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)]. Using steady-state data generate in bioreactor experiments, flux analysis is performed through a freely available software. 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 has been 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 were done to validate metabolic models and to propose possible strategies to improve polymer process production. Chemostat cultures exploring the regions of multiple nutrient limitations were done. The resulting steady states enable the study of different physiological situations and result on a snapshot representing each situation.

The genome sequencing performed in the studied bacteria (*Pseudomonas LFM 046*) enabled the construction of the model in genomic scale improving the metabolic modeling studies.

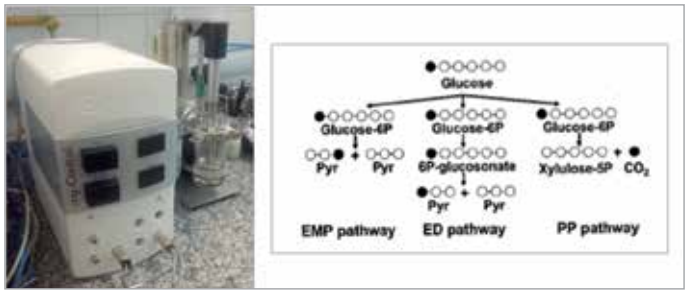

Figure 2. Minibioreactor (100 ml working volume) utilized on experiments with labeled carbon source. Illustration of how measurement of the ^{13}C enrichment patterns can be used to identify active pathways. EMP (Embden-Meyerhof-Parnas), ED (Entner-Doudoroff), PP (Pentose phosphate)

MAIN PUBLICATIONS

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