cost estimations for this unit were obtained from Vataavuk et al., [54]. Adsorption and desorption times are important for determining the required AC and can be calculated based on adsorption theory. This is also important for establishing desorption auxiliary equipment and utility requirements [54]. In this study, one shift at 8 h/d was considered for adsorption and one 16 h/d shift was considered for desorption or standby.

Two different approaches are available for estimating the required amount of AC in adsorbing systems. One approach considers the unsteady-state energy and mass transfer phenomena occurring in the adsorbent bed. In the other approach, the carbon requirement is estimated based on the nature of the VOC and other parameters in the absorbers. In this study, the amount of required carbon is considered based on the former approach. Also, 10% of fresh carbon make up is considered for the system. Microwave regeneration of AC is suggested to reduce downstream expenses for limonene recovery [94]. The adsorber sizing procedure will be determined via the bed surface loading rate. In this study, a standard 4-foot carbon bed depth with a maximum gas loading of rate of 100 cfm per square foot of bed surface is maintained. This will ensure adequate gas contact and sufficient time to reach adsorption equilibrium. Adsorption capacity, which is defined as the amount of target product capable of being adsorbed on a unit weight of activated carbon, is dependent on characteristics of the target product, the temperature of the gas stream, and also on the type of AC in use. Typical adsorption capacities range from 5% to 30% of the weight of the carbon [55]. Based on a 30% adsorption capacity of the applied AC, the amount of required activated carbon was calculated.

As mentioned previously, the annual limonene production of the 1st scenario was 32,433 L/yr, and the annual limonene production of the 2nd scenario assumed a 100-fold increase in productivity (Table 2). The electricity required for condensation and adsorbers as well as the microwave power consumption required for the regeneration of activated carbon would be proportional to the produced limonene and thus was considered 100-fold higher in the 2nd scenario. The electricity required to operate PBRs and the nutrients required for cultivation in both scenarios were considered to be identical. The increase in limonene productivity has no impact on biomass production as limonene is excreted from the cell, which is why these parameters are identical. The water necessary for cyanobacterial cultivation and the energy required by the PBRs for cultivation were also considered to be identical. The theoretical required CO₂ and H₂O fixed in the biomass retentate is identical in both scenarios as the biomass retentate is assumed to be identical in both scenarios (Table 2). However, the theoretical required CO₂ and H₂O fixed in limonene molecules would be 100-fold higher in the 2nd scenario. This has been calculated based on the stoichiometry of the limonene production. The energy required for supplying CO₂ to the production facility was considered to be identical in both scenarios as the enriched gas will be supplied and recycled through the system. The additional CO₂ needed in the 2nd scenario would be supplied from a neighboring plant and was considered to be equal to the amount of carbon fixed in the limonene. The biomass production in both scenarios is also identical, which results in equal energy inputs in the harvesting unit, including electricity required for mixing, energy required by the DAF unit, and the energy required by the EC unit. As the biomass retentate, which is supplied to the AD unit was considered to be identical in the 1st and 2nd scenarios, the biogas production and corresponding input-output values were also considered to be equal in both scenarios.

4. Results and discussion

FARM simulated 10 years of operation for the production facility using 2 different scenarios. The 1st scenario used actual production data from a limonene producing filamentous cyanobacteria strain [11]. The 2nd scenario assumed a 100-fold increase in limonene productivity compared to the 1st scenario. The summary statistics for the key output variables (KOVs) from the 1st and 2nd scenario are shown in Table 3 and 4, respectively. The fractional mean of production costs, taxes, dividend costs, and non-production costs are shown in Figure 2.

Table 3: Summary statistics for KOVs used in the 1st scenario.

Iteration (1st scenario)	Mean	StDev	CV	Min	Max
Production Costs (M\$)	107.933	5.816	5.388	90.578	131.429
Interest (M\$)	35.293	6.785	19.225	20.975	55.412
Dividends (M\$)	0.080	0.000	0.000	0.080	0.080
Principal	0.162	0.000	0.025	0.162	0.162
Replacement	0.029	0.000	0.091	0.029	0.029
Taxes	0.000	0.000	0.000	0.000	0.000
Total Costs (M\$)	143.466	9.035	6.298	119.591	173.614
Prod Cost Fraction	0.754	0.037	4.844	0.638	0.844
Interest Fraction	0.245	0.037	14.942	0.154	0.360
Dividends Fraction	0.001	0.000	6.277	0.000	0.001
Principal Fraction	0.001	0.000	6.277	0.001	0.001
Replace Fraction	0.000	0.000	6.276	0.000	0.000
Taxes Fraction	0.000	0.000	0.000	0.000	0.000
TC (\$/Ton of Biomass) Yr 5	14.335	1.176	8.205	11.713	19.208
Bio Prod Yr 5 (tons)	10242208.358	474725.713	4.635	8203675.851	11224538.020
Rec (M\$) 5	2.247	0.431	19.178	1.612	5.193
NCI (M\$) 5	-140.979	9.044	-6.415	-171.255	-117.520

478 **Table 4: Summary statistics for KOVs used in the 2nd scenario.**

Iteration (2nd scenario)	Mean	StDev	CV	Min	Max
Production Costs (M\$)	107.918	5.584	5.174	88.346	127.168
Interest (M\$)	0.876	0.156	17.858	0.505	1.361
Dividends (M\$)	17.896	6.776	37.860	7.540	65.255
Principal	0.162	0.000	0.025	0.162	0.162
Replacement	0.029	0.000	0.091	0.029	0.029
Taxes	35.105	15.810	45.036	10.940	145.609
Total Costs (M\$)	161.954	22.934	14.161	121.927	313.310
Prod Cost Fraction	0.676	0.080	11.810	0.324	0.851
Interest Fraction	0.005	0.001	21.176	0.003	0.009
Dividends Fraction	0.108	0.023	21.241	0.057	0.208
Principal Fraction	0.001	0.000	11.868	0.001	0.001
Replace Fraction	0.000	0.000	11.864	0.000	0.000
Taxes Fraction	0.209	0.058	27.603	0.086	0.465
TC (\$/Ton of Biomass) Yr 5	12.675	0.977	7.705	10.701	17.174
Bio Prod Yr 5 (tons)	10236132.713	488529.290	4.773	8258798.428	11002757.950
Rec (M\$) 5	227.566	45.171	19.850	153.018	536.786
NCI (M\$) 5	118.773	45.170	38.031	49.732	434.500

479

480

A

B

Figure 2: Fractional mean of production costs, taxes, dividend costs, and non-production costs. (A: 1st scenario; B: 2nd scenario).

The mean annual production cost of both scenarios at year 5 was \$107.9 million. The reason that production costs were identical for both scenarios even though limonene productivity was increased 100-fold in the 2nd scenario is that the amount of biomass that needs processed does not change. Limonene is excreted from the cyanobacterial cell and can be collected without disrupting the cell. Also, the equipment used in the production facility was selected based on the limonene productivity of the 2nd scenario, thus equipment costs were identical. However, in the 1st scenario it constituted 75% of the total OPEX, while in the 2nd scenario it constituted 68% of the total OPEX. In the 1st scenario, the non-production costs constitute 24% of the total OPEX, compared to 0.7% in the 2nd scenario. Non-production costs consist of principal, replacement of machinery, and interest costs. The reason that the 1st scenario has a larger percentage of the OPEX consisting of non-production costs is due to interest still being paid at year 5 in the 1st scenario. Both scenarios evaluated would produce ~11 billion tons of biomass at year 5. The production cost for the 1st scenario at year 5 would be \$15.10 per L limonene produced, compared to \$0.15 per L limonene produced for the 2nd scenario. The model estimated the commodity price of limonene at year 5 will be \$4.66 per L, which shows why the 2nd scenario is economically feasible and the 1st scenario is not.

The average probability of economic success and NPV at year 5 for the 1st and 2nd scenarios assuming fractional reductions in CAPEX and OPEX is shown in Table 4 and 5, respectively. Each scenario was simulated for 100 combinations reducing CAPEX and OPEX in 10% increments from 0 to 90% [21]. In the 1st scenario, every scenario in the 10x10 matrix yields a 0% probability of economic success at year 5. Assuming no fractional reduction in CAPEX or OPEX, the average NPV at year 5 was -\$588 million. Reducing the CAPEX and OPEX by 0.9 would still yield a 0% probability of economic success and the average NPV was -

\$48 million. In the 1st scenario, every scenario in the 10x10 matrix yields a 100% probability of economic success at year 5. Assuming no fractional reduction in CAPEX or OPEX, the average NPV at year 5 was \$392 million. Reducing the CAPEX and OPEX by 0.9 increases the average NPV to \$695 million. Reductions in CAPEX have little impact in increasing the NPV of either scenario because the total CAPEX for both scenarios is \$1.5 million. Thus, fractional reductions have little effect on the NPV of each scenario, which is -\$588 million for the 1st scenario and \$392 million for the 2nd scenario when no fractional reductions are considered. In the model, it was determined that a limonene productivity of 1.02 mg/L/d is needed to yield an NPV of 0 dollars at year 5.

Table 5: Average probability of economic success and NPV at year 5 for scenario assuming fractional reductions in CAPEX and OPEX for the 1st scenario.

Probability of Economic Success and Net Present Value (M\$)

ARID Fraction OPEX	Fractional Reductions in the CAPEX									
	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%
	-588	-588	-588	-588	-587	-587	-587	-587	-587	-587
	0.1	0%	0%	0%	0%	0%	0%	0%	0%	0%
	-528	-528	-528	-528	-528	-527	-527	-527	-527	-527
	0.2	0%	0%	0%	0%	0%	0%	0%	0%	0%
	-468	-468	-468	-468	-468	-468	-467	-467	-467	-467
	0.3	0%	0%	0%	0%	0%	0%	0%	0%	0%
	-409	-408	-408	-408	-408	-408	-408	-407	-407	-407
	0.4	0%	0%	0%	0%	0%	0%	0%	0%	0%
	-349	-349	-348	-348	-348	-348	-348	-348	-347	-347
0.1	0.5	0%	0%	0%	0%	0%	0%	0%	0%	0%
	-289	-289	-289	-288	-288	-288	-288	-288	-288	-287
	0.6	0%	0%	0%	0%	0%	0%	0%	0%	0%
	-229	-229	-229	-229	-228	-228	-228	-228	-228	-228
	0.7	0%	0%	0%	0%	0%	0%	0%	0%	0%
	-169	-169	-169	-169	-169	-168	-168	-168	-168	-168
	0.8	0%	0%	0%	0%	0%	0%	0%	0%	0%
	-109	-109	-109	-109	-109	-109	-108	-108	-108	-108
	0.9	0%	0%	0%	0%	0%	0%	0%	0%	0%
	-50	-49	-49	-49	-49	-49	-48	-48	-48	-48

Table 6: Average probability of economic success and NPV at year 5 for scenario assuming fractional reductions in CAPEX and OPEX for the 2nd scenario.

Probability of Economic Success and Net Present Value (M\$\$s)

ARID Fraction OPEX	Fractional Reductions in the CAPEX									
	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0	100% 392	100% 392	100% 392	100% 392	100% 392	100% 392	100% 392	100% 393	100% 393	100% 393
0.1	100% 425	100% 425	100% 425	100% 426	100% 426	100% 426	100% 426	100% 426	100% 426	100% 426
0.2	100% 459	100% 459	100% 459	100% 459	100% 459	100% 459	100% 460	100% 460	100% 460	100% 460
0.3	100% 492	100% 492	100% 493	100% 493	100% 493	100% 493	100% 493	100% 493	100% 493	100% 494
0.4	100% 526	100% 526	100% 526	100% 526	100% 526	100% 527	100% 527	100% 527	100% 527	100% 527
0.5	100% 559	100% 560	100% 560	100% 560	100% 560	100% 560	100% 560	100% 560	100% 561	100% 561
0.6	100% 593	100% 593	100% 593	100% 593	100% 594	100% 594	100% 594	100% 594	100% 594	100% 594
0.7	100% 627	100% 627	100% 627	100% 627	100% 627	100% 627	100% 627	100% 627	100% 628	100% 628
0.8	100% 660	100% 660	100% 660	100% 661	100% 661	100% 661	100% 661	100% 661	100% 661	100% 661
0.9	100% 694	100% 694	100% 694	100% 694	100% 694	100% 694	100% 694	100% 695	100% 695	100% 695

A summary of operating expenses for the hypothetical limonene production facility at year 5 of the 10 year simulation is provided in Appendix A. CAPEX values are identical for both scenarios even though limonene productivity is 100-fold greater in the 2nd scenario because the limonene productivity does not change the amount of biomass produced. Also, the equipment used in the production facility was selected based on the limonene productivity of the 2nd scenario, thus equipment costs were identical. These values were calculated by FARM, which has been used extensively for modeling studies involving photoautotrophic microorganisms [14-16, 18]. A summary of capital expenses for the production facility is provided in Appendix B. Again, for the reasons explained above, these expenses would be identical for both scenarios.

Comparisons can be made between this model, specifically in the 1st scenario which uses actual production data, and an algal farm scenario that was also modeled using the FARM. The algal farm scenario was considered to be the most promising of the seven scenarios evaluated for the NAABB [15]. It should be noted that this is not an ‘apples to apples’ comparison. The analysis in this study evaluates the best case scenario for many aspects of the operation while the algal study evaluated technologies that were currently available and have been proven at a scaled up rate, using actual yields verified by field values, not maximum theoretical yields. The cyanobacterial model had a 100% probability of economic success compared to a 0% probability of economic success for the algal model. There are several factors that caused the cyanobacterial model to have a much greater chance of economic success than the algal model. The total CAPEX for the algal model was \$1.1 billion compared to \$1.5 million for the cyanobacterial model. The reason for the large difference is the cost of land (\$35.9 million), construction (\$158.98 million), and liners (\$156.9 million) for the algal model, which cultivates the microbe outdoors and needs large amounts of land for cultivation. In the cyanobacterial model, there is an 82-fold increase in liter of product per acre of land compared to the algal model. In the cyanobacteria model, cultivation takes place inside PBRs, thus there was no need for liners and the cost of land combined with construction was only \$0.063 million. The algal model also had a larger OPEX value compared to the cyanobacterial model at year 5 of the simulation, \$261.1 million vs. \$162.0 million, respectively. The major operating cost that causes the substantial difference between the algal scenario and the best case cyanobacterial scenario is interest. In the algal model at year 5, interest was \$102.2 million compared to \$0.5 million in the cyanobacterial model. The majority of OPEX at year 5 of the cyanobacterial model is from costs associated with the EC unit (\$83.0 million).

By altering the limonene productivities in the model, we were able to determine that a productivity of 1.02 mg/L/d is needed to yield an NPV of 0 dollars at year 5. This suggests that in the production facility described in this study, limonene productivity greater than 1.02 mg/L/d would yield an economically feasible production facility. However, if the productivity facility was changed in any way, such as the amount of PBRs used to cultivate cyanobacteria, the productivity needed to obtain an NPV of 0 would also probably change. Since the current limonene productivity of the genetically engineered cyanobacteria strain is only 0.018 mg/L/d, this study strongly suggests that the production facility described in this study would not be economically feasible at this time.

5. Conclusions

Developing, renewable sustainable sources of chemicals and next-generation biofuels is becoming increasingly important as the global population continues to grow. Cyanobacteria have received a considerable amount of attention from industrial microbiologists due to their ability to produce these chemicals and fuels using minimal nutrient inputs. Filamentous cyanobacteria strain development is an area that needs to be improved to achieve the goal of an economically successful next-generation biofuel facility. Biofuels, such as limonene, are typically toxic to the microbe that produces them [95, 37, 96-98]. Thus, for a production process to become economically feasible, it will be necessary to develop strains able to tolerate the titers of chemicals that they are producing. This can be done via several mutagenesis techniques, including directed evolution [99]. Developing renewable, sustainable sources of fuel is becoming increasingly important.

In the hypothetical production facility described in this study, limonene was produced by genetically engineered filamentous cyanobacteria. The reason that limonene was chosen for this study was because production data was available [11]. However, this production facility would be appropriate for processes in which cyanobacteria produce other VOCs that could be utilized as 4th generation biofuels. It is clear that limonene productivity by the cyanobacterial strains needs to be substantially increased in order to achieve a economically feasible process. While there are several studies that have evaluated large scale biofuel processes involving algae, to the best of our knowledge this is the first study detailing a large scale biofuel production process from filamentous cyanobacteria. This study shows strong evidence that a next-generation biofuel production facility utilizing genetically engineered strains of filamentous cyanobacteria could become economically feasible in the future.

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Declaration of authors' contributions

All of the authors of the manuscript were involved in the conception and design of the study, or acquisition of data, or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, and final approval of the version to be submitted.

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