Algal Research 34 (2018) 116–125

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Pilot-scale production of poly-β-hydroxybutyrate with the cyanobacterium *Synechocystis* sp. CCALA192 in a non-sterile tubular photobioreactor

Clemens Troschl[a,*], Katharina Meixner[a], Ines Fritz[a], Klaus Leitner[a], Alejandra Palacios Romero[a], Adriana Kovalcik[b], Petr Sedlacek[b], Bernhard Drosg[a]

[a] *Institute of Environmental Biotechnology, Department of Agrobiotechnology, IFA Tulln, University of Natural Resources and Life Sciences Vienna, Konrad-Lorenz Strasse 20, 3430 Tulln, Austria*

[b] *Faculty of Chemistry, Brno University of Technology, Purkynova, 118 Brno, Czech Republic*

ARTICLE INFO

Keywords:
Cyanobacteria
Synechocystis sp. CCALA192
PHB
Tubular photobioreactor

ABSTRACT

The biopolymer poly-β-hydroxybutyrate (PHB) can be used as a promising bioplastic. It has a broad range of applications and is degraded relatively rapidly by soil organisms. Like many prokaryotes, the cyanobacterium *Synechocystis* sp. CCALA192 produces this biopolymer as a storage compound, especially under nutrient limitation.

In a 200-L tubular photobioreactor, we cultivated *Synechocystis* sp. CCALA192 semi-continuously over a period of 75 days with CO_2 as sole carbon source. A two-stage cultivation strategy was performed, where after 5–7 days nitrogen was depleted and the culture started to produce PHB and gradually turned from blue-green to yellow. After 16–20 days, 90% of the culture were harvested and the residual 10% were used as inoculum for the following cycle. The harvested culture had an average biomass concentration of 1.0 g/L with an average PHB content of 12.5% of cell dry weight. After restarting with fresh nutrients, the yellow culture turned blue-green again and degraded the PHB within 24–48 h. When nitrogen of the medium was consumed, PHB was produced again and the cycle continued. In the late stage of each production cycle, a ripening process was observed, where no CO_2 was consumed but the PHB concentration was still rising at the expense of the existing glycogen rich biomass.

Establishing a stable *Synechocystis* sp. CCALA192 culture under non-sterile conditions turned out to be difficult, as this small unicellular organism is very sensitive and easily grazed by protozoa. Therefore, a special cultivation strategy with partially anoxic conditions was necessary.

1. Introduction

According to the Intergovernmental Panel on Climate Change (IPCC), scientific evidence for anthropogenic carbon dioxide (CO_2) as the main driver for climate change is unequivocal [1]. Therefore, more and more effort has been made to utilize CO_2 as raw material and transform it into different products [2]. Apart from the chemical possibilities, there are also biotechnological ways using CO_2 as a substrate. Cultivating microalgae with CO_2 as carbon source is one promising approach [3, 4] and there is a wide variety of microalgal products, ranging from fuels like biodiesel [5–8] and bioethanol [9, 10] over platform chemicals like isoprene [11] to high-value products like polyunsaturated fatty acids or astaxanthin [12, 13].

Another interesting product synthesized by cyanobacteria, formerly known as blue-green algae, is the bioplastic poly-β-hydroxybutyrate (PHB). PHB is the most common polymer in the group of polyhydroxyalkanoates (PHAs) and the only PHA produced, when using CO_2 as sole carbon source. It has favorable mechanical properties similar to polypropylene and can be processed by thermoplastic methods, including fiber spinning or injection molding. But in opposite to petroleum-based polypropylene, it is compostable and degraded rapidly by soil organisms [14]. This could avoid serious environmental problems caused by spillage and litter from persistent plastics. For example, in the Austrian Danube river, plastic litter had temporarily outnumbered fish larvae. The small plastic particles are ingested by a wide range of organisms with yet unknown consequences [15].

Cyanobacteria produce PHB as an intracellular energy and carbon storage compound. The most important trigger for PHB production is nutrient deprivation, especially nitrogen limitation. The role of PHB in cyanobacteria is still not clear; besides its storage function, it is

* Corresponding author.
E-mail address: clemens.troschl@boku.ac.at (C. Troschl).

https://doi.org/10.1016/j.algal.2018.07.011
Received 17 April 2018; Received in revised form 8 June 2018; Accepted 15 July 2018

supposed that it serves as an electron sink and helps to restore $NADP^+$ and maybe supports the cell's stress resistance [16]. Generally, PHB concentrations in nutrient-deprived, photoautotrophic cyanobacteria range between 5 and 20% of cell dry weight (cdw), which is still quite low, compared to heterotrophic PHB producers with concentrations above 70% of cdw. However, in contrast to heterotrophic bacteria, cyanobacteria do not consume sugars, that are responsible for an estimated 50% of the total production costs [17]. Furthermore, cyanobacteria do not depend on agricultural crops, what makes them even more attractive as sustainable biomass producers.

There are numerous reports about PHB production with cyanobacteria: Kamravamanesh et al. produced 16.4% PHB of cdw with *Synechocystis* sp. PCC6714 [18]. Panda et al. produced 11.2% PHB of cdw with *Synechocystis* sp. PCC6803 [19]. Khetkorn et al. produced 26% PHB of cdw with a genetically modified *Synechocystis* sp. PCC6803 [20]. An exceptional high PHB content of 55% of cdw was produced with the thermophilic *Synechococcus* sp. MA19 [21]. Generally higher PHB contents could be produced with adding carbon sources like acetate or glucose. With this strategy, Bhati et al. produced 78% PHB of cdw with *Nostoc Muscorum* Agardh [22]. However, the addition of organic carbon sources will change the metabolism from autotrophic growth to heterotrophic growth. For further examples we recommend the latest reviews about this topic [23–26].

Almost all of the reported experiments were performed under sterile laboratory conditions and there are hardly any reports about pilot-scale production of PHB under non-sterile conditions. Apart from *Arthrospira* sp., which can be grown in a highly alkaline environment, cyanobacteria are not grown in an industrial scale. *Arthrospira* sp. produces comparable low amounts of PHB, therefore other strains like *Synechocystis* sp. or *Synechococcus* sp. are more promising. There are next to no reports about full-scale production plants with these small cyanobacteria. Indications are strong that the primary reason may be the difficulty of establishing a stable process – avoiding disturbances or overgrowth by other microorganisms. The main difference of large photobioreactors compared to lab-scale systems is the impossibility of sterilization. Therefore, large photobioreactors are susceptible to biological pollutants. Contamination problems due to protozoa are a major drawback when cultivating microalgae in an industrial scale [27].

This work investigates the pilot-scale production of PHB with *Synechoystis* sp. CCALA192 in a non-sterile photobioreactor with a volume of 200 L. Over a period of 75 days, four production cycles were performed with a special cultivation strategy of partially anoxic conditions. Several parameters such as biomass concentration, PHB concentration, glycogen concentration and CO_2 consumption were measured. The culture was routinely examined under the microscope and occurring contaminants are described. Furthermore, a new operation mode is proposed for a more efficient PHB production with cyanobacteria.

2. Material and methods

2.1. Organism and culture conditions for strain maintenance

Synechocystis sp. CCALA192 was ordered from the Culture Collection of Autotrophic Organisms, Institute of Botany, Trebon, Czech Republic. This strain was chosen as it proved to be the most promising PHB producer within a previously performed screening of roughly 30 strains. For strain maintenance in the laboratory, the strain was cultivated in BG11 medium [28] supplemented with 1 g/L sodium hydrogencarbonate ($NaHCO_3$) with a resulting pH of 8.5. Shaking flasks containing 50 mL of medium were used, light intensity was 1000 lx with a light/dark cycle of 16/8 and a temperature of 25 °C.

2.2. Photobioreactor and online analytics

The photobioreactor used in this study is situated in a small glass house at the coal power plant in Dürnrohr, Austria. It is a tubular reactor built with glass tubes from the Schott AG, Germany, with an inner diameter of 60 mm. The volume of the reactor is 200 L and the total length is 80 m. The basic design of the reactor is described elsewhere [29]. A centrifugal pump was used for circulating the medium with a velocity of 0.7 m/s. A bubble column with a height of two meter served as degasser. Pure nitrogen gas (N_2) with a constant flowrate of 500 mL/min served for oxygen removal. Pure carbon dioxide (CO_2) with variable flowrate was injected over a PI (proportional-integral) operated mass flow controller to keep the pH value. This allows online monitoring of CO_2 consumption as well as precise pH control. The pH value and oxygen concentration were continuously measured with probes (CPS11D and COS51D, both Endress + Hauser GmbH, Austria). The photosynthetically active radiation was measured with a PAR sensor (Theodor Friedrichs & Co., Germany). An additional artificial light was supplied with four 250 W gas discharge lamps (2× Philips Maser HPI-T and 2× Philips Master SON-T) and 50 m of LED strips (60 SMD-LED, 14.4 W per meter, 2700–3000 K). The LED strips were mounted directly on the glass tubes. The artificial illumination was installed to somehow compensate for the unfavorable situation, that the power plant was partially shading the glasshouse. The light situation in the glass house was therefore very complex and several PAR sensors would have been necessary for acquisition of adequate data for calculations. PAR is therefore only used in Fig. 5. The temperature in the glasshouse was controlled with an air conditioning system to 25 °C ± 2 °C. The data from all probes was captured by a data acquisition board (National Instruments) and connected to a computer for measurement and control.

2.3. Culture medium and cultivation strategy in the photobioreactor

Synechocystis sp. CCALA192 was cultivated in the photobioreactor with a modified BG11 medium. This BG11 contained no citric acid and was supplemented with 0.5 g/L $NaHCO_3$ and 0.5 g/L Na_2CO_3. The pH was adjusted to pH 10 (production cycle 1 and 2) and pH 9 (production cycle 3 and 4), respectively. 0.4 g/L instead of 1.5 g/L $NaNO_3$ were used, leading to self-limitation of the culture. After 16–20 days of cultivation, 90% of the culture were harvested and the remaining 10% were used as inoculum for the next production cycle.

Due to degassing with pure nitrogen gas, the oxygen concentration in the reactor decreased below detection limit during night. This approach was necessary for contamination control and led to stable growth conditions.

2.4. Analytical methods

For cell dry weight (cdw) determination, 50 mL of the culture were centrifuged at 4000*g* for 10 min. The pellet was washed with deionized water, centrifuged again and dried at 105 °C overnight. After weighing, the dried pellet was then used for PHB determination.

The determination of PHB concentration in the biomass was performed with a modified method after Karr et al. [30]. The dried pellet was heated at 95 °C for 4 h in 1 mL of concentrated sulfuric acid. This step converts PHB to crotonic acid. After diluting to 25 mL with deionized water, crotonic acid was measured on a HPLC system (Agilent 1100, column: Transgenomic CARBOSep COREGEL 87H). Commercially available PHB and pure crotonic acid were used as standards.

Volumetric PHB production rate was calculated as a mean value for each production cycle.

$$\textit{Volumetric PHB production rate} = \frac{\textit{Final volumetric PHB concentration}}{\textit{Duration of production cycle.}}$$

The determination of glycogen concentration was performed with a modified method after Maurer et al. [31]. 10 mL of the culture were centrifuged and the pellet was resuspended in 2 mL 6 M hydrochloric acid and heated at 95 °C for 2 h for total breakdown of the cells and