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Validation of Fluorescence Microbead for Biofilm Labeling Method for Tracking Biofilms Growth and Interaction during The Treatment of Wastewater

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ABSTRACT

A Method for understanding the granular sludge-flocs interactions during aerobic granular formation was developed in this study. The developed method was subjected to a validation test. The objective of the validation test was for measuring the reproducibility and consistency of the fluorescent microbeads methods for biofilms labelling and quantifying the coverage percentage of the fluorescence microbeads from both the labelled flocs and granules. The fluorescence microbeads were used as tracer for observing and understanding the interactions between granular seeds and floccular activated sludge during the aerobic granular sludge formation. Two difference microbeads (color red, size 4 μm , with sulphate group / relatively hydrophobic and yellow green fluorescence, size 10 μm , carboxylic group / relatively hydrophilic microbeads were used to label crushed granule, intact granule and floccular sludge. A serial concentrations of fluorescence microbeads and lenght of time incubation for achieving the optimal biofilm microbead coverage were investigated. The attachment and coverage percentage of biofilm by the microbeads were obserbed by Confocal Laser Scanning Electron Microscope (CLSM). Digital images were analysed by digital image analysis in microbial ecology (DAIME). The results indicated majority of the beads evenly attached to the flocs since day one, while the hydrophilic microbeads attached to the surface of the crushed granule after 5 and 7 days of incubation (covering around 25% to 40% of the particle). After 24 hours incubation the hydrophobic beads covered almost 70% of the floc surfaces. This study suggested the method potential application for investigation of aerobic granular sludge development.

Keywords: Fluorescence microbeads, Aerobic Granular Sludge, Floccular Sludge, CLSM, DAIME.