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Life cycle analysis of a large-scale limonene production facility utilizing filamentous N_2-fixing cyanobacteria

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ABSTRACT

Due to the adverse effects of fossil fuel use, it is becoming increasingly important to produce next-generation biofuels from renewable, sustainable sources. Filamentous N_2-fixing strains of cyanobacteria have emerged as promising industrial microorganisms capable of producing a range biofuels and chemicals using CO_2, water, and sunlight. In this study, a life cycle analysis (LCA) was conducted on a hypothetical production facility that uses a genetically engineered strain of filamentous cyanobacteria to produce the cyclic hydrocarbon limonene. Two scenarios were evaluated in which the only difference between the scenarios was the limonene productivity of the engineered cyanobacteria strain. In Scenario 1, the cyanobacterium was assumed to produce limonene at a rate of 1.8 mg/L/h, resulting in an annual production of 32,727 L/yr of limonene. In Scenario 2, limonene productivity was 55.5 mg/L/h, resulting in annual production of 1,000,000 L/yr. Both scenarios were assumed to produce the same amount of cellular biomass, that was converted to biogas by anaerobic digestion and the biogas was converted by gas turbines into electricity to power the facility. Excess electricity was assumed to be sold to the grid. The major environmental burdens of the facility, which were measured in eco-points and calculated based on the Eco-indicator 99 method, were the cyanobacteria nutrient supply (especially sodium nitrate) and the photobioreactor (PBR) electrical requirements. The lower output of limonene in Scenario 1 meant that less energy was required for product recovery, leaving more electricity for sale to the grid. Even though a higher limonene productivity will worsen the environmental profile of the process, both scenarios described in this study have less of a negative environmental impact than biodiesel production. This study strongly suggests both scenarios of the theoretical limonene production facility described herein holds great potential as a future solution for producing next-generation biofuels directly from solar energy.

1. Introduction

Developing renewable, sustainable sources of biofuels is necessary to decrease the environmental burden created by the extensive use of fossil fuels. Fossil fuel reserves are finite and the adverse effects of fossil fuel-generated greenhouse gases are well established [1–3]. Biofuels can be categorized based on the type of feedstock used and/or the type of fuel produced. Each new generation of biofuel has been developed to overcome limitations or disadvantages of prior generations. This categorization has led to 4 generations of

Abbreviations: AC, activated carbon; AD, anaerobic digestion; AISIM, Algae Income Simulation Model; CIP, clean-in-place units; DAF, dissolved air flotation; EC, electrocoagulation; FARM, Farm-level Algae Risk Model; FU, functional unit; GWP, global warming potential; LCA, life cycle analysis; mPt, milipoint; NAABB, National Alliance for Biofuels and Bioproducts; NREL, National Renewable Energy Laboratory; PBR, photobioreactor.

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biofuels being defined [4]. While each biofuel type has its own advantages and disadvantages, together they have begun to decrease the burden of global fossil fuel consumption.

First generation biofuels were developed in the 1970s and 80s and consist of either: 1) ethanol produced via fermentation of sugar (primarily from sugar cane) or hydrolyzed starch (primarily from corn), or 2) biodiesel produced via trans-esterification of vegetable oil (primarily soybean oil or animal fats). The fuel ethanol process is well established and consists of feedstock pretreatment (milling, crushing, and solubilizing in water), saccharification (converting starch into sugars for the corn ethanol process), fermentation, distillation, and co-product recovery [5,6]. First generation biofuels have three major disadvantages: production costs, market access, and competition for arable land with food crops. Because 1st generation biofuel feedstocks are also used for food, the feedstock usually accounts for more than 33% of total production costs, and this situation is unlikely to change as the world population and food demand continue to rise [7]. Second generation biofuels are typically defined as ethanol or other biofuels produced from lignocellulosic biomass, and includes a diverse range of by-products, wastes, and dedicated feedstocks [8]. The sustainability of 2nd generation biofuels is limited by land availability and competition for land use [9–12]

Due to the drawbacks associated with 1st and 2nd generation biofuels, 3rd and 4th generation biofuels have been developed. These are fuels derived from the fixation of CO_2 by photosynthetic algae and cyanobacteria [13], where the photosynthetic organism serves as both the photocatalyst and producer of biofuel [14]. At this time, algae and cyanobacteria appear to be the only sources of biofuel capable of meeting the global demand for transportation fuel [15–18]. While algal oil can potentially be used directly as a fuel, in most cases the oil is subsequently processed through traditional oil refinery or biodiesel technologies into biofuels [19–21]. Therefore, many researchers now suggest that the definition of 3rd generation biofuels be altered to photoautotrophic conversion of CO_2 into oil or algal biomass that is subsequently converted into biofuels [22]. This conversion step is a limitation to 3rd generation biofuels that does not exist with 4th generation biofuels. Fourth generation biofuel is the term used for the production of 'drop-in' biofuels directly from genetically engineered algae or cyanobacteria [19–21]. The benefit of using drop-in biofuels is that they can be mixed with crude derivatives without the need to develop new fuel infrastructures [23].

Heterocyst-forming filamentous cyanobacteria have the ability to fix atmospheric nitrogen, meaning that the cultivation medium does not need a combined nitrogen source, which is a considerable expense. This is one of the reasons that industrial microbiologists have focused on engineering filamentous N_2-fixing cyanobacteria to produce next-generation biofuels and high-value chemicals [24], including limonene [25], farnesene [26], myrcene [27], and linalool [28].

The biofuel producing strain of filamentous cyanobacteria evaluated in this study was previously described by Halfmann et al., [25]. In that study, a genetically engineered *Anabaena* sp. PCC 7120 (herein referred to as LimS-DXP *Anabaena*) produced limonene (Fig. 1) photosynthetically and it was postulated that this strain could be used for the large-scale production of limonene as a next-generation biofuel. However, production would need to be increased substantially before an economically feasible process could be achieved. Previously, our research group [29] performed an economic feasibility analysis on a theoretical limonene production facility which used the genetically engineered filamentous cyanobacteria as the limonene producer. This study showed that an economically feasible process is currently not possible due to the low limonene productivity of the cyanobacterial strain. However, if productivity was increased, an economically feasible process would be possible. Data from the Halfmann et al., [25] study and our previous study are [29] were used as the basis for substantial parts of this LCA study.

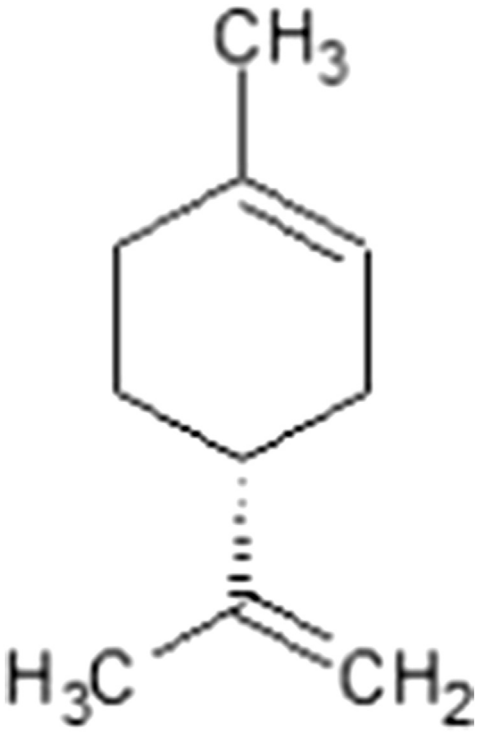

Fig. 1. Molecular structure of limonene. (Structure drawn with ACD/ChemSketch Freeware).

The aim of this study was to evaluate the environmental profile of a hypothetical, next-generation biofuel production facility that uses genetically engineered cyanobacteria to produce limonene [29]. To evaluate the environmental profile of the theoretical production facility, an LCA was conducted. LCA is a method commonly used to evaluated the environmental impacts of a process by quantifying resource demands, energy demands, and the resulting emissions [30]. LCAs have been commonly used to evaluate environmental profiles of algal and cyanobacterial chemical production facilities [30–32]. In this study, a cradle-to-gate strategy was applied to define the systems boundaries. Scenario 1 was defined based on a hypothetical production facility described by Johnson et al., in which 32,727 L/yr limonene was produced [29]. Scenario 2 was based on a hypothetical facility described by Halfmann et al., that produced 1,000,000 L/yr of limonene [25]. The only difference between Scenario 1 and Scenario 2 was the total annual limonene production, due to different limonene productivities of the engineered cyanobacteria. The environmental profiles of both scenarios were then compared to the conventional production of fossil-based diesel environmental profile. The goal of this study was to provide evidence as to what the expected environmental effect of increased limonene production by the theoretical facility described below will be. Minimizing the negative environmental impact of this facility while maintaining economic feasibility will be essential.

2. Data and methods

2.1. Production system overview

A process flow diagram of the limonene production process is shown in Fig. 2, while Table 1 lists process inputs and parameters for both