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# Life cycle net energy and greenhouse gas emissions of photosynthetic cyanobacterial biorefineries: Challenges for industrial production of biofuels

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## ABSTRACT

The net energy ratios (NERs) and greenhouse gas (GHG) emissions of a Photosynthetic Cyanobacterial Biorefinery (PSBR) were evaluated using a Life Cycle Assessment (LCA) approach. This study assessed engineered cyanobacterium cultures in which biosynthetic biofuels are directly secreted. Biofuels researched in this study include bisabolane, a biosynthetic substitute for D2 diesel; heptadecane, a substitute for diesel; and ethanol, a substitute for gasoline. Results demonstrate that cyanobacteria-based ethanol has higher yields, lower NER, and lower GHG emissions than bisabolane and heptadecane products in *Synechocystis* sp. PCC6803. By performing a sensitivity analyses of the life cycle NER and GHG emissions responses of the system, we derived the improvements in system biology, metabolic engineering, and process engineering that would be required to minimize the environmental impacts at an industrial scale. The NER and GHG emissions of the biofuel products were found to be the most sensitive to organism-level biofuel productivities, the energy consumption of vapor compression and distillation, and the energy consumption of culture mixing. This LCA will serve as a baseline for stakeholders; including policy makers, cyanobacterial refineries, and researchers, to establish the goals to engineer cyanobacteria and sustainable bioprocesses.

## 1. Introduction

Photoautotroph-based biofuels are considered one of the most promising renewable resources to meet the global energy requirements for the transportation system [1]. Long-term research and development has resulted in demonstrations of microalgae areal oil productivities that are higher than crop-based biofuels, about 10 times that of palm oil and about 131 times that of soybean [1–4]. Cyanobacteria is reported to have ~4 times the areal productivity of microalgae on an equivalent energy basis [5]. Downstream of the cultivation process, the cyanobacteria biomass and bioproducts are supplied to biorefineries producing feed, biomaterials, biosynthetic chemicals, and biofuels [6].

Biofuel technologies, especially microalgae, have been extensively researched to model their environmental impacts, techno-economics, net energies and greenhouse gas (GHG) emissions [7–16]. None of these studies have evaluated the biofuels bisabolene and heptadecane produced by cyanobacteria. For cyanobacteria-based ethanol, previous research reports net energy ratios (NERs) ranging from 0.20 to 0.55 MJ consumed:(MJ produced)<sup>-1</sup> and GHG emissions ranging from 12.3 to 19.8 g CO<sub>2eq</sub>:MJ<sup>-1</sup> [17]. Although this previous research report presents an in-depth review of the processes involved in the proposed

technology, productivities of ethanol were based on a cost-effective range established by the authors rather than being based on near-term or physically realizable productivities. In this LCA, we consider the current data regarding biofuel production including biomass productivities, biofuel productivities and process energy consumptions. This LCA is novel in that the lifecycle impacts of the two biofuels investigated in this study, bisabolene and heptadecane, from genetically modified cyanobacteria will be evaluated in comparison to more conventional ethanol production schemes.

This research seeks to model the NER and GHG emissions for photosynthetic biorefineries growing cyanobacteria, where bisabolene and heptadecane biofuels are compared to ethanol so as to understand the biological and process engineering challenges for industrial scale production.

## 2. Methods

### 2.1. Goals and scope

Life Cycle Assessment (LCA) is a framework for evaluating the energy use, emissions and impacts of direct, indirect, and supply chain

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processes [18]. A LCA model was developed in this study to assess these aspects of a cyanobacteria-based biofuel facility. In developing the goals and scope of this project, we seek to compare a bisabolane or heptadecane production to cyanobacteria-based ethanol production, a near-term and commercially promising technology. Cyanobacteria-based ethanol is chosen as the baseline for comparison because these organisms display the highest productivities and rates of carbon partitioning [19], and could potentially meet the environmental goals as for renewable fuels in the U.S. [20].

The primary audience for this LCA includes cyanobacteria researchers, policy makers, and process engineers. The outputs of this study are direct lifecycle comparisons of the environmental and energy impacts of these cyanobacteria based biofuel systems.

This LCA considers biomass productivities and biofuel yields of *Synechocystis* sp. PCC6803. Current biofuel yields of biosynthetic bisabolane and heptadecane reported in the literature serve as the baseline in a sensitivity analysis at the system level. The biosynthetic bisabolane and heptadecane biofuels secreted by *Synechocystis* sp. PCC6803 are compared to state-of-the-art cyanobacteria-based biofuel, ethanol. Cyanobacteria-based ethanol is the cyanobacteria-based biofuel which reaches the highest carbon partitioning assimilated, 63%, during cultivation of *Synechocystis* sp. PCC6803 [19], and therefore has the highest commercialization potential.

This study considers a cyanobacteria production system located in Fort Collins, CO, USA. Weather conditions, including temperature and incident radiation, are used to model real-world biomass productivities.

### 2.1.1. Impacts considered

The two sustainability metrics and impacts considered in this study are net energy ratio (NER) and lifecycle GHGs, and were elicited from a set of surveyed stakeholders.

The production of biofuel as an energy carrier is the primary goal of any potential biofuel technology. Therefore, net energy ratios (Eq. (1)) were the first metric of interest for this LCA.

$$\text{NER} = \frac{E_{\text{consumed}}}{E_{\text{produced}}} \quad (1)$$

NER are defined in this study by normalizing the energy consumed ( $E_{\text{consumed}}$ ) in the cyanobacteria growth, fuel extraction, and conversion processes by the energy produced ( $E_{\text{produced}}$ ) by this system as embedded in the lower heating value of the biofuel.

Various economic and policy incentives have been developed to incent the production of fuels with low net GHG emissions [20]. Therefore, the second metric of interest is lifecycle greenhouse gas (GHG) emissions (Eq. 2).

$$\text{GHG emissions}_{\text{fuel/energy}} = FC * EF_{\text{fuel}} * P_{\text{technology}} \quad (2)$$

Lifecycle GHG emissions ( $\text{GHG emissions}_{\text{fuel/energy}}$ ) are defined by the Intergovernmental Panel on Climate Change (IPCC) as the direct or indirect amount of fuel or energy consumed ( $FC$ ) by the emission factor based on the type of fuel or energy technology ( $EF_{\text{fuel}}$ ), and the penetration ( $P_{\text{technology}}$ ) or fraction of the energy source of a given energy technology [21].

### 2.1.2. Functional unit

The functional units for this study are the energy produced from biofuels to displace petroleum fuels, in MJ (Fig. 1).

### 2.1.3. System boundary

The boundaries of the combined growth, extraction and conversion systems to be researched in this LCA are illustrated and summarized in Fig. 2. The processes considered for this study start with the growth stage of the cyanobacteria, and end at the point of conversion of the bioproducts to a biofuel which can displace conventional fuels. The system includes the direct energy requirements of the facility. The embedded GHG emissions for each of the energy sources are included.

The water and nutrient requirements will be supplied by recycled commercial water and commercial/industrial fertilizers. Carbon dioxide is assumed to be obtained from waste streams from local industrial CO<sub>2</sub> facilities including power plants, amine natural gas treatment plants, and fermentation plants.

Three novel cyanobacteria-based biofuel production systems will be evaluated as independent systems. The impacts of these biofuels will be compared to those of the conventional fuels they would displace. Bisabolane will replace conventional jet fuel from crude oil, heptadecane will replace low-sulfur diesel from crude oil, and ethanol will replace gasoline blendstock from crude oil. The distribution of biofuels and the end use (combustion of these biofuels) will not be taken into account to avoid misinterpretations when comparing different cyanobacteria-based biofuel systems.

## 2.2. LCA tools

The PSBR systems were modeled in the GaBi 6 software by constructing three comparable models to describe the function of these three cyanobacteria-based biofuels. GaBi is a tool that allows for the estimation of the lifecycle energy and emissions output of a process as a function of the energy, material consumed for that process. The GaBi model was used to calculate the lifecycle, material consumption, net energy use, and GHG emissions for the lifecycle of the cyanobacteria-to-biofuel process.

In evaluating the life cycle energy consumption of the cyanobacteria-to-biofuel process, the biomass that is not converted to fuel can be considered as a co-product. For this study, the cyanobacteria co-product credits are allocated using the displacement method. The displacement method assumes that the co-product displaces a preexisting conventional product. The displacement co-product credits represent the lifecycle energy and GHG emissions that would be required to produce the displaced product. Co-product credits are subtracted from the overall energy and GHG emissions of the cyanobacteria-to-biofuel process.

## 2.3. Cyanobacteria cultivation and biofuel concentration systems

The genetically engineered cyanobacteria, *Synechocystis* sp. PCC6803, that are the subject of this study, are cultivated in enclosed photobioreactors to protect them from contamination and to enable the collection of the biofuel from the photobioreactor media and headspace. The batch bioprocess is carried out in flat photobioreactors providing a total culture volume of 126,000 m<sup>3</sup>. For validation purposes of the growth stage subsystem of this LCA, we performed experimental work in a bench scale flat photobioreactor with surface to volume ratio of 112 m<sup>2</sup>·m<sup>-3</sup>. Cultures were mixed by sparged air at the bottom of the photobioreactor at 0.5 m<sup>3</sup> of air per minute per cubic meter (VVM) ( $\pm 0.3$ ). Photobioreactors were inoculated with *Synechocystis* sp. PCC6803 cells at 0.107 g·l<sup>-1</sup> ( $\pm 0.061$ ). The cultures were grown using a high pressure sodium (HPS) lighting system with a spectrum ranging from 400 to 700 nm at extreme conditions, sunny day at noon or a Photosynthetic Active Radiation (PAR) over 1600  $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . Cyanobacterium biomass was harvested upon quasi-steady state conditions, reaching a productivity of 0.128 g·l<sup>-1</sup>·d<sup>-1</sup> ( $\pm 0.033$ ) (Supplementary Information).

The cyanobacteria were grown in BG11 media. CO<sub>2</sub> enriched air (2% CO<sub>2</sub>) is sparged through the bioreactor to provide carbon and active mixing of the culture. Mixing by sparge is performed during periods of photosynthetically active growth and when bioavailable nitrogen is present in the media. Industrial forms used in our bioprocess modeling include sodium nitrate and monopotassium phosphate [22]. Thermal regulation of the photobioreactors is performed by a temperature controlled heat exchanger coil, set at 29 °C, supplying tap water. U.S. average grid electricity is used to power pumping and sparging of the cultivation process.

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