Quantification of Sludge Morphology through the Aerobic Granulation Process ReNUWIt REU 2020

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Introduction

Water resource recovery facilities (WRRFs) across the United States predominantly use the low-cost and well-established technology of conventional activated sludge (CAS) to remove nutrients and organic matter from wastewater. Wastewater is mixed with activated sludge, a low density, loose structure of microorganisms, that degrades carbon, nitrogen, and phosphorus. Then in the secondary clarifier, the settled solids, or flocs, are separated from the water through gravity settling. At times CAS may be dominated by filamentous bacteria, resulting in slow settling sludge. Subsequentially, WRRFs require longer hydraulic residence times to meet effluent solids discharge limits. In comparison, aerobic granular sludge (AGS) is composed of large, dense, and spherical floc, that allows rapid settling and provides enhanced nutrient removal. However, the implementation of AGS requires complex operating procedures and a minimum reactor depth of 18 ft, not common at WRRFs [1].

Alternatively, hydraulic selection (process of selectively removing poor-settling bacteria from the bioreactor by means of hydrodynamic forces) can be implemented to place a constant environmental stress on the microbial community to promote the growth of fast settling floc (biosolids). Researchers at Colorado School of Mines proposed a hydraulic selector device utilizing this process (Figure 1), that successfully improved sludge settling velocities of activated sludge, enabling AGS formation. The hydraulic selector is located above the sludge settling blanket, and removal of poor-settling floc is dependent on the velocity gradients developed by the selector outflow.

While the hydraulic selector has been successful in the past, understanding when the velocity gradients need to be increased will be key to the hydraulic selector's success in any activated sludge system. In order to rapidly and accurately evaluate the hydraulic selection performance during the granulation process, analysis of phase contrast images can be performed to gain visual indicators of the microbiology present and allow for quantification of floc morphology characteristics. Specifically, aspect ratio (AR) is the ratio of the major axis to the minor axis of the floc and floc diameter is the measure of the floc major axis.



Figure 1. Schematic of hydraulic selector technology at different operating outflows.

Research Objective

The main objective of the study was to quantify changes in activated sludge morphology of bioreactors with and without hydraulic selector technology and relate sludge morphology characteristics to sludge settling properties.

Approach

Three pilot-scale, 120 L bioreactors were operated at the Mines Park testbed for 80 days in order to evaluate different solid wasting mechanisms. A control bioreactor (BR1) followed the traditional wasting mechanism, wasting purely from the bottom of the bioreactor, bioreactor two (BR2) combined hydraulic selector wasting and traditional wasting, while bioreactor three (BR3) wasted completely through the hydraulic selector. The solid retention time of all bioreactors was maintained between 10 and 15 days and the food to microorganism ratio (F:M) was kept between 0.15-0.2 g COD/g VSS. Every week, samples of the activated sludge were collected from each bioreactor during the aeration phase and analyzed under a phase contrast microscope (Olympus CX41, Center Valley, PA) at 100x and 40x magnification. 100x images were used to calculate filamentous area while 40x images were used to evaluate floc morphology. Between 15 and 50 images were captured per activated sludge sample and then stitched together to create the overall profile of the sludge.

ImageJ, an open source image processing program, was used to perform image analysis (Figure 2) [2]. Direct brightness thresholding was first performed to segment filamentous bacteria from the flocs. Then each image was processed to remove background noise to warrant accurate detection of flocs and



Figure 2. (A) Example stitched image (B) Outline of floc in red and outline of filament in green

filaments (Figure 2B). Floc diameter, aspect ratio, floc area and filamentous length were then calculated and transferred to an excel data file. Floc diameter is related to density of the floc-floc diameter of 250-500 µm are most desirable. The AR information yields a range of values; 1 indicates a perfect circular floc and values >1 indicate the deviation of a floc structure from circle/sphere. As а AR

approaches 1 and the structure is less elongated, it is assumed that settleability will increase, observed by lower SVI measures and higher sludge settling velocities [3]. Lastly, total filament length and total floc area were used to calculate relative filament abundance.

50%

Results and Discussion

In the first 35 days of the experiment, all three BRs experienced a rapid reduction in relative filament abundance. This was caused by the high F:M that prevented growth of filamentous bacteria (Figure 3).

From day 21 to 59 the AR of BR 1-3 did not change greatly, with the mean AR remaining between 1.5 and 2 (Figure 4A). However, the mean floc diameter in Figure 4B did show an increase in floc size in BR2 and BR3. approximately 110 and 140 µm on day 21 and then 190 and 230 µm on day 59, respectively. Comparing aspect ratio with floc diameter measurements provides more insight to the sludge settling characteristics. For example, on Day 41, BR1 has the lowest AR and could be concluded to have the best performing sludge, but by combining this with the mean floc diameter, the low aspect ratio is not an indicator of better settling sludge because the flocs are still small in comparison to BR2 and BR3.

Conclusion

Image capture under a phase contrast microscope can be a viable tool to gauge sludge settling performance. However, the quantity of images captured would need to be increased to gain confidence in the overall sludge profile and its corresponding resulting information. Shape descriptor information from 40x magnification capture along with floc diameter information can give indication of settling performance. Moving forward, images will continue to be captured and analyzed to correlate a relationship between sludge morphology and operating conditions.

Filament Abundance BR1 40% BR2 30% **▲**BR3 20% 10% 2 0% 7 14 21 28 35 42 0 **Experimental Time (days)** Figure 3. Captured at 100x magnification, Relative

filament abundance, total filament length compared to total floc area, throughout the expirement.



Figure 4. Captured at 40x magnification. (A) Shape descriptor, aspect ratio, over the experiment amongst all bioreactors. (B) Average floc diameter over the experiment amongst all bioreactors.

References

- [1] T.R. Kent, C.B. Bott, Z.W. Wang, State of the art of aerobic granulation in continuous flow bioreactors, Biotechnol. Adv. 36 (2018) 1139–1166. https://doi.org/10.1016/j.biotechadv.2018.03.015.
- [2] W. Burger, K. Krysiak-Baltyn, P.J. Scales, G.J.O. Martin, A.D. Stickland, S.L. Gras, The influence of protruding filamentous bacteria on floc stability and solid-liquid separation in the activated sludge process, Water Res. 123 (2017) 578–585. https://doi.org/10.1016/j.watres.2017.06.063.
- [3] R. Jenné, E.N. Banadda, G. Gins, J. Deurinck, I.Y. Smets, A.H. Geeraerd, J.F. Van Impe, Use of image analysis for sludge characterisation: Studying the relation between floc shape and sludge settleability, Water Sci. Technol. 54 (2006) 167–174. https://doi.org/10.2166/wst.2006.384.