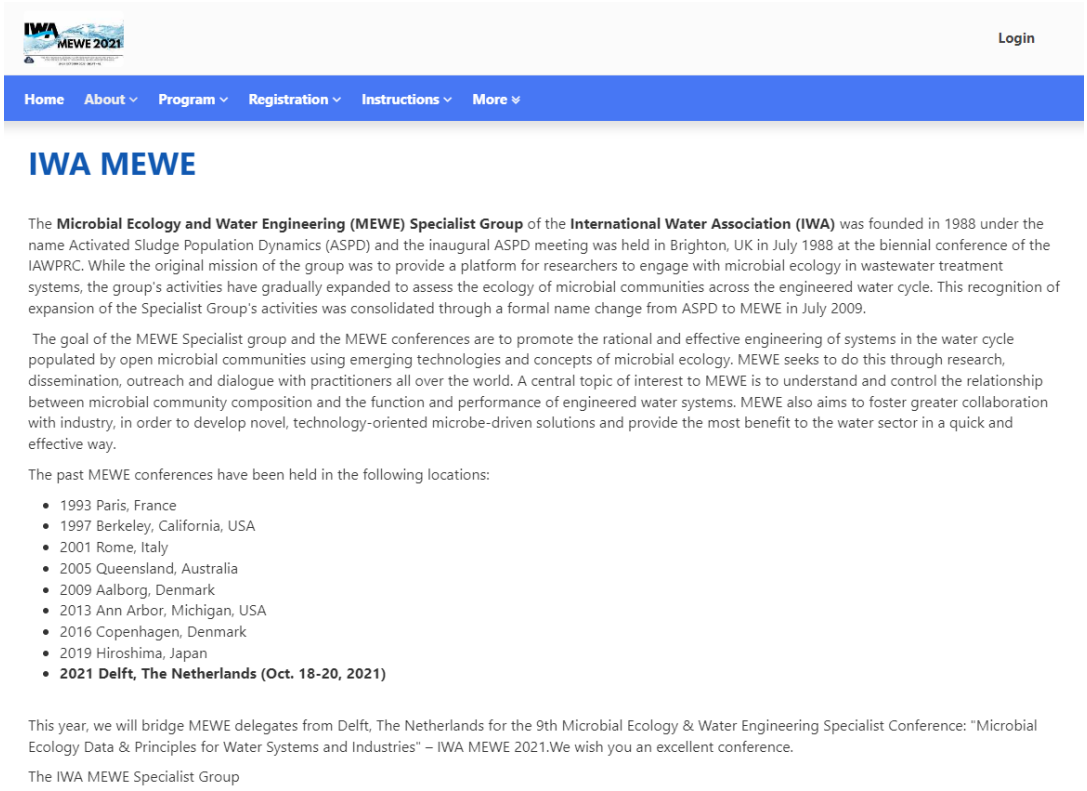

Use of fluorescence microbeads to understand aerobic granule formation for activated sludge wastewater treatment

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Aerobic granular sludge is an emerging technology in wastewater treatment. Aerobic granules form larger biofilm aggregates, settle much faster, and can maintain higher biomass levels compared to conventional sludge floccular biofilms. Thus, the application of aerobic sludge granules has potential operational and financial advantages. However, long start-up periods are required to develop aerobic granules from a floccular-based system, and loss of biomass can occur. In a recent study using an innovative seeding strategy, addition of crushed granules to a floccular sludge significantly reduced the start up period (Pijuan et al. 2011). However, currently there is a poor understanding of how granules form, which is required for identifying optimal start-up strategies.

The study aims to elucidate the mechanisms of granule formation and to understand the accelerated process using the crushed granule seeding strategy mentioned above. Previously, fluorescent microbeads have been used as tracers for particle movement to examine the transport of particulate species within biofilms (Drury et al. 1993; Tijhuis et al. 1994), and also for investigating the dynamics of spatial distributions of particulate components in mixed

population biofilms (Okabe et al. 1997). To the best of our knowledge, this is the first study using fluorescent microsphere labelling to monitor granule growth in a Sequencing Batch Reactor (SBR) treating real wastewater.

This new method was applied to study mechanisms of granule formation and understand the accelerated process. Granular and floccular biofilms were labelled with differently coloured fluorescent microbeads (4 µm diameter). These were then added to a laboratory scale wastewater treatment reactor. During the reactor operation period individual granules were removed and examined by confocal laser scanning microscopy, incorporating image analysis using the Daime program.

Labelled biofilms with a median diameter size of 200 µm were used to seed the reactor. During reactor operation the median size increased to 900 µm by day 80. Labelled biofilm particles were successfully detected in samples from the activated sludge reactor over the 80-day period. In the early stage (first 26 days) there was evidence that flocs were attaching to the surface of the granules (Figure 1), and further analysis indicated this attachment was permanent as can be seen from the ratio of green and red beads over the 80 day period (Figure 2).

The results imply that the granules act as nuclei for floccular particle attachment, which accelerates the granule formation. This provides important supporting evidence for this innovative strategy and for the full-scale application of this technology. Additionally, the approach of using fluorescent microbeads to monitor biofilm dynamics over an extended period in a reactor is novel and could be extended to understanding the growth of other biofilm systems.

Keywords: aerobic granulation, crushed granules, flocs, fluorescence microbeads.

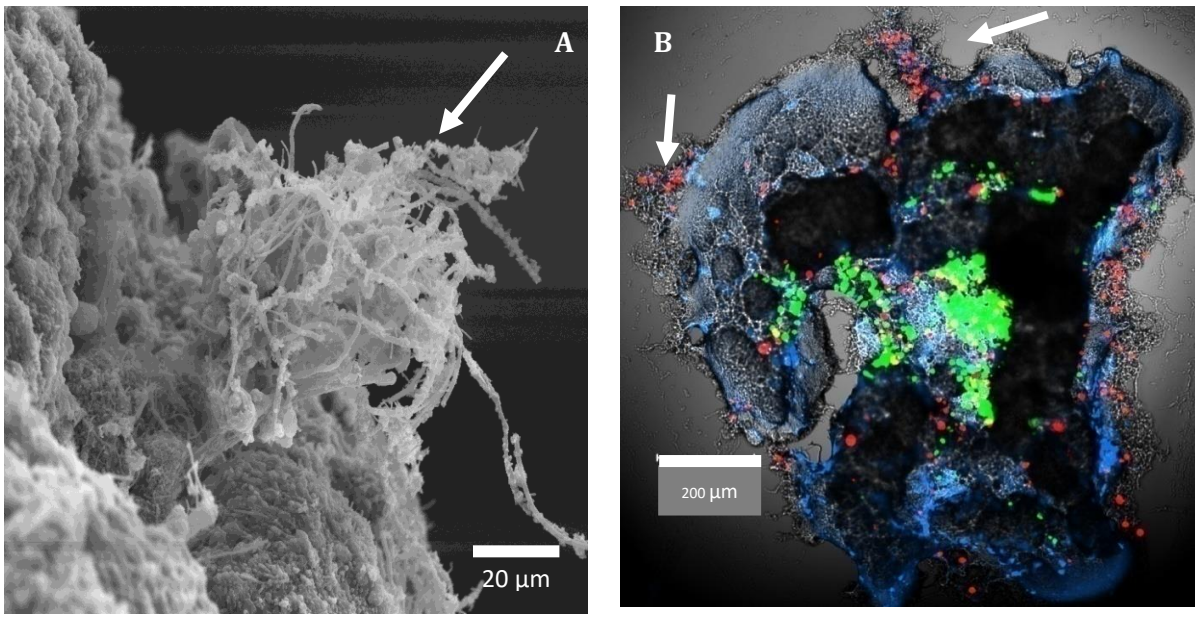

Figure 1. Evidence of attachment of flocs onto granule surfaces on day 26 of reactor operation. An SEM image of the granule surface with what appears to be protruding floc material, arrowed (A). Brightfield CLSM images showing the attachment of labelled flocs (red) to the surface of the labelled granules (green). Blue signal represents the EPS staining of the granules.

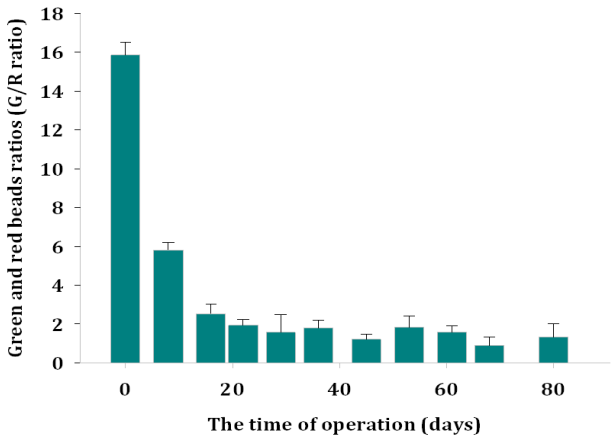

Figure 2. Ratio of green and red beads in the growing granules during 80 day period. Error bars are the standard error of 25-30 analysed images.

References

Drury WJ, Stewart PS, Characklis WG (1993) Transport of 1-μm latex particles in *Pseudomonas aeruginosa* biofilms. Biotechnology and Bioengineering 42:111-117

Okabe S, Yasuda T, Watanabe Y (1997) Uptake and Release of Inert Fluorescence Particles by Mixed Population Biofilms. Biotechnology and Bioengineering 53:459-469

Pijuan M, Werner U, Yuan Z (2011) Reducing the start up time of aerobic granular sludge reactots treating nutrient rich wastewater.

Tijhuis L, Van Benthum WA, van Loosdrecht MCM, Heijnen JJ (1994) Solid retention time in spherical biofilms in a biofilm airlift suspension reactor. Biotechnology and Bioengineering 44:867-879