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Instrumental Characterization of Cyanobacteria as Polyhydroxybutyrate Producer

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Abstract : Cyanobacteria are gram-negative prokaryotes belonging to a group of photosynthetic bacteria. In comparison with heterotrophic microorganisms, cyanobacteria utilize atmospheric nitrogen and carbon dioxide without any additional substrates. This ability of these microorganisms could be employed in biotechnology for the production of bioplastics, concretely polyhydroxyalkanoates (PHAs) which are primarily accumulated as a storage material in cells in the form of intracellular granules. In this study, there two cyanobacterial cultures from genera Synechocystis were used, namely Synechocystic sp. PCC 6803 and Synechocystis salina CCALA 192. There were optimized and used several various approaches, including microscopic techniques such as cryo-scanning electron microscopy (Cryo-SEM) and transmission electron microscopy (TEM), and fluorescence lifetime imaging microscopy using Nile red as a fluorescent probe (FLIM). Due to these instrumental techniques, the morphology of intracellular space and surface of cells were characterized. The next group of methods which were employed was spectroscopic techniques such as UV-Vis spectroscopy measured in two modes (turbidimetry and integration sphere) and Fourier transform infrared spectroscopy (FTIR). All these diverse techniques were used for the detection and characterization of pigments (chlorophylls, carotenoids, phycocyanin, etc.) and PHAs, in our case poly (3-hydroxybutyrate) (P3HB). To verify results, gas chromatography (GC) was employed concretely for the determination of the amount of P3HB in biomass. Cyanobacteria were also characterized as polyhydroxybutyrate producers by flow cytometer, which could count cells and at the same time distinguish cells including P3HB and without due to fluorescent probe called BODIPY and live/dead fluorescent probe SYTO Blue. Based on results, P3HB content in cyanobacteria cells was determined, as also the overall fitness of the cells. Acknowledgment: Funding: This study was partly funded by the projectGA19-29651L of the Czech Science Foundation (GACR) and partly funded by the Austrian Science Fund (FWF), project I 4082-B25.

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