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INTRACELLULAR POLY-P ASSESSMENT BY DAPI STAINING AND IMAGE ANALYSIS

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In wastewater treatment, enhanced biological phosphorus removal (EBPR) is considered a well-established process to remove phosphate (P). EBPR is based on the activity of polyphosphate-accumulating organisms (PAOs) able to take up and store large amounts of P as intracellular (poly-P) granules. However, monitoring poly-P in mixed cultures is usually performed by a laborious and time consuming off-line chemical analysis. Thus, there is a clear need to develop new techniques to rapidly monitor these processes, such as image analysis coupled to sample staining and microscopy inspection.

A lab-scale sequencing batch reactor (SBR) was fed with synthetic wastewater containing acetate and propionate as main carbon sources and an orthophosphate solution was added. A COD/P ratio of 10 mg COD mg P-PO₄⁻¹ was used to provide selective advantages to PAOs. The SBR was operated with a cycle time of 6 h: 120 min anaerobic including 5 min feed, 180 min aerobic and 60 min wasting/settling. Biomass samples were collected at the end of the aerobic stage. Bulk P concentration was determined by segmented flow analysis and total P concentration was similarly measured following acid digestion at 100°C. Intracellular poly-P concentration was determined by subtracting the bulk P from the total P. Intracellular poly-P granules were observed in epifluorescence microscopy using DAPI staining with a 25 µg mL⁻¹ DAPI solution. A long pass filter was used with an excitation bandpass of 365–370 nm and emission cut off at 421 nm. A specially developed program in Matlab was used for image analysis.

A total of 41 samples were collected. Two thirds were fed as training data to the partial least squares (PLS) model and the remaining used for validation. Both absolute (in mg poly-P / L) and relative (in mg poly-P / g MLSS) intracellular poly-P concentrations were studied. This procedure was found to predict, at some extent, the relative intracellular poly-P concentration (real poly-P = 0.971 x predicted poly-P, R² of 0.744). Regarding the absolute intracellular poly-P concentration, a total of 3 samples needed to be discarded in order to obtain a similar result (real poly-P = 1.005 x predicted poly-P, R² of 0.731).

References: Mesquita, D.P., Leal, C.S., Cunha, J.R., Oehmen, A., Amaral, A.L., Reis, M.A. & Ferreira, E.C. 2013. Prediction of intracellular storage polymers using quantitative image analysis in enhanced biological phosphorus removal systems. *Anal. Chim. Acta* 770, 36–44.