THE CYANOBACTERIA ROUTE TO PRODUCE POLY-β-HYDROXYBUTYRATE

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The amount of plas(cs produced and released in the environment is drama(cally increased during the last decades. The plas(cs produc(on takes up a large frac(on of fossil resources and their disposal leY worrying traces in the environment. A solu(on to both issues is the biodegradable plas(c: i) they are produced from renewable resources; ii) they may be disposed without to compromise the environment. Polyhydroxyalkanoates (PHAs) are widely adopted as building blocks for bioplas(c produc(ons and they may be produced by microorganisms. The poly-β- hydroxybutyrate (PHB) is the most widespread and thoroughly characterized PHA found in bacteria. It is accumulated by various microorganisms as carbon and/or energy storage source when operated under harsh condi(ons. In par(cular, cyanobacteria are poten(al host systems for the PHB produc(on. This contribu(on reports a join research between the University of Napoli and the University of Amsterdam on the feasibility of PHB produc(on by means of autotrophic cyanobacteria cultures in photobioreactors. *SynechocysDs* PCC 6803 was inves(gated. Tests were carried out in shacked flasks and photobioreactors. Batch tests were aimed at the op(miza(on of the growth condi(ons. The growth medium was BG11 (1.5 g/L $NaNO_3$; 0.04 g/L K_2HP_4; 0.075 g/L $MgSO_4$; 0.036 g/L $CaCl_2$; 0.006 g/L Citric Acid; 0.006 g/L Ammonium Ferric Citrate; 0.001 g/L Na_2EDTA; 0.02 g/L $NaCO_3$). Nitrate and phosphate concentra(ons in the medium were tuned to find the best growth condi(ons and subsequently PHB best produc(vity. Inclined bubble column photobioreactors were adopted: volume of 800 mL. Light/dark cycle was adopted: 12 h light at 150μEm^{-2}s^{-1}; 12 h dark. Gas flow rate was set at 4 vvm. CO_2 concentra(on in the air stream was set at 2%v. Cultures were characterized in terms of biomass, PHB content, pH, nitrate consump(on and phosphate consump(on.

INTRODUCTION

In today's modern era of science and technology, plas(cs are among the materials widely used over the world. Although mechanical-technological features of plas(cs have been worldwide recognized, plas(cs have long been vilified because they are environmentally unfriendly, i.e. they are not biologically degradable

Bioplas(cs are materials that are both *biodegradable* and produced from *biomasses (*biobased materials). "Biodegradable materials" means that microorganisms are able to convert them into natural substances (e.g. water, carbon dioxide, compost, ..).

Polyhydroxyalkanoates (PHAs) produced by microorganisms are fundamental building blocks for bioplas(c produc(ons. Poly- β-hydroxybutyrate is the most widespread and thoroughly characterized PHA found in bacteria. It is accumulated as carbon and/or energy storage material in various microorganisms under harsh condi(ons: typically under N/P deple(on stresses provided the presence of the carbon source (Panda et al., 2006). PHB is a poten(al bulding block for plas(cs and it fits the new waste management strategies (Balaji et al., 2013).

PHBs have been produced by heterotrophic bacteria. Cyanobacteria are poten(al host systems for the PHB produc(on because of the minimal nutrient requirements and the photoautotrophic nature: cyanobacterial species accumulate the homopolymer of PHB under photoautotrophic condi(ons.

Cyanobacteria - known as blue-green algae - are Gram nega(ve photoautotrophic bacteria. Cyanobacteria are characterized by short duplica(on (me. For the growth, they need simple inorganic nutrients such as phosphate, nitrate, magnesium, and calcium as macronutrients and ferrous, manganese, zinc, cobalt, and copper as micronutrients. 6803 is a unicellular non-nitrogen (N2)-fixing

The cyanobacteria that I use in this study is *SynechocysDs* sp. PCC6803 (Fig. 1). *SynechocysDs sp. PCC* cyanobacterium and a ubiquitous inhabitant of fresh water (Ikeuchi and Tabata,2001). It can grow autotrophically or heterotrophically in the absence of light. This organism shows impressive growth characteris(cs: it is a rela(vely fast growing (minimal doubling (me seven to eight hours) cyanobacterium, with no specific nutri(onal demands. Thus, it can grow fully photoautotrophically, mixotrophically, and chemoheterotrophically.

PHB is an intracellular product (Fig. 2). An extrac(on step/process to recover PHB from the cells is required. The sonica(on method was used tu cells rupture and the propanolisys method was used to obtained PHB. The procedure to detect PHB in cyanobacteria extract, proposed by Nagamani et al. (2011) consist in a slight modifica(on of the gas chromatographic method of Riis and Mai (1988).

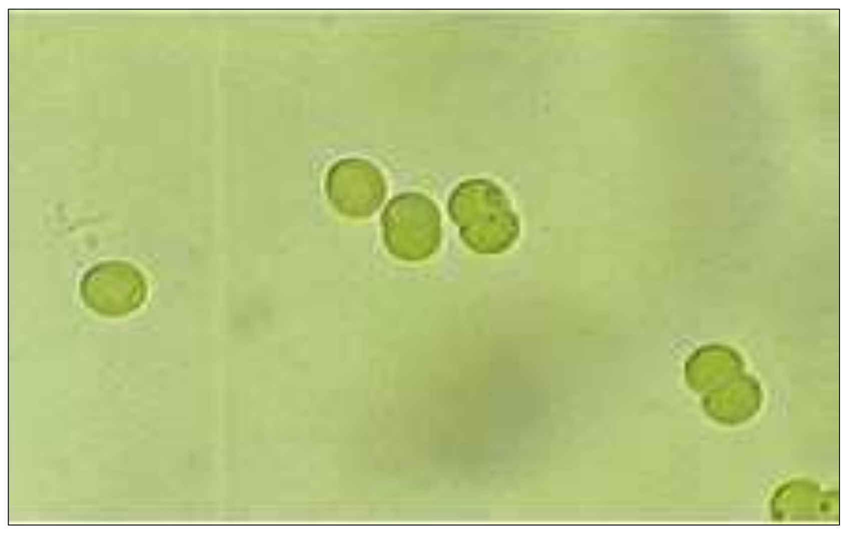

Fig. 1- SynecochysDs PCC 6803

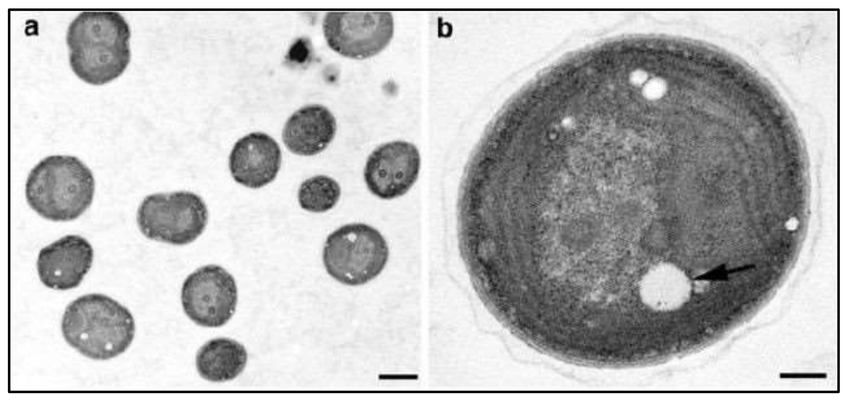

Fig. 2- Intracellular granules of PHB

MATERIALS AND METHODS

- **GROWTH MEDIUM:** the growth medium was BG11 (1.5 g/L $NaNO_3$; 0.04 g/L K_2HP_4; 0.075 g/L $MgSO_4$; 0.036 g/L $CaCl_2$; 0.006 g/L Citric Acid; 0.006 g/L Ammonium Ferric Citrate; 0.001 g/L Na_2EDTA; 0.02 g/L $NaCO_3$). Nitrate and phosphate concentra(ons in the medium were tuned to find the best growth condi(ons and/or PHB best produc(vity.
- **PRELIMINARY BATCH TESTS:** batch tests were aimed at the op(miza(on of the growth condi(ons (Fig. 3).
- **REACTOR CONFIGURATION:** inclined bubble column photobioreactors were adopted: volume of 800 mL (Fig. 4). Light/dark cycle was adopted: 12 h light at 150μEm^{-2}s^{-1}; 12 h dark. Gas flow rate was set at 4 vvm. CO_2 concentra(on in the air stream was set at 2%v. Cultures were characterized in terms of biomass, PHB content, pH, nitrate consump(on and phosphate consump(on.
- **SONICATION:** PHB was accumulated in cyanobacteria cells. Sonicator (Sonics Vibracell 500) was used for cells rupture.
- **PROPANOLYSIS METHOD:** PHB was quan(fied using a slight modifica(on of the gas chromatographic method proposed by Riis and Mai (1988): i) sample sonica(on; ii) weight of the precipated polymer; iii) mixing with 1,2-Dichloroethane (DCE), n-Propanol, hydrochloric acid (HCl), 200 μl internal standard (benzoic acid); iv) incubated for 4 h in a water bath at 85⁰C under intermioent shacked condi(ons; v) mixing with water at room temperature and incuba(on under shacked condi(ons for 20 – 30 s; vi) the DCE-Propanol phase was analyzed by gas chromatograph (Agilent 6890 equipped with FID).

Fig. 3 – Batch test apparatus.

Fig. 4- Photobioreactor configuraDon.

RESULTS

- Fig. 5 reports a growth curve of Synechocys(s sp. PCC 6803 in a photobioreactor.
- Fig. 6 shows the PHB accumulated at the booom of vials at the ened of the recovery procedure.

EXPECTED RESULTS

- Iden(fica(on of a spectrum of poten(al cyanobacterial strains for PHB produc(on via biotechnological route.
- Iden(fica(on of best photobioreactor configura(on for cyanobacteria growth and PHB produc(on.
- Collec(on of data (kine(cs, yield, …..) to support the feasibility study of the PHB produc(on process by adop(ng the selected cyanobacterial strains.
- Development of conversion model to support the design of bioreactors for the PHB produc(on process by adop(ng the selected cyanobacterial strains.
- Engineered cyanobacterial strain characterized by high performances.

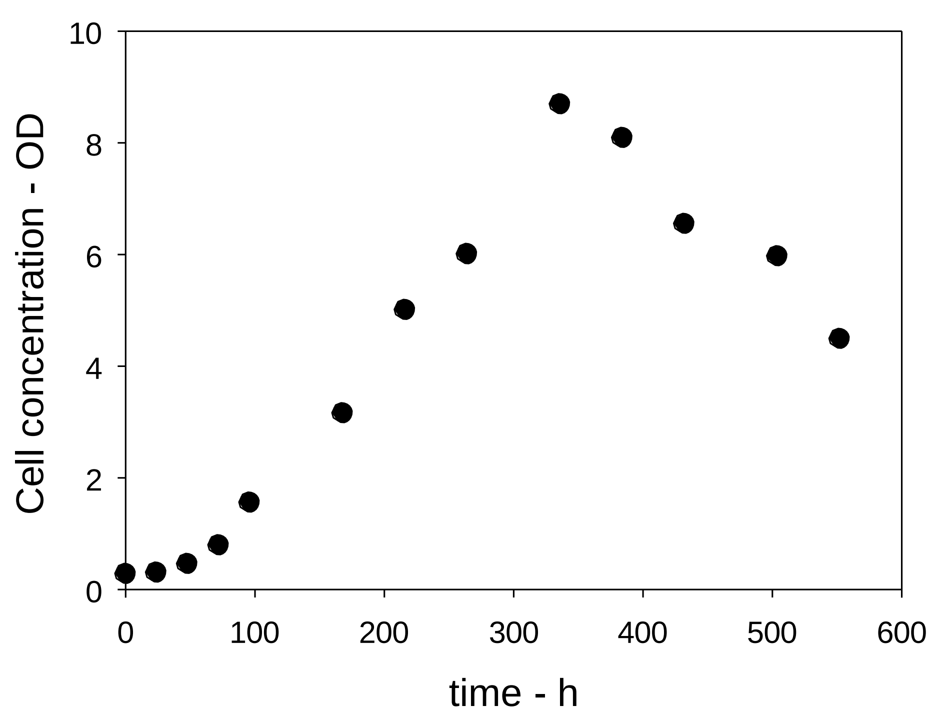

Fig. 5- Growth of SynechocysDs sp. PCC

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Fig. 6 – PHB from SynechocysDs sp. PCC cultures

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