Aerobic granular sludge (AGS) technology depends on the growth of a self-immobilizing microbial community to form granules, typically achieved through environmental and operational selective pressures. Long star-up periods and granules stability in long-term operation are described as the main drawbacks of this technology. During granulation, it is expected that most of the sludge achieve a granular form however often this is not the case. Microbial flocs and filamentous bacteria may endure in the system, potentially affecting granulation and/or granules stability.

Quantitative image analysis (QIA) has helped researchers to understand microbial population dynamics in activated sludge, e.g. identifying bad settling properties phenomena. In this work, we used QIA to monitor morphological changes of suspended sludge during an aerobic granulation process with two sodium acetate concentrations; i.e. R1: 250 mgCOD.L⁻¹; R2: 500 mgCOD.L⁻¹. R1 and R2 reached good settling properties, achieving sludge volume index (SVI5) of 36 and 55 mL.g⁻¹, respectively. However, granules were only visually observable in R1. QIA showed differences in aggregates with equivalent diameter of 100 µm ≤ Deq ≤ 650 µm, between both reactors in early stages of granulation. After 18 days, these aggregates were similar in key parameters such diameter, length and width, day from which a higher increase was observed in R1 than in R2. After 22 days, these aggregates represented 94% and 62% of the total projected area in R1 and R2, respectively. Interestingly, differences in aggregates appeared earlier in the reactors operation by morphological descriptors. In fact from day 11 onwards, aggregates in R1 exhibited higher compactness and robustness than in R2. Furthermore, the prevalence of filamentous bacteria in R2 might have been the reason why microbial aggregates could not achieve granular form. In R1, the total length of filaments (TL) remained below 3.3 mm.L⁻¹ from day 25, whereas in R2, TL continued to increase up to 16.3 mm.L⁻¹.

Overall, results showed that QIA could be used to monitor morphological changes of activated sludge during an aerobic granulation process. This could be particularly useful to decrease the long-periods required for granulation, and also to monitor AGS system stability in long term operations.