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Polymer accumulation in mixed cyanobacterial cultures selected under the feast and famine strategy

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ABSTRACT

In this study, a sequencing batch reactor (SBR), operated with transient carbon availability (feast and famine) and different nutrients loads, was used to select cyanobacteria accumulating poly (3-hydroxyalkanoate) (PHB) and carbohydrates from a mixed wastewater-borne microbial culture. The SBR was operated with 12 h aerobic light and 12 h anaerobic dark phases, evaluating the effect of three different operational conditions consisting on; 1) carbon limitation, 2) carbon and phosphorus limitation and 3) phosphorus limitation. Once a steady state was reached in each operational period of the SBR, part of the biomass was collected and submitted to separate batch tests in order to investigate the maximum PHB and carbohydrates accumulation levels. Batch tests were performed during 24 h of illuminated aerobic condition and 24 h of dark anaerobic condition, while inorganic carbon was constantly present. During the SBR operation, inorganic carbon was mostly used for biomass and carbohydrate production, showing very low PHB accumulation levels (< 1%). Notwithstanding, in subsequent batch tests, PHB was accumulated after a complete depletion of nitrogen, reaching almost 4%. Concerning carbohydrates, it was found that phosphorus limitation (with and without carbon limitation) led to a culture mostly dominated by cyanobacteria and higher levels of carbohydrate content (43%–48%) than the culture with carbon limitation and high loads of nitrogen and phosphorus (29%). Such contents were obtained in only 24 h of incubation under aerobic illuminated conditions. Hence, these encouraging results indicate that carbon uptake and the consequent polymers production from cyanobacteria can be enhanced through carbon and nutrient feeding strategies.

1. Introduction

Cyanobacteria are prokaryotes capable to perform oxygenic photosynthesis and they can be found in almost every environment on earth [1]. During the last decades, they have received much attention as a rich source of polymers, being considered as one of the most promising group of organisms to produce them [2]. Cyanobacteria are able to accumulate both carbohydrates in form of glycogen and polyhydroxyalkanoates (PHA), e.g., poly (3-hydroxyalkanoate) (PHB). Carbohydrates and PHB are attracting increasing interest due to their potential as a biofuel substrate and as a bioplastic, respectively. Although those polymers are also accumulated in other photosynthetic and non-photosynthetic bacteria, the studies that have been done thus far have based their polymers production on the utilization of organic molecules as C source [3]. In the case of cyanobacteria, their mechanism for polymer production is based on carbon storage through oxygenic photosynthesis implying simple requirements for cultivation and the utilization of CO_2 as carbon source [4]. This ability for CO_2 fixation and conversion into biopolymers is nowadays significantly attractive due to the worldwide concern with the CO_2 impact in climate change.

Until now, experiments on carbohydrates and PHB production from cyanobacteria have been performed through pure strains and genetically modified species [5–9], implying strictly controlled processes leading to high production costs, and subsequently expensive products [10]. In this context, a more sustainable alternative for the production of polymers from cyanobacteria could be the use of wastewater-borne cultures. This approach implies the lack of sterilization of substrates or reactors and cheaper equipment that could reduce the production costs compared to pure culture processes. Nevertheless, in spite of being an attractive alternative, the utilization of mixed cyanobacterial cultures to produce biomass and polymers strictly depends on the composition

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of the culture. Indeed, a conventional mixed wastewater-borne culture is composed by a mixture of cyanobacteria, other bacteria (which also could accumulate carbohydrates and PHB) and eukaryotic microorganisms, such as green algae, diatoms, metazoa and protozoa, which are unable to produce both polymers. Hence, a certain control of the consortium composition would be necessary in order to achieve favorable yields.

Previous studies, mainly carried out in lakes and reservoirs [11–14], but also in wastewater systems [15,16], highlighted that absolute nutrients concentration and ratio (N:P) are the two most important factors influencing the competition of cyanobacteria with other species (i.e., green algae) [12,17]. More specifically, the dominance of cyanobacteria has been related to their high affinity for N and P and capacity to store them intracellularly [18].

Concerning the polymer accumulation capacity of cyanobacteria, it has been demonstrated that nutrient limitation coupled with carbon excess are determining factors to increase polymer accumulation [19]. Thus, due to their high tolerance to nutrient changes and carbon availability, cyanobacteria polymer production usually requires prolonged periods. The low carbon uptake efficiency turns the polymer production into a slow process compared with processes involving heterotrophic bacteria. Indeed, the maximum accumulation of polymers in cyanobacteria usually takes > 9 days of incubation for carbohydrates and > 11 days for PHB accumulation [20–22]. This fact highlights the need for new strategies to improve the efficiency of inorganic carbon (IC) uptake in cyanobacteria and its transformation into polymers.

Considering the example of mixed bacterial cultures, one of the most feasible strategies to select specific accumulating microorganisms and improve PHB and carbohydrate production is the application of unbalanced growth, also called feast and famine [23]. This process consists of a transitory carbon supply, in which the biomass is subjected to a period of carbon availability and a subsequent absence of carbon. With this process, cell growth and storage products are enhanced while the microorganisms able to store carbon and utilize their own reserves are selected.

In this work, feast and famine is proposed as a strategy for the selection of autotrophic cyanobacteria accumulating polymers. To the authors' knowledge this is the first time that this strategy is employed to select cyanobacteria from a wastewater-borne culture using inorganic carbon as substrate to produce value-added polymers. In the present study, a mixed wastewater consortium was cultivated in a sequencing batch reactor (SBR), evaluating the effect of different nutrients ratios and loads under transient carbon availability on polymer production during the intercalation of aerobic and anaerobic phases. In addition to the effect of those factors on polymer production, the effects of other parameters such as microbial composition, nutrient uptake and oxygen production are also considered and discussed.

2. Material and methods

2.1. Sequencing batch reactor set-up

For the enrichment of cyanobacteria producing PHB and carbohydrates, a double jacket acrylic reactor with a working volume of 2 L was used. The reactor was operated as a non-sterile sequencing batch reactor (SBR). The inoculum utilized consisted of a consortium of green algae, cyanobacteria, bacteria and protozoa, obtained from a pilot photobioreactor described elsewhere [16].

The SBR was operated with a hydraulic retention time (HRT) of 2 days and sludge retention time (SRT) of 10 days. The reactor operation was based on 24 h cycles according to the following scheme (Fig. A1):

1. Light aerobic phase (12 h): i) 9:30 am, nutrient uptake period, starting with the addition of 1000 mL of growth medium to the reactor (6 h), ii) 3:00 pm, carbon uptake period (carbohydrate accumulation), starting with a pulse of 6 mL of 0.442 g L^{-1} of Na_2CO_3 (0.050 g C L^{-1}).

2. Dark anaerobic phase (12 h): iii) 9:30 pm, start of the anaerobic phase in which argon was sparged into the culture (250 mL/min for 20 min every 2 h) in order to remove oxygen, iv) 8:50 am, effluent withdrawal phase (2 min) in which 200 mL of the mixed liquor was removed from the culture, v) 9:00 am, settling phase (30 min without stirring), vi.) Supernatant withdrawal phase in which 800 mL were removed from the medium.

During the light phase, the SBR was continuously illuminated with two external LED lamps (23 W) placed on the two sides of the SBR giving a light intensity of 91 W/m^2, corresponding to a volumetric light intensity of 2.1 W/L.

Throughout the whole cycle (with the exception of the settling and supernatant withdrawal phases), the medium was stirred using a magnetic stirrer (VELP scientific, USA). The reactor temperature was controlled at 27 °C by means of a water jacket and a thermostat bath (Julabo, Germany). The pH of the reactor was maintained at 8.2 using dosages of 0.01 M NaOH and 0.05 M HCl.

The SBR was operated for 6 months during which three different conditions were tested as shown in Table 1. In the first condition high N and P loads were applied to the culture while it was exposed to a low carbon load. In the second condition a low carbon load was coupled with a low phosphorus load. Lastly, in the third condition, the effect of only low phosphorus load was tested. Those conditions tested the influence of loads on cyanobacteria dominance, growth and production of PHB and carbohydrates. Each condition was tested when the reactor reached steady state. The reactor was considered to be in steady state when the concentration of total suspended solids (TSS) at the end of the cycle showed stable results or after the system reached three SRTs.

Table 1
Experimental operating conditions of the SBR.

Operation period	C load (mg L^{-1} d^{-1})[a]	N load (mg L^{-1} d^{-1})[a]	P load (mg L^{-1} d^{-1})[a]	C:N:P ratio
Condition 1	50	12.5	4.2	12:3:1
Condition 2	50	12.5	1	50:12.5:1
Condition 3	100	12.5	1	100:12.5:1

[a] Volumetric load per volume of reactor.

The growth solution added at the beginning of the light phase in each cycle consisted in growth medium containing: 0.049 g L^{-1} NH4Cl, 0.072 g L^{-1} $CaCl_2{\cdot}2H_2O$, 0.001 g L^{-1} Na_2EDTA, 0.075 g L^{-1} $MgSO_4{\cdot}7H_2O$, 0.6 g L^{-1} $C_6H_8FeNO_7$ (ammonium ferric citrate), 0.006 g L^{-1} $C_6H_8O_7$ (citric acid), and 1.0 mL^{-1} of trace elements: 2.86 g H_3BO_3, 0.39 g $Na_2Mo_4{\cdot}2H_2O$, 1.8 g $MnCl_2{\cdot}4H_2O$, 0.08 g $CuSO_4{\cdot}5H_2O$, 0.22 g $ZnSO_4{\cdot}7H_2O$ and 0.05 g $Co(NO_3)_2.6H_2O$. Depending on the operation of the SBR, K_2HPO_4 concentration varied from 0.24 g L^{-1} in operation 1 and 0.0056 g L^{-1} for operations 2 and 3. Na_2CO_3 was added independently as the inorganic carbon source by the addition of 1 pulse of 6 mL containing 0.442 g L^{-1} (0.050 g C L^{-1}) at the beginning of the carbon phase during operations 1 and 2, and 2 pulses of 0.442 g L^{-1}, one at the beginning and the other in the middle of the light phase in condition 3.

2.2. Batch test for polymer accumulation

For each operation period, when steady state was reached, 200 mL of mixed liquor were collected and placed in a separate batch reactor, filled to 400 mL with deionized water and used for polymer production batch experiments. In these tests, the operation of the reactor was based on a 48 h cycle, starting with 24 h of light aerobic condition followed by 24 h of dark anaerobic condition. During the light aerobic phase, the reactor worked as an open photoreactor while in the anaerobic phase the reactor was hermetically closed. This strategy was performed in order to accumulate high carbohydrate content during the light aerobic