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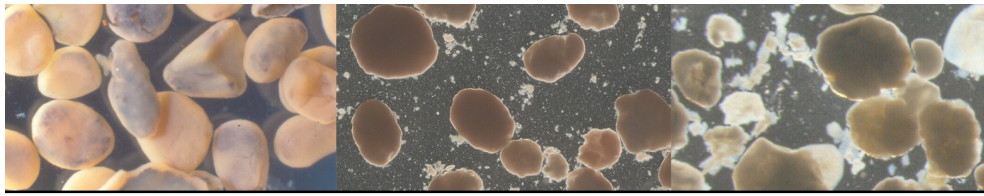
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Cyanobacterial abundance drives photogranulation

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Abstract: Oxygenic photogranules couple oxygen production through photosynthesis to heterotrophic respiration. They may be used in bioprocess engineering applications like wastewater treatment. We demonstrate that photogranulation does not require hydrodynamic shear and washout but also happens under static, batch conditions. Instead, it appears that the formation of these granules strongly depends on the initial presence and abundance of filamentous, motile cyanobacteria. In replicated augmentation experiments, we tested additions of cyanobacteria over a wide concentration gradient. The addition of small numbers of cyanobacteria statistically improved photogranulation, while too many added cyanobacteria led to the formation of microbial mats. Photogranulation is thus driven by entirely different factors than those typically postulated for anaerobic and aerobic granules.

Keywords: oxygenic photogranules; role of cyanobacteria; augmentation

Session 2 – Mechanisms of granular sludge formation

Introduction

Oxygenic photogranules have recently been described in the literature as a potentially interesting granule type for bioprocess engineering (Arcila and Buitron, 2016; Tiron et al., 2017). These granules possess the ability to produce oxygen by photosynthesis that can be immediately utilized as terminal electron acceptor by heterotrophic activity in the presence of a suitable carbon source, e.g., wastewater (Abouhend et al., 2016). One important feature during the development of these granules is the ample production of phototrophic biomass. In the wastewater treatment context, the use of photogranules may be one way to recover chemical energy contained in wastewater in the form of an easily biodegradable biofeedstock (Park et al., 2015), possibly allowing energy-positive wastewater treatment (Milferstedt et al., 2017).

How oxygenic photogranules form is far from understood. It appears, however, that filamentous and motile cyanobacteria play an essential role in photogranulation (Milferstedt et al., n.d.). One phylogenetic group of organisms as a key to successful granulation has not been reported in other granule-based ecosystems and hints towards a unique mechanistic role of these bacteria. We demonstrate here that typical drivers for granulation, e.g., hydrodynamic shear (Liu and Tay, 2002) and washout (Tijhuis et al., 1994), are not required for photogranulation. We further show how the success of photogranulation can be controlled by augmentation with cyanobacteria.

Material and Methods

Photogranules were cultivated under static, batch conditions, i.e., in the absence of hydrodynamic shear without any washout, in individual 10 ml serum bottles closed with a rubber stopper or in 24 well-plates. The only major carbon source was an initial batch of

activated sludge, augmented with different concentrations of a cyanobacterial concentrate that we obtained from a previous cultivation. The microbial communities (i.e., bacteria (excluding cyanobacteria), cyanobacteria and eukaryotic algae) were analysed using inventories of 16S and 23S rRNA gene fragments after Illumina MiSeq sequencing. The concentration of cyanobacteria in the augmentations was quantified using qPCR. Progress of granulation was measured and quantified using time-lapse scanning (Epson Perfection V500) coupled to automated image analysis (custom imageJ and R scripts).

Results and Conclusions

We were able to generate oxygenic photogranules under static batch conditions, i.e., in the absence of traditionally postulated environmental factors driving granule formation in other systems (Figure 1.1 A). Photogranules obtained from static batch cultivations shared important morphological and ecological properties with photogranules obtained in sequencing batch reactors (Figure 1.1 B). In both cases, a dense mat-like layer of interwoven filamentous cyanobacteria encased a non-phototrophic core that was in the case of static granules unconsolidated. The photolayer appeared to be the structure-giving element as granules typically disintegrated when the layer was compromised. Apart from the spherical shape, a cross section through a photogranule resembled the structure of microbial mats.

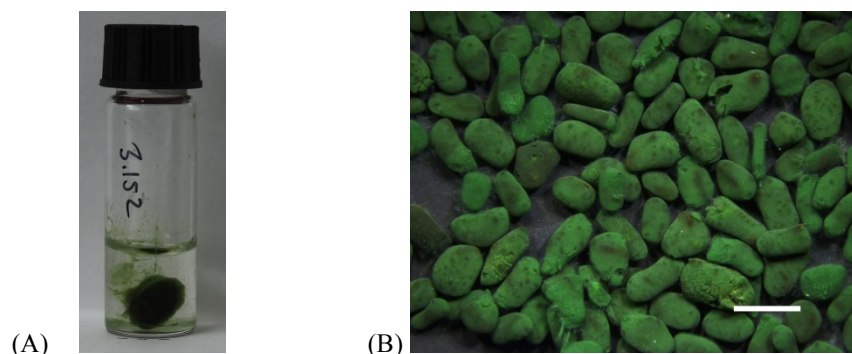
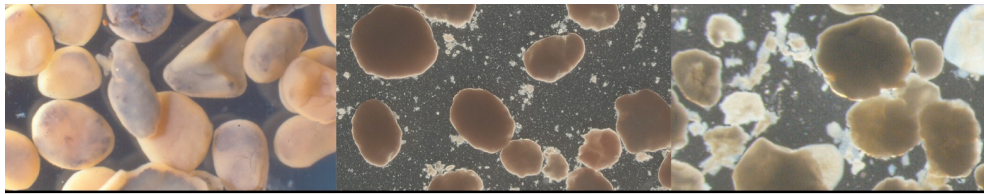


Figure 1.1 Statically formed photogranule (A) and photogranules generated in a sequencing batch reactor (B). The outer diameter of the vials in (A) is 15 mm. The scale bar in (B) represents 3 mm.

Whether the photolayer develops spherically around the initial activated sludge or whether a microbial mat develops during static cultivations is controlled by the initial concentration of cyanobacteria when starting the static batch cultures. In Figure 1.2, we show the progress of photogranulation as a function of the number of cyanobacterial cells added at the beginning. In the figure, the heading for each panel lists the additional 16S rRNA copy number resulting from cyanobacteria augmentation as a proxy for added cells. Granulation progress is expressed as a decrease in diameter of the biomass detected on images taken through the bottom of 24 well plates. Initially, the sludge bed covered the entire bottom of the wells, i.e., a diameter of 1.6 cm, equalling the diameter of the well. During successful photogranulation, the sludge bed contracted until a well-formed granule with a minimum diameter of 0.7 cm was formed.

We systematically observed in replicated experiments that already small additions of cyanobacteria (Figure 1.2 “ $3 \cdot 10^2$ copies ml^{-1} ”) statistically significantly decreased the diameter of a granule compared to untreated control cultivations (Figure 1.2 “0 copies ml^{-1} ”). Even though the absolute difference may not appear relevant, it is noteworthy that these additions had an effect. At a concentration of $3 \cdot 10^4$ copies ml^{-1} , the final diameter statistically



significantly increased again, eventually leading to complete granulation failure (Figure 1.2 “ $3 \cdot 10^6$ ”) and the formation of microbial mats.

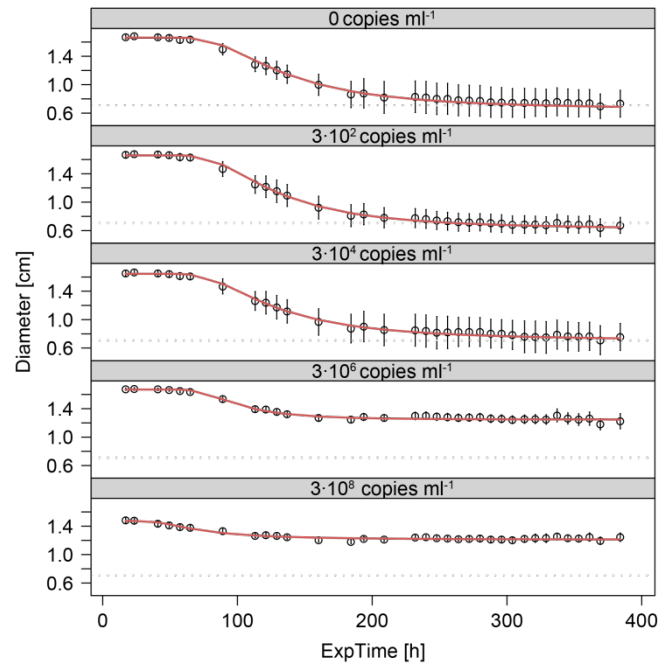


Figure 1.2 Progress of photogranulation as a function of added cyanobacteria indicated as copies ml^{-1} in the panel headings. Photogranulation is quantified as the decrease in diameter of the initial activated sludge bed. Initially, the activated sludge covered the entire bottom of the cultivation well, i.e., 1.6 cm. After successful photogranulation, a well-contracted granule formed with a minimum diameter of approximately 0.7 cm (0 , $3 \cdot 10^2$ and $3 \cdot 10^4$ copies ml^{-1}). High additions of cyanobacteria led to granulation failure and the development of microbial mats ($3 \cdot 10^6$ and above).

We conclude that not only the presence but also the abundance of cyanobacteria drives the development of photogranules, leading to exciting hypotheses on their mechanistic role in photogranulation to be tested in mathematical models.

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