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# Use of Fluorescent Microbeads to Understand Aerobic Granule Formation for Activated Sludge Wastewater Treatment

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## INTRODUCTION

- ❑ Aerobic granular sludge is an emerging technology for wastewater treatment
- ❑ Potential advantages
  - Fast settling and high biomass
  - Operational and cost savings
- ❑ Potential disadvantages
  - Long start-up periods
  - Loss of biomass
  - Poor understanding of granule formation
- ❑ Floccular sludge is generally used as the starting material
- ❑ This study uses a novel method to improve the understanding of the granule formation process

## HOW DO GRANULES FORM?

### Method

Fluorescent microbeads are used to label flocs (red) and small granules (green)



Mixture of labeled flocs (75%) and small granules (25%) were used to seed a reactor operated for wastewater treatment for 80 days

### Hypothesis

1. Flocs attached to the small granules
2. Flocs attached together
3. Small granules grow
4. Small granules attach to bigger ones



### Growing granules



### Cross Section

## RESULTS

- ❑ Labeled biofilms were successfully detected in samples from the activated sludge reactor over the 80-day period (Figure 1 and 2).
- ❑ In the early stage (first 20 days) there was evidence that flocs were attaching to the surface of the granules, and further analysis indicated this attachment was permanent (Figure 3).

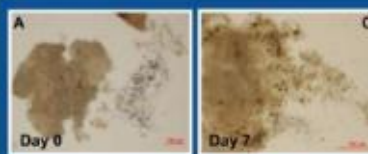


Fig 1. Pairs of light microscopy (A & C) and epifluorescent microscopy (B & D) images of labeled flocs and small granule interactions.

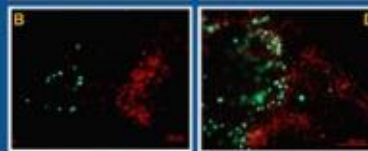


Fig 2. Granules formation detected by Confocal Laser Scanning Microscopy (A) and Scanning Electron Microscopy (B).

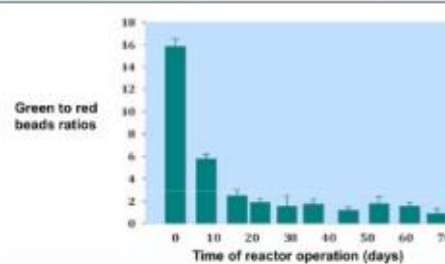


Fig 3. Ratio of green and red beads on granules examined during the reactor operation. In the first 20 days, flocs become attached to the small granules

## CONCLUSIONS

- ❑ A novel approach using fluorescent microbeads labelling was used to monitor biofilm dynamics within a wastewater treatment reactor.
- ❑ The results imply that the granules act as nuclei for floccular particle attachment, which accelerates the granule formation.
- ❑ This provides important conceptual understanding for the innovative application of aerobic granules for wastewater treatment.

## ACKNOWLEDGEMENTS

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# Development of flocs and granules labelling method with fluorescence microbeads to understand aerobic granule formation for activated sludge wastewater treatment

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Aerobic granular sludge is an emerging technology for wastewater treatment. Aerobic granules form larger biofilm aggregates than conventional sludge floccular biofilms, settle much faster than flocs, and can maintain higher biomass levels.

Thus, there are potential operational and cost saving advantages using aerobic granules. However, long start-up periods are required for the development of aerobic granules from a floccular-based system, and loss of biomass and 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, submitted).

However, currently there is poor understanding of how granules form and such conceptual information is important for improving start-up strategies.

This study aims to determine mechanisms of granule formation and understand the accelerated process.

A novel methodology was used to follow the granule formation. Granular and floccular biofilms were labelled with differently coloured fluorescent microbeads (4 µm diam); these were then added to a laboratory scale wastewater treatment reactor. Confocal laser scanning microscopy, incorporating image analysis using the daime program, was used to monitor the granule formation period. Labelled biofilms were successfully detected in samples from the activated sludge reactor over the 80-day period. In the early stage (first 20 days) there was evidence that flocs were attaching to the surface of the granules, and further analysis indicated this attachment was permanent.

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, our novel use of fluorescent microbeads to monitor biofilms for an extended period in a reactor, provides foundation methodology for studies of biofilm dynamics.

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

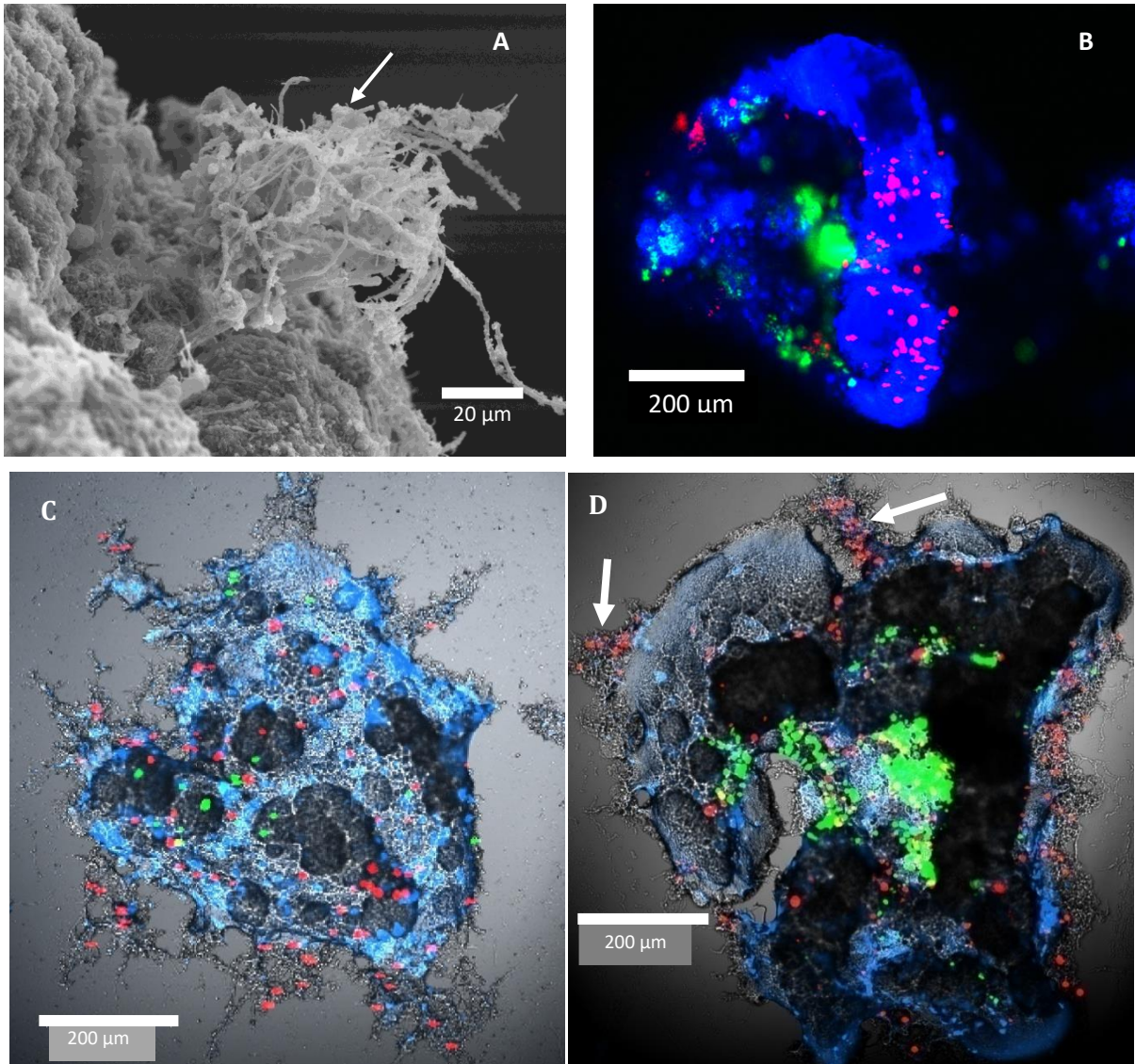


Figure 1. Evidence of attachment of flocs onto granule surfaces on day 26 of reactor operation. An SEM image of the granule surface with what appears to be protruding floc material, arrowed (A). The CLSM images of a section of a granule (B). Brightfield CLSM images showing the attachment of labelled flocs (red) to the surface of the labelled granules (green) (D and E).