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Producing next-generation biofuels from filamentous cyanobacteria: An economic feasibility analysis

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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.

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

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.

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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 CO_2 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 N_2-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

Fig. 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 Fig. 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