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# Outlook on the Potential of Cyanobacteria to Photosynthetically Produce High-Value Chemicals and Biofuels at an Industrial Scale

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## Abstract

As the need to develop sustainable and renewable sources of chemicals and energy is increasing, cyanobacteria have emerged as an attractive industrial microorganism. Using energy from sunlight, $H_2O$, and $CO_2$, cyanobacteria can either produce naturally or be genetically engineered to produce high-value chemicals and next-generation biofuels.

The list of chemicals that cyanobacteria have produced is extensive and constantly growing, however the titers of the chemicals produced by cyanobacteria are generally low.

Thus, before an economically feasible large-scale chemical production process can be achieved, cyanobacterial production titers must be increased. Also, costs associated with cultivating cyanobacteria at an industrial scale must be decreased.

In this communication, our research group's progress towards enhancing the industrial potential of cyanobacteria is summarized and potential future targets for research are discussed. Cyanobacteria as industrial microorganisms holds a great deal of potential towards developing high-value chemicals and next-generation biofuels.

**Keywords:** Cyanobacteria; Biofuel; Bioenergy

## Introduction

The world is currently facing two pressing problems: fossil fuel depletion and global climate change due to elevated $CO_2$ emissions.

The need for developing renewable, sustainable sources of chemicals and biofuels is increasing as the global population grows and the burden on fossil fuel reserves increases.

Using microorganisms to produce these compounds, either naturally or via genetic engineering, has garnered a great deal of interest from researchers due to the relative ease and speed of which many microbes can be cultivated.

Genetically engineering cyanobacteria to become cellular factories (sometimes referred to as cyanofactories) that can directly convert $CO_2$ into high value chemicals and next-generation biofuels holds tremendous potential as a novel, industrially feasible, platform technology (Figure 1B) [1-4].

Through genetic alteration of targeted metabolic pathways, the carbon flow of cyanobacteria can be redirected from producing stored bioenergy precursors (i.e., lipids and polysaccharides) to the direct production of excreted products such as hydrocarbons, hydrogen gas, alcohols, ammonia, etc.

Excretion of these compounds from the cell will enable continuous product recovery from the culture fluid, while maintaining a viable cell "factory" in a recirculating photobioreactor (PBR) system.

This proposed cyanofactory bypasses the energy-intensive, multi-step processes currently used to produce biofuels from plant biomass or algal oil (Figure 1A).

Figure 2 shows a more specific example of a cyanofactory. This schematic illustrates a hypothetical facility that produces limonene using a genetically engineered strain of $N_2$-fixing cyanobacteria specifics of this facility are described below.

At this time, more than 20 chemicals have been synthesized by genetically engineered cyanobacteria and this number is expected to grow [4]. The highest volume application of genetically engineered microorganisms is transportation fuels [5], and cyanobacteria (along with microalgae) are likely the only renewable resource capable of meeting the global demand for these fuels [6,7].

In order for a biofuel production process from cyanobacteria to become economically feasible, the cost of the biofuel must be similar or less than fossil-derived fuels.

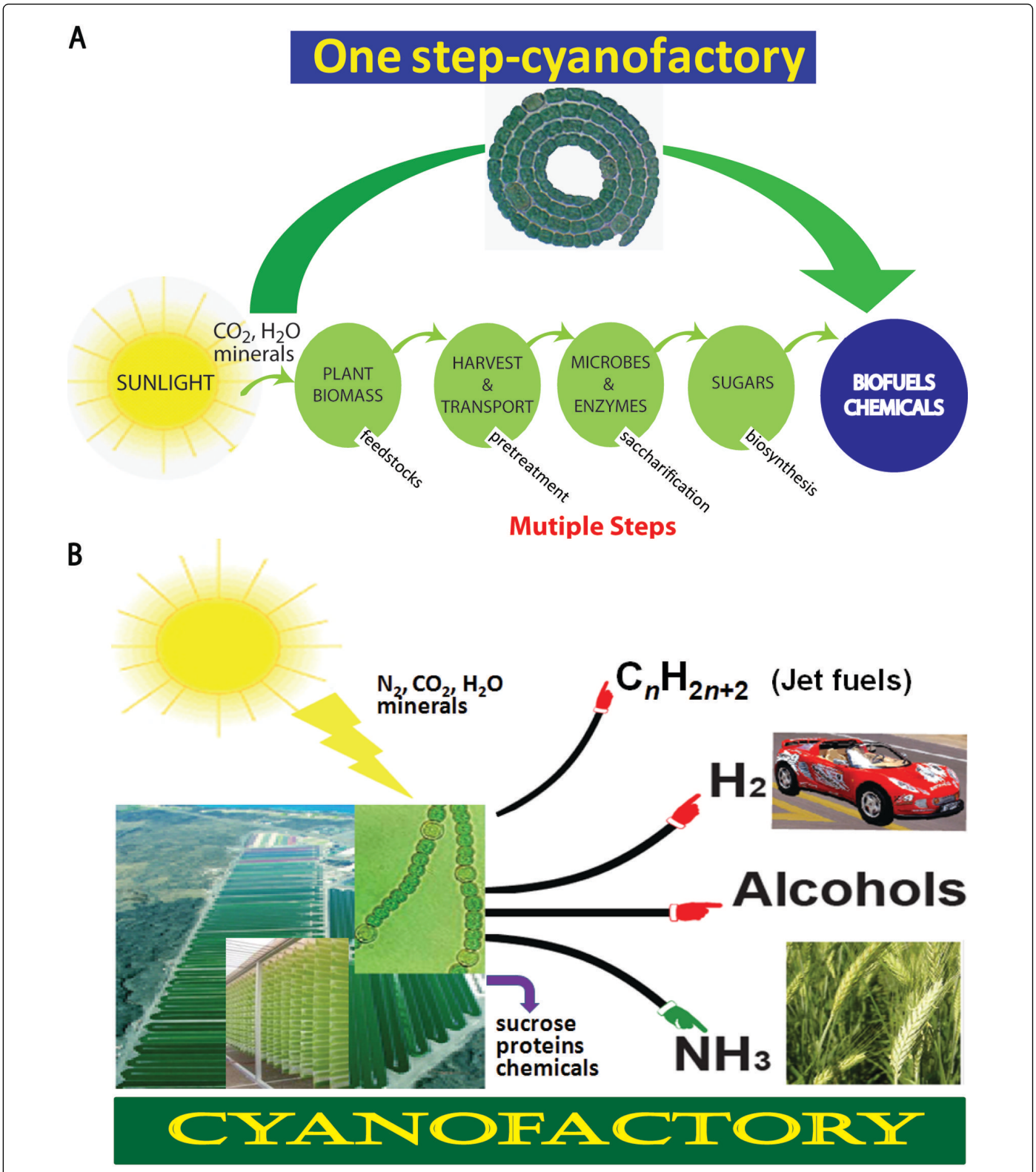


**Figure 1:** Diagrams illustrating the ability of genetically engineered cyanobacteria to convert sunlight, $CO_2$, and $H_2O$ into high-value chemicals and drop-in biofuels.
**A:** one step-cyanofactory (top) vs conventional, multiple-steps biomass model. **B:** Proposed cyanofactory for production of biochemicals.

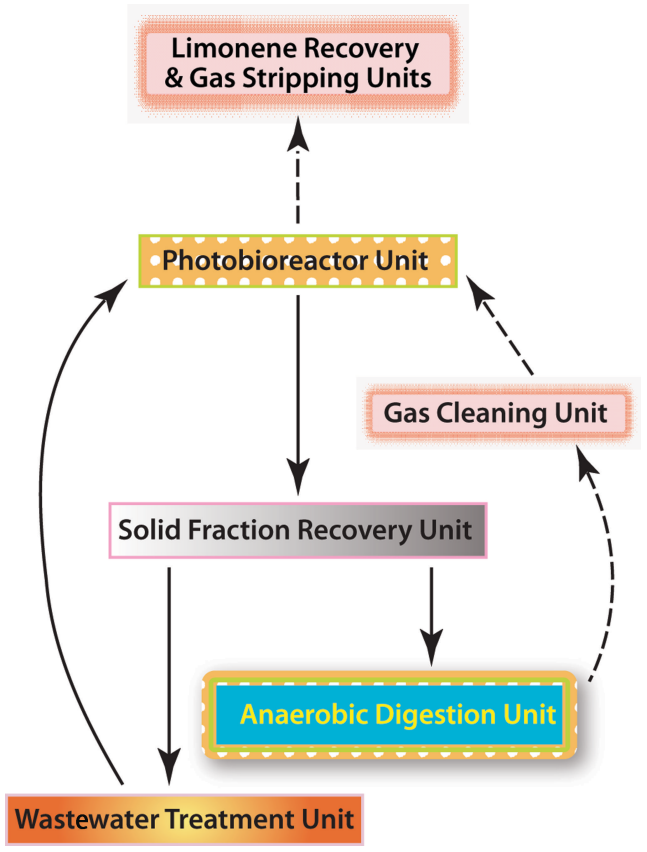


**Figure 2:** Schematic of a theoretical cyanofactory which uses a genetically engineered strain of cyanobacteria to produce limonene [20]. Solid lines=solid steam (i.e., biomass and water). Dashed lines=gas stream.

Unfortunately, the currently low biofuel productivities of cyanobacteria result in high production costs. Therefore, products other than fuels are being targeted to develop economically feasible processes. To highlight recent research focused on cyanobacteria as industrial microorganism, this communication summarizes the current outlook on cyanobacterial processes capable of producing high value chemicals and next-generation biofuels.

Along with cyanobacteria, algae have been investigated for their potential to produce high value chemicals and biofuels directly from $CO_2$. In many peer-reviewed publications, cyanobacteria and microalgae are incorrectly considered synonymous; however, cyanobacteria are prokaryotes and algae are eukaryotes [8]. Some studies consider the use of algae to be advantageous because they naturally produce oils that can used as a feedstock for biodiesel production [9]. The disadvantage of this system is that the unit operations to recover algal cells, extract oil from the cells, and convert the oil into biodiesel are costly and energy intensive [10].

On the other hand, target compounds produced by cyanobacteria are typically excreted from the cell, and can be harvested directly from the culture medium or from the headspace gas (depending on product volatility). Cyanobacteria also have the advantage of being much easier to genetically manipulate, since they are prokaryotic. The genetic tools for cyanobacteria are also more advanced [11].

The list of high-value compounds that cyanobacteria can produce is extensive and includes: alcohols, [12], alkanes [13], alkenes [14] free fatty acids (FFAs) [15], sugars [16], and terpenoids [17]. While many of these compounds can be used as biofuels, many have other higher value applications. For example, limonene ($C_{10}H_{16}$) is a cyclic hydrocarbon that both genetically engineered unicellular [18] and diazotrophic filamentous cyanobacteria [17] can produce. It has potential as a biodiesel and a jet fuel, but also has applications in flavoring and pharmaceuticals [17,19]. Our research group conducted an economic feasibility analysis using limonene production data from a genetically-engineered cyanobacteria strain that had a limonene productivity of 0.018 mg/L/d [17]. In this proposed facility, cyanobacteria would be cultivated in a batch mode so that the batch would be completed before the microorganisms reached decline phase and biofuel production would become hindered. The sum of capital expenditures (CAPEX) for this facility was 1.5 million dollars and the sum of operating expenditures (OPEX) at year 5 of the 10-year model was 162 million dollars. This analysis concluded that productivity would need to be increased approximately 57-fold to achieve break-even based on biofuel prices (i.e., a net present value (NPV) of zero dollars at year five of a ten-year simulation) [20].

Therefore, for this facility to be economically feasible, limonene productivity would need to be substantially increased, and/or limonene would need to be marketed at a much higher price in food or pharmaceutical markets. This hypothetical cyanobacterial production facility cannot be directly compared to established biodiesel and ethanol facilities because at this time several components are theoretical at a large scale (i.e., the chemical separation process). Several strategies can be used to increase chemical production by cyanobacteria. Generally, biofuels and high-value chemicals are toxic to the microorganism that produces them [21,22]. As such, it has been hypothesized that increasing the tolerance of the microbe to these chemicals may increase production. While there are examples of microbial strains being developed with increased tolerance to target chemicals and exhibiting increased production [23,24], there are also examples where increased tolerance led to a negligible or decreased production of the target chemical [25,26].

Our group performed several proof-of-concept studies to determine whether directed evolution is a viable method for increasing tolerance to high value chemicals and next-generation biofuels in cyanobacteria. We generated an *Anabaena* sp. PCC 7120 strain that, when compared to wild-type, exhibited a 220% increase in tolerance to farnesene ($C_{15}H_{24}$). We also developed an *Nostoc punctiforme* ATCC 29133 strain with a 20% increase in tolerance to linalool [27]. Next we will genetically engineer these strains to produce the chemicals they have increased tolerance to. This sequence of strain development is preferred, since the method used to develop tolerance is based on introducing random mutations throughout the chromosome. If synthase genes are engineered into the chromosome first, they would be subject to random mutation (and potential inactivation) in subsequent directed evolution work.

Decreasing the costs associated with supplying nutrients to cyanobacterial cultures is another area of active research with much potential to improve the economic feasibility of chemical production processes. Nitrogen, in particular, is a major cost of cultivating cyanobacteria at an industrial scale. The manner in which nitrogen is supplied to large-scale production systems will play an essential role in achieving environmental sustainability and economic feasibility [28]. For this reason, there has been a considerable amount of attention paid

to using diazotrophic, filamentous strains of cyanobacteria as high-value chemical and next-generation biofuel producers. The ability of these strains to fix atmospheric nitrogen makes them promising candidates for industrial applications. These strains can even be cultivated in tap water, although the maximum biomass concentration they can achieve is significantly lower than when grown in BG11 (standard cyanobacteria media) [29].

While diazotrophic strains of filamentous cyanobacteria are capable of nitrogen fixation, it is an energetically expensive process that requires eight electrons and at least 16 adenosine triphosphate (ATP) molecules per mole of fixed nitrogen [30]. If this cost significantly decreases chemical production, it may not be preferred to cultivate these microorganisms in nitrogen-free medium. To address this, we compared four combined nitrogen sources with atmospheric nitrogen for large-scale cultivation of diazotrophic, filamentous cyanobacteria [31]. By analysing cyanobacterial growth and the environmental impact, via a life cycle analysis (LCA), associated with large-scale processes using the different nitrogen sources, it was determined that ammonium chloride is the best nitrogen source for the large-scale cultivation of filamentous cyanobacteria.

This study provided evidence that while cultivating diazotrophic, filamentous cyanobacteria in medium with no combined nitrogen source would be cheaper, the substantially reduced growth would make it very difficult to develop an economically feasible process. A potential future direction of this research would be to evaluate the impact on chemical production of using lower concentrations of nitrogen. This could be performed similar to a study by Wu et al. [32], who optimized biomass production of *Scenedesmus* sp. LX1 using several models that described the combined effects of different nutrients and light intensity on growth rate. Diazotrophic, filamentous strains of cyanobacteria may still be considered promising industrial microorganisms if it is determined they can produce target chemicals in medium supplemented with lower concentrations of nitrogen than non-nitrogen fixing strains of cyanobacteria.

## Conclusion

While there have been many studies showing the technical feasibility of high value chemical and next-generation biofuel production from cyanobacteria, it is still unclear if any of these processes could become successful at an industrial scale. Rather than engineering strains of cyanobacteria to produce one chemical, it may be a better strategy to develop a suite of cyanobacteria strains capable of producing several chemical compounds. This proposed 'cyanofactory' would have a greater probability of economic success if various commodity chemical market values decreased. It would also be beneficial to target chemicals that have multiple applications, such as fuels and solvents. One of the benefits of cyanofactories is that they could mitigate $CO_2$ emissions by utilizing flue gases from ethanol and coal-fired power plants, however the impact these factories would have on the global carbon footprint won't be able to be accurately calculated until more large-scale processes are operational. Currently, research focused on enhancing the industrial potential of cyanobacteria is in its infancy, however much progress has been made in moving towards the goal of developing industrial strains of cyanobacteria capable of high-value chemical and next-generation biofuel production.

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