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POLYHYDROXYALKANOATE GRANULES QUANTIFICATION IN MIXED MICROBIAL CULTURES: SUDAN BLACK B *VERSUS* NILE BLUE A STAINING

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Polyhydroxyalkanoates (PHAs) are intracellular granules found in a wide variety of microorganisms under limited nutrient conditions when carbon source is available in excess. These polymers, usually from lipid nature, are used as carbon and energy sources for metabolic synthesis and growth. Despite the important role of PHAs in cell physiology, they are regarded as potential substitutes of traditional petrochemical plastics with the additional advantage of being completely biodegradable and produced from mixed microbial cultures (MMC). PHA quantification is regularly accomplished using a digestion step prior to chromatography analysis which is a labor and time-consuming technique.

To overcome these limitations in polymers quantification, the present work investigates two methods for PHA granules identification based on quantitative image analysis (QIA) procedures in an enhanced biological phosphorus removal (EBPR) system operated for three months. MMC were analyzed for PHA granules detection by Sudan Black B (SBB) and Nile Blue A (NBA) staining using bright-field and epifluorescence microscopy, respectively. The captured color images were evaluated through QIA and the image analysis data was further processed using multivariate statistical analysis. Quite satisfactory partial least squares (PLS) regressions (R^2) of 0.85 for NBA and 0.86 for SBB were established between PHA concentrations predicted from QIA parameters and determined by the standard analytical method. Although SBB staining procedure was found to provide a somewhat higher estimation of PHA concentrations in MMC, the consistency between PLS results allowed to conclude that both SBB and NBB staining methods combined with QIA procedures demonstrated the capability to estimate PHA concentrations. Concluding, both staining procedures are promising alternative for a faster PHA assessment relatively to the laborious standard PHA quantification.