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Polyphosphates and poly-β-hydroxybutyrate granules identification through quantitative image analysis in enhanced biological phosphorus removal systems

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Enhanced biological phosphorus removal (EBPR) is a widely implemented technique to remove phosphate from wastewater treatment processes, being cost effective and more reliable than traditional chemical methods. EBPR is performed by operating the system sequentially with anaerobic and aerobic conditions. Several studies have already been performed ranging from different strategies for the competition between polyphosphate accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs) to modeling both types of bacterial activities. Until now, little attention has been given to the development of newer, faster, simpler, and better suited monitoring techniques for this type of system. This work is focused on the development of fluorescence based image analysis techniques for polyphosphates and poly-β-hydroxybutyrate granules detection in EBPR systems since off-line analyses are labor intensive and difficult to apply in full-scale plants. A lab-scale sequencing batch reactor fed with synthetic wastewater containing volatile fatty acids (VFAs) and orthophosphate was used. The reactor had a working volume of 4 L and was operated with a cycle time of 6 h consisting of 2 h anaerobic, 3 h aerobic, 50 min settling and decanting, and 5 min wasting. In each cycle, 2 L of synthetic wastewater was fed to the reactor in the first 5 min of the anaerobic period, resulting in a hydraulic retention time (HRT) of 12 h. The pH was controlled during both the anaerobic and aerobic periods around 7, and the temperature was controlled at 30 ºC in order to provide selective advantages to GAOs over PAOs. The ratio between chemical oxygen demand (COD) and P in the feed was kept at 10 (g COD/g P). Biomass samples were collected at the end of the anaerobic and aerobic phases and fixed with phosphate buffer saline solution (PBS) and ethanol. Two fluorescence staining methods were used: (1) DAPI for poly-P identification; and (2) nile blue for poly-β-hydroxybutyrate granules. So far, promising results were achieved regarding the quality of images obtained by these fluorescence staining methods which are later treated by image analysis procedures.