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Cyanobacterial factories for the production of green energy and value-added products: An integrated approach for economic viability

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ABSTRACT

Cyanobacteria are Gram-negative photosynthetic prokaryotes and are well known for their role in global carbon dioxide and nitrogen fixation. Recent developments in synthetic biology and metabolic engineering tools for these prokaryotes have allowed designing of novel metabolic pathways in these organisms which makes them potential candidates for the commercial production of fourth generation of biofuels. However, cost associated with large scale cultivation, harvesting, downstream processing and continued decrease in the price of crude oil in recent years has threatened the economic viability of biofuels production at commercial level. Cyanobacteria are known to produce wide range of secondary metabolites having biotechnological, biomedical and industrial importance. Cyanobacterial secondary metabolites can be industrially exploited as natural sunscreens, algaecides, herbicides, insecticides, antibiotics, antifungal, immunosuppressant, anticancer, antiviral and anti-inflammatory agents. Here, we propose an integrated approach for economic viability of biofuel production using cyanobacterial chassis by combining the production of value-added products synthesized by these organisms to attain a reasonable price for biofuels per barrel which can compete with current and/or future market price.

1. Introduction

Cyanobacteria are photoautotrophic microorganisms that are well known for their role in global CO_2 and nitrogen fixation. Cyanobacteria have very simple growth requirements and can be cultivated using the solar or artificial light energy, water and source of essential elements like C, P, S, N, K and Fe [1]. Cyanobacteria can also be cultivated using nutrient rich agro-industrial wastes and wastewater which subsides the requirement of large amount of fertilizers and water for their large scale cultivation [2]. Cyanobacteria possess chlorophyll-containing photosystems and phycobilisomes for the absorption of light energy which is finally converted into chemical energy through photosynthesis. Similar to other photosynthetic organisms, cyanobacteria utilize Ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) enzyme for the assimilation of carbon, i.e., CO_2, into three-carbon compound which is further allocated to different biochemical processes, including biosynthesis of different metabolic compounds and accumulation of biomass [3,4]. Rubisco possess both carboxylase and oxygenase activity depending on the ambient levels of CO_2 and O_2; later activity of Rubisco is associated with photorespiration which results in loss of carbon [5]. Therefore, to overcome photorespiration, cyanobacteria possess well developed carbon concentrating mechanisms which increases the ambient level of CO_2 around Rubisco in a proteinaceous compartment called carboxysome [4]. Cyanobacteria can alter the size and composition of their main light harvesting complex, i.e., phycobilisomes, according to the available quality and quantity of light at different depths in a water system, and thus have well developed photoadpative or photoprotective mechanism [3].

Post-industrialization developments have raised several concerns related to the health of global climate, especially global CO_2 emission and ozone depletion. Recently, United Nations Climate Change Conference 2015 held at Paris got so much public attention where scientists and law makers from 196 countries reached to a global agreement called Paris agreement to control the emission of CO_2. There is a general consensus that emission of carbon dioxide from combustion of fossil fuels has contributed to the global climate change [6,7]. However, natural reservoirs of fossil fuels are limited and are prone to depletion due to ever-increasing demand of energy. Thus, over combustion of fossil fuels poses threat to both depletion of classical source of energy and global climate change. This scenario has driven the recent development of sustainable renewable energy, i.e., biofuels, from microalgae and cyanobacteria which can help in reducing the

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global levels of CO_2 by recycling it into usable biomass, fuels and other green products. Biofuels are derived from a biomass which is the outcome of photosynthesis [8].

Time to time there have been developments in the field of biofuels production and based on source of raw materials and technologies, there are four generations of biofuels. First and second generations biofuels are derived from food crops and non-edible materials, respectively, however, these biofuels have major disadvantage of competing with crop plants for fertile land [9]. Furthermore, catalytic efficiency of cellulose and hemicelluloses biomass processing requires significant improvement, and development of acidic catalysts has been proposed to overcome this major limitation [10]. Third and fourth generation biofuels are based on photosynthetic aquatic microorganisms such as microalgae and/or cyanobacteria and their metabolically engineered strains that do not compete for agricultural land [11,12]. Both microalgae and cyanobacteria are equally important for the production of renewable energy, however, cyanobacteria have advantage over microalgae due to their fast growth, simple growth requirements, plasticity of their metabolism and availability of well developed tools for their genetic modification [11–14]. Biofuels production using cyanobacteria requires large scale outdoor cultivation which can be achieved through closed photobioreactors or open ponds, however, cost associated with large scale cultivation, harvesting and downstream processing for biofuels production is too high that pose threat to the economic viability of biofuels production using microalgae and cyanobacteria [15–17]. Furthermore, continuous decrease in crude oil price has created a competitive atmosphere for biofuels industry before its commercialization.

The conversion of cellulose and hemicelluloses into biofuels and value-added chemicals has been proposed for sustainable production of biofuels. The catalytic conversion of levulinic acid to fuels and valuable chemical commodities such as diphenolic acid and delta-amino levulinic acid has been well appreciated in the context of economic viability of biofuel industry [10]. Similarly, furfural offers a promising chassis for the production of lignocellulosic biofuels and value-added chemicals such as 2-methyl furan and 2-methyltetrahydrofuran, furfuryl alcohol, tetrahydrofurfuryl alcohol, furan, tetrahydrofuran, cyclopentanol and cyclopentanone [18]. However, further research is required in the field of hydrolysis of lignocellulosic biomass; mainly in terms of catalyst properties and reaction conditions for better economic and sustainability of cellulose and hemicelluloses based biofuel industry [10,18]. Cyanobacteria are source of wide range of biologically active high value compounds [19,20] and large scale production of these bioactive compounds for industrial application can be integrated with biofuels production to reduce the cost associated with cultivation, harvesting and downstream processing. In this review, we propose an integrated approach for economic viability of biofuels production by discussing number of biologically active cyanobacterial secondary metabolites that can be combined with biofuels production to achieve the economic sustainability.

2. Cyanobacterial chassis for the production of bioenergy and value-added products

Photosynthesis carried by microalgae including cyanobacteria provides a sustainable platform for the production of energy-rich biofuels. There are different biological, chemical and physical processes such as anaerobic digestion, fermentation, transesterification, pyrolysis and hydrothermal liquefaction that can be utilized to convert the photosynthetically produced biomass to biofuels [21]. However, conversion of photosynthetically active radiation (400–700 nm) to biomass by photosynthesis is limited, and therefore, large quantities of biomass are required for supporting the demand and attaining economic viability of biorefineries. Cyanobacteria have evolved the phenomenon of chromatic acclimation to maximally utilize the available light photons for driving the photosynthesis [22]. The effective CCM of cyanobacteria can be utilized in diverting photosynthetically fixed CO_2 for the synthesis of various fuels and chemicals. Therefore, simultaneous production of variety of co-products in addition to biofuel is proposed to provide value to biorefineries. Recent developments in application of synthetic biology and/or metabolic engineering tools have led to the successful constructions of novel pathways in cyanobacterial chassis for the production of various green fuels and chemicals [23–26]. The application of genetic and metabolic engineering has led to the introduction of novel pathways in cyanobacteria for the production of value-added products such as alcohols and diols, hydroxyl acids, alkanes, fatty acids, sucrose, glucosylglycerol, isoprenoids and farnesene [26]. Recently, tail gas reactive pyrolysis (TGRP) of *Spirulina* biomass was found to give better result in comparison to lignocellulosic materials for the production of fuels and different chemicals [27]. Metabolic engineering of cyanobacteria focusing on modular optimization of multiple gene expressions resulted in photosynthetic conversion of CO_2 to farnesyl diphosphate-derived phytochemicals of economic importance [28]. Ethylene, which serves as a petrochemical source for various plastics, textiles and chemicals production, can be renewably produced by photosynthetic CO_2 fixation by expressing bacterial ethylene-forming enzyme in cyanobacteria [29]. In addition to newly introduced pathways for novel compounds, cyanobacteria are natural source of wide range of metabolites of potential economic importance; explained in following sections, which could also add to the economic viability of biorefineries.

3. Cyanobacteria as a source of bioenergy

The need of energy at global level has been steadily increasing due to better economic growth and ever increasing population. However, classical fossil fuel sources that are currently fulfilling our energy demand are limited and their combustion pose threat to our environment. Therefore, there is a need to develop some alternate source of energy which is both sustainable and environment friendly. Cyanobacteria and microalgae are very promising candidates that can fill our energy hunger in a sustainable and environment friendly manner [12,30]. Both microalgae and cyanobacteria contain hydrocarbon chains that can replace fossil fuel hydrocarbons and their derivatives [31]. Cyanobacteria are simple and fast growing photosynthetic prokaryotes with comparatively small genome size, which can be easily manipulated. Therefore, cyanobacteria have all the potential to act as a platform for the development of fourth-generation biofuel [12]. Following are the some advantageous properties of cyanobacteria that make them better candidate for their exploitation in sustainable energy program development.

- Cyanobacteria can be cultivated in non-fertile waste land, and therefore, do not compete for fertile land area.
- Cyanobacteria grow very rapidly than land plants or other eukaryotes, and therefore, can give better biomass per meter square of land used for cultivation.
- Cyanobacteria have very simple growth requirements, and can be cultivated using solar light, water, CO_2 and minimal nutrients or wastewater obtained from domestic, commercial or industrial source. Some cyanobacteria also have the ability to fix atmospheric N_2 and do not require nitrogen supplement for their growth.
- Cyanobacteria are easily accessible to manipulate genetics and associated metabolic pathways.

Genetic manipulation of several cyanobacteria has been standardized, and therefore, it is possible to manipulate metabolic pathways in cyanobacteria by removing or adding genes in these organisms. Genome of number of cyanobacteria has been completed or in progress. Table 1 summarizes the information for genome size, number of plasmids and total number of genes encoded by genomic and extrachromosomal DNA in various sequenced cyanobacteria which is