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Development of image analysis methodologies to quantify intracellular PHA, polyphosphate and glycogen within wastewater treatment plants

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In wastewater treatment plants (WWTP), enhanced biological phosphorus removal (EBPR) processes are performed by mixed cultures containing polyphosphate (PAO) and glycogen accumulating organisms (GAO). In these processes, it is of crucial importance to monitor the intracellular metabolism, namely glycogen, polyhydroxyalkanoates (PHA) and polyphosphate (polyP) inclusions, to determine its efficiency. However, traditional monitoring, carried out through off-line chemical analyses, is laborious and time-consuming. Therefore, there is a clear need to develop new techniques to promptly quantify these intracellular polymers, with image analysis emerging as a promising tool.

The use of staining methodologies with specific fluorescent dyes is widespread in EBPR research, including Nile blue for PHA and DAPI for polyP. Although rarely applied in EBPR studies, Aniline blue is a fluorescent stain that can be used for glycogen determination. Furthermore, these fluorescent stains have generally been employed for qualitative rather than quantitative analysis. Therefore, this study aim focused on developing fluorescence-based staining methodologies for glycogen, and on acquisition, processing and image analysis procedures for PHA, polyP and glycogen. Image analysis data was then correlated with traditional analytical data by multivariable statistics.

Regarding the determination of the glycogen intracellular concentration, results have been promising, presenting a good correlation (R2 of 0.915) between analytical and image analysis data. The staining and image analysis procedures for the determination of the intracellular concentration of PHA and polyP are currently being optimized. This study will provide a quantitative means to assess PAO and GAO metabolic activity *in-situ* in WWTP, facilitating the optimisation of these processes.

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