METABOLIC ENGINEERING APPLIED TO BIOPOLYMER PRODUCTION

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Figure 1. Bioreactor bacterial cultivation collecting data in the project.

Metabolic flux analysis (MFA) and other tools of metabolic and biochemical engineering are currently being applied for the improvement of the production of biodegradable polymers belonging to the polyhydroxyalkanoates family (PHA), particularly poly-3-hydroxybutyrate-co-3-hydroxyalkanoate of medium-chain-length [P(3HB-co-HAmcl)]. Flux analysis is based on a free access software and experimental data generated in bioreactor. Bacterial cultures in a continuous mode operation, employing the technique of multiple nutrient limitation to induce polymer accumulation is another strategy to study the role of such nutrients and to generate data to MFA. Metabolic models will be proposed, simulated and validated utilizing the experimental data from bioreactor cultivations. The analysis of the metabolic models will provide information to propose genetic modifications and process strategies, to obtain higher yields and productivities. It is expected that, by the end of this project, the metabolic engineering approach emphasizing the integration of different methodologies and procedures will be established. As a result, a Metabolic Engineering Group is being created in the Microbiology Department at ICB USP, in such a way that processes involving other bioproducts could be approached in
a similar manner.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Analysis of the tracing pattern on C-labelled PHA has been performed, evidencing for the first time the extreme effectiveness of this approach to determine fluxes distribution in the central metabolism involved on PHA production. Associated with elementary mode analysis of the metabolism, this procedure allowed the identification of biochemical engineering and/or genetic approaches to improve production of these polymers and to characterize bacterial strains for the production of other compounds.

Different strains were characterized with respect to PHA production. Those genes that showed promising performance and interest have been cloned. Thereby strains producing copolymers of P(3HB-co-HAmcl) in a controlled manner were successfully obtained. This group of copolymers has properties that have attracted great interest as they permit to obtain plastic films.

Bioreactor experiments are underway to validate metabolic models to propose possible strategies to improve polymer process production. Chemostat cultures exploring the regions of multiple nutrient limitations are scheduled. The resulting steady states will enable the study of different physiological situations and will result on a snapshot representing each situation.

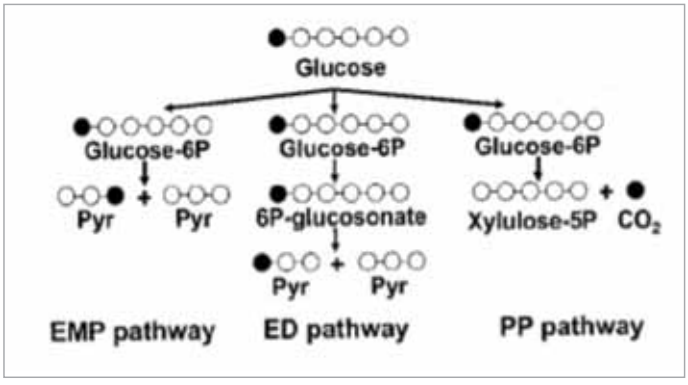

Figure 2. Illustration of how measurement of the 13C enrichment patterns can be used to identify active pathways. EMP (Embden-Meyerhof-Parnas), ED (Entner-Doudoroff), PP (Pentose phosphate).

MAIN PUBLICATIONS

Kawai LA, Taciro MK, Rozzo YPG, Silva LF, Fonseca GG, Gomez JGC. 2011. Metabolic Flux analysis of polyhydroxialkanoates produced from *Pseudomonas sp* LFM 046 utilizing glucose labeled. 26th Brazilian Congress of Microbiology 134-G, Foz do Iguaçu, Paraná, Brasil.

Lício DCP. Screening of PHA producing bacteria and molecular characterization of the PHA synthase enzyme. 2011. Master dissertation (Biotecnology) – University of São Paulo.

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