

PREDICTION OF INTRACELLULAR STORAGE POLYMERS USING QUANTITATIVE IMAGE ANALYSIS IN ENHANCED BIOLOGICAL PHOSPHORUS REMOVAL SYSTEMS

D.P. Mesquita¹, C. Leal², J.R. Cunha¹, A. Oehmen³, A.L. Amaral^{1,2},
M.A.M. Reis³, E.C. Ferreira¹

¹*IBB-Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal*

²*Instituto Superior de Engenharia de Coimbra, Instituto Politécnico de Coimbra, Rua Pedro Nunes, Quinta da Nora, 3030-199 Coimbra, Portugal*

³*CQFB/REQUIMTE, Chemistry Department, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
e-mail: daniela@deb.uminho.pt*

Intracellular polymers, such as polyhydroxyalkanoates (PHA) synthesized by microorganisms for energy and carbon storage, can be commercially used as biodegradable plastics in a wide range of applications. The possibility of obtaining high PHA contents (where the most common monomers found are poly-hydroxybutyrate (PHB) and poly-hydroxyvalerate (PHV)) from inexpensive inocula and raw materials emerges as a promising and commercially interesting alternative. For this purpose, mixed cultures operated under feast/famine cycles are most frequently used. PHA is an important storage polymer in the metabolism of microorganisms involved in enhanced biological phosphorus removal (EBPR) systems. It is stored by polyphosphate accumulating organisms (PAO) and glycogen accumulating organisms (GAO), as described in previous publications [1,2]. Monitoring intracellular storage polymers in bacteria is usually performed through laborious and time consuming off-line chemical analyses. Thus, there is clearly a need to develop new techniques in order to promptly monitor these processes. Image analysis techniques have the potential to be a non-invasive and rapid means of assessing the amount of different storage polymers inside microbial cells, providing the evaluation of these important biotechnological processes. The present study focuses on predicting intracellular storage polymers in EBPR systems. For that purpose, quantitative image analysis techniques were developed and partial least squares (PLS) were used to model PHA results. An EBPR fed with synthetic wastewater containing volatile fatty acids (VFAs) and orthophosphate was used. Biomass samples were collected at the end of the anaerobic and aerobic phases. Analytical PHA quantification was performed by biomass digestion and gas chromatography analysis. In the concurring image analysis methodology Nile blue was used as a fluorescence staining method for PHA granules identification. The results from image analysis allowed establishing a PHB prediction ability presenting a regression value (R^2) of 0.854, a PHA prediction regression value (R^2) of 0.843 and a PHV prediction regression value (R^2) of 0.779. The lower prediction ability for PHV could be explained since this parameter presented only a small contribution to the overall PHA. The analysis performed based on the variable importance in the projection (VIP) established a core of three image analysis parameters (granules total area, granules total intensity and image intensity) as the most important regarding PHA, PHB and PHV prediction.

Acknowledgement: Fundação para a Ciência e a Tecnologia (FCT) in Portugal is gratefully acknowledged through the project PTDC/EBB-EBI/103147/2008. DP Mesquita would like to acknowledge FCT for a post-doctoral grant (SFRH/BPD/82558/2011).

References:

- [1] A. Oehmen, A.M. Saunders, M.T. Vives, Z. Yuan, J. Keller, *Journal of Biotechnology*, 123, 22 (2006).
- [2] A. Oehmen, P.C. Lemos, G. Carvalho, Z. Yuan, J. Keller, L.L. Blackall, M.A.M. Reis, *Water Research*, 41, 2271 (2007).