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A quantitative evaluation of ethylene production in the recombinant cyanobacterium *Synechocystis* sp. PCC 6803 harboring the ethylene-forming enzyme by membrane inlet mass spectrometry

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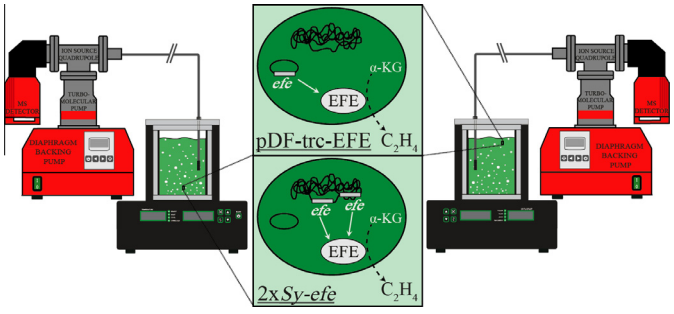
HIGHLIGHTS

- The ethylene production by *Synechocystis* sp. PCC 6803 is evaluated under changing lights.
- For ethylene quantitative monitoring, membrane inlet mass spectrometer was used.
- Ethylene was produced with rates ranging between 0.07 and 0.26 mmol (C_2H_4) gDW^{-1} h^{-1}.
- Photochemical conversion varied between 0.1% and 7.4% under high and low light.
- Carbon partitioning was estimated as 9.8–16.5% for two *Synechcoystis* strains.

GRAPHICAL ABSTRACT



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ABSTRACT

The prediction of the world's future energy consumption and global climate change makes it desirable to identify new technologies to replace or augment fossil fuels by environmentally sustainable alternatives. One appealing sustainable energy concept is harvesting solar energy via photosynthesis coupled to conversion of CO_2 into chemical feedstock and fuel. In this work, the production of ethylene, the most widely used petrochemical produced exclusively from fossil fuels, in the model cyanobacterium *Synechocystis* sp. PCC 6803 is studied. A novel instrumentation setup for quantitative monitoring of ethylene production using a combination of flat-panel photobioreactor coupled to a membrane-inlet mass spectrometer is introduced. Carbon partitioning is estimated using a quantitative model of cyanobacterial metabolism. The results show that ethylene is produced under a wide range of light intensities with an optimum at modest irradiances. The results allow production conditions to be optimized in a highly controlled setup.

1. Introduction

The predicted detrimental effects of climate change caused by almost exclusive utilization of fossil resources for the production of transportation fuels and bulk chemicals provides compelling

Abbreviations: MIMS, membrane inlet mass spectrometer; EFE, ethylene forming enzyme; LOD, limit of detection; LOQ, limit of quantification; k_La, mass transfer coefficient between liquid and gas phase within the photobioreactor cuvette.

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arguments for the development of genuinely sustainable technologies for its renewable production. Fossil resources are not being used only for energy production but serve also as a source for everyday products. During processing, enormous amounts of CO_2 are released into the environment. Renewable energy production coupled with CO_2 sequestration is required for long term sustainability. The utilization of photosynthetic microorganisms, including cyanobacteria, in renewable bioenergy production has been studied for decades and recent studies show substantial progress in the field (Wijffels et al., 2013). Among cyanobacteria, *Synechocystis* sp. PCC 6803 (*Synechocystis* hereafter), has gained prominent status as a model organism with the first genome sequenced (Kaneko et al., 1996), possibilities of natural transformation (Grigorieva and Shestakov, 1982), high carbon partitioning into product over biomass (Ungerer et al., 2012) and tolerance to high concentrations of end-products (Kämäräinen et al., 2012).

Ethylene is the most widely produced and utilized petrochemical originating from oil with an annual worldwide production exceeding 140 million tons in 2014. Substantial energy requirements for its production (units of gigajoules per 1 ton of ethylene (Worrell and Phylipsen, 2000)) are connected with vast carbon emissions (up to 2 tons of CO_2 per 1 ton of ethylene (Eggleston et al., 2006)). During ethylene production from traditional resources such as naphtha, ethane or ethanol, also extensive amount of toxic compounds is co-produced (Ghanta et al., 2013). This altogether make efforts towards enabling renewable production of this compound truly compelling. Ethylene production in photosynthetic organisms coupled with light utilization as an energy source offers a sustainable alternative to conventional production. Ethylene is naturally produced in higher plants as a hormone with functions in growth and stress response regulation (Lin et al., 2009) and it is also produced by a wide range of microorganisms (Eckert et al., 2014). In photosynthetic microorganisms, ethylene production has been introduced in the cyanobacteria *Synechococcus* sp. PCC 7942 and *Synechocystis* sp. PCC 6803 (Fukuda et al., 1994; Guerrero et al., 2012; Takahama et al., 2003; Ungerer et al., 2012). An illustration of the metabolic pathways related to ethylene production is shown in Fig. 1. Also, natural ethylene producing cyanobacteria have been identified (Huang and Chow, 1984), though the metabolic pathway remains unknown.

In the recent years, great progress has been achieved in constructing stable ethylene-producing cyanobacteria and several factors that influence high ethylene yields have been optimized. For example, two different *efe* genes have been expressed in *Synechocystis* sp. PCC 6803 (incorporated in a self-replicating plasmid (Guerrero et al., 2012) or in the chromosome (Ungerer et al., 2012)), resulting in production rates of approximately 200 nl (C_2H_4) $ml^{-1}_{culture}$ h^{-1} $OD^{-1}_{730/750}$ with the highest reported productivity of 171 mg (C_2H_4) $L^{-1}_{culture}$ d^{-1} achieved using dense cultures (Ungerer et al., 2012). Optimization of EFE expression levels through modulation of the ribosome binding site of the expression construct has also been shown to increase ethylene production (Xiong et al., 2015). However, there is still considerable room for improvement (actual yield vs. theoretical yield in the best case) and little is still known about the biochemical and process factors that limit ethylene production.

Light, as the only energy source for photosynthesis-driven processes, is one of the fundamental parameters in biotechnology and cyanobacterial physiology. The effect of light intensity on ethylene production using cyanobacteria harboring the ethylene forming enzyme has been studied with the strain *Synechococcus* IEK2-2. It has been reported that ethylene production exhibits a photoinhibition pattern and a model for production rates in low and high density cultures under varying light intensities has been derived (Wang et al., 1999). An evaluation of light effects on ethylene production by *Synechocystis* has only been examined in one study using dense cultures (Ungerer et al., 2012) and only under a limited range of conditions.

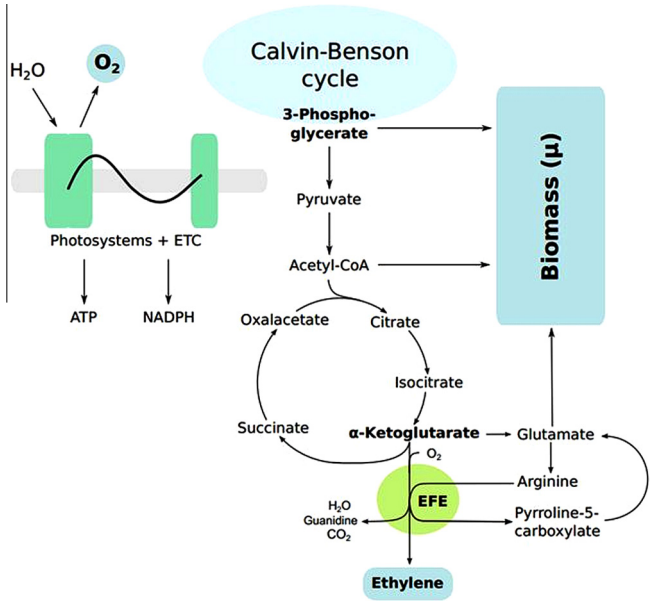

Fig. 1. Schematic representation of the main metabolic pathways related to photoautotrophic growth, ethylene production and oxygen evolution in the cyanobacterium *Synechocystis* sp. PCC 6803 harboring the ethylene forming enzyme (EFE). Stoichiometric coefficients of all reactions are neglected and the actual stoichiometry of the EFE reaction is listed in the text. ETC = electron transport chain.

In this study, a novel setup allowing quantitative evaluation of ethylene (and other volatile compounds) production in a quasi-continuous culture of photosynthetic microorganisms is presented. The ethylene production by two strains of a model cyanobacterium *Synechocystis* sp. PCC 6803 under a wide range of light intensities is evaluated. Using a stoichiometric model of cyanobacterial metabolism, it was possible to relate ethylene evolution to other cellular light-driven processes including growth and photosynthesis. Parameters for ethylene and biomass production were identified and carbon partitioning and cellular energetic balance were estimated. Making use of culture parameters obtained under highly controlled conditions, the model provides additional information on cellular physiological states under varying light intensities. The analysis significantly improves upon previous estimations of ethylene evolution in cyanobacterial cultures and allows for a stringent and quantitative evaluation of optimal culture conditions.

2. Methods

2.1. Strains and inoculum conditions

Two ethylene-producing recombinant strains of *Synechocystis* sp. PCC 6803 were used for the experiments: *Synechocystis* sp. PCC 6803 2x *Sy-efe* (Ungerer et al., 2012) and *Synechocystis* sp. PCC 6803 pDF-trc-EFE (Guerrero et al., 2012). Both strains were harboring the ethylene forming enzyme (EFE) from *Pseudomonas syringae*; the *efe* regions were integrated in a self-replicating plasmid in case of strain pDF-trc-EFE and in a chromosome in case of strain 2x *Sy-efe*. The strain 2x *Sy-efe* contained two copies of the *efe* gene in contrast to the strain pDF-trc-EFE which contained only one copy. Both genes were codon-optimized for expression in *Synechocystis* sp. PCC 6803; further details can be found in the related studies.