UNDERSTANDING YOUR MICROBIAL ZOO: USING MICROSCOPY TO ASSIST CONTROL OF ACTIVATED SLUDGE PROCESSES

Curtis Gordon, *Integration Optimisation Engineer,* Icon Water
Laura Fuhrman, *Senior Process Compliance Engineer,* Icon Water
Garry Charker, *Engineering Coordinator,* Icon Water

ABSTRACT

Successful operation of any treatment plant requires monitoring to ensure the process remains within its operating limits and achieves treatment objectives. Monitoring can take different forms, including online monitoring using instrumentation, grab sampling for bench tests and visual monitoring of asset and process performance.

Microscope observation of activated sludge is a form of process monitoring which provides insights into the health of a biological ecosystem, and therefore the effectiveness of the biological treatment process. Understanding what normal, healthy sludge looks like will help to identify when abnormal conditions occur and can be used to catch and diagnose process upsets before they take hold.

Microscope observation and reporting skills were implemented in Icon Water's Operations team at the Lower Molonglo Water Quality Control Centre (LMWQCC), by using instructions with photographic examples to allow for comparative analysis in a simple reporting template. Regular microscopy reports allowed for a familiarity and interest in the microbial health of the plant within the Operations team, and daily reporting during extreme wet weather events allowed for instantaneous process feedback to safely push the plant to its limits.

1.0 INTRODUCTION

The activated sludge process is commonly used in the secondary wastewater treatment stage, and biological treatment forms the foundation of this process. Standard monitoring techniques can be used to collect process information, however the living ecosystem allows for a unique form of additional monitoring: direct observation of the 'Microbial Zoo' of organisms living in the activated sludge by viewing them under a microscope.

Microscope analysis of activated sludge requires only a few pieces of unique equipment, along with procedures for sample preparation and microscope operation to generate a weekly 30-minute task that can deliver valuable information on the health of the biological treatment stage. Collected data can be used to provide instant feedback on process changes, to develop an understanding of how the biomass in the plant changes seasonally and periodically, and to give treatment staff a new perspective the dynamic and complex ecosystem that lives within an activated sludge plant.

Icon Water's primary wastewater treatment facility, the Lower Molonglo Water Quality Control Centre (LMWQCC), treats average dry weather flows of 100 ML/d, using pre-treatment, primary sedimentation and incineration of solids, secondary treatment using an Activated Sludge process, and tertiary filtration and disinfection stages. In 2020 a procedure for Microscope analysis was formalised to allow for standardised methods to sample, prepare and analyse activated sludge. The materials and methods are described in this paper, along with a case study of how Microscopy was used to assist process control during a wet weather event.

2.0 DISCUSSION

2.1 Materials

The Microscope: a few pieces of specialised equipment are required to perform microscope analysis, with the main upfront cost being of course the microscope itself. Microscopes can vary in price and features, but any sort of upright light microscope will work for this procedure. A few microscope features that are important include:

- Ability to set the microscope oculars and objectives to achieve one or more total magnification settings between 100x - 400x.
- Ability to adjust and move the sample stage to be able to scan different areas of the microscope slide.
- Adjustable light settings (dim, contrast etc.). A **phase contrast light setting** (as opposed to bright field) is highly recommended for clear viewing of activated sludge samples.
- Optional, but highly recommended: A camera attachment that allows for photography of samples through the microscope lens.
- Optional: computer software that allows for analysis and data capture of microscope photos.

Microscope Slides: basic lab equipment is also required to prepare the activated sludge microscope slides:

- Glass microscope sample slides – must be suitable for making 'wet mount' slides.
- Glass cover slips – sized to fit your chosen sample slide.
- Pipette – simple disposable plastic pipettes are fine.

Sample Equipment: some common sample equipment is used to collect the activated sludge sample from the plant:

- 100 mL sample bottles.
- Suitable sample location – a location as close to the end of the aerated zone as possible, that is safe and easy to access.
- Sample equipment – i.e. sample pole and container.
- PPE – gloves, safety glasses etc.

2.2 Sampling Methodology

Sample Collection: Sampling of activated sludge should occur at the end of the aerated zone in the biological treatment stage, where microbial activity and populations will be at their greatest. Samples can also be taken from other locations as required to troubleshoot specific problems (i.e. bulking foam sample to identify problem filamentous bacteria). Samples should be collected in a small sample bottle, leaving an air gap at the top to allow for aerobic conditions to persist. Analysing the sample sooner will give the best results, however sludge can be left for a few hours hours at room temperature without major effects.

Microscope Slide Preparation: A minimum of 2 'wet mount' slides per sample should be prepared to ensure a good representation of the sample is viewed. The sample bottle should be gently mixed, and then using a pipette, place 2 – 3 drops of sample towards the base of each slide. A glass cover slip is placed on top, evenly spreading the sample to all edges of the slide without trapping any air. Label the slides, noting down the sample time and location. Slides last for about 30 minutes before starting to dry out.
The sample slide is then placed on the microscope stage and viewed under the phase contrast light setting. The observer will scan the slide from edge to edge in a systematic manner and can make observations on different properties within the sample.

2.3 Microscope Reporting

Within the activated sludge ecosystem, the heavy lifting of the biological nutrient removal is undertaken by different types of bacteria, which are too small to view under even the highest magnification settings of a standard microscope. This makes direct quantitative observations about biomass activity impractical and is better left to instrumentation such as DO probes or N and P analysers.

Instead, microscope reporting can provide useful qualitative data about other indicators within the sludge that are in turn used to infer the health of the bacterial population, and hence the performance of the biological treatment stage. This information can be recorded in a reporting template and saved to provide a database that over time can provide an understanding of the quality and health of the biomass alongside the context of other process data.

Three areas should be observed and analysed during microscope analysis:

1. Flocs
2. Filaments
3. Higher Organisms

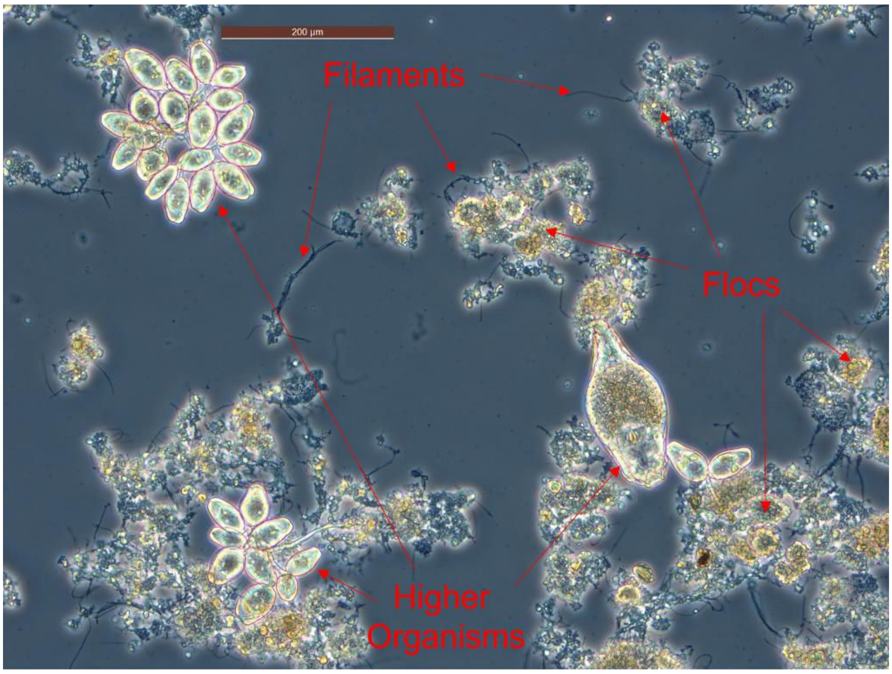

Figure 1: Features of an Activated Sludge microscope slide

Flocs are comprised of aggregated solid material, containing organic matter, inert particles, bacteria and filaments, all held together by bacterial polymeric slime and chemical forces of attraction. The size, shape, and density of a floc will contribute to its settleability, which directly influences the performance of later clarification and filtration stages. 'Good' flocs generally have the following properties:

- Firm – flocs are well formed and have clearly defined boundaries within the surrounding solution. There is no bleeding of loose particulate matter around the floc's edges.
- Round – the more circular a floc's shape is, the better. More jagged or irregular shapes will increase a floc's surface area and reduce settleability.
- Compact – the denser a floc is, the faster it will settle. Whilst floc density cannot be measured directly, the colour of a floc can be observed, with darker, fuller flocs indicating a higher density.

- Size – whilst not essential, it is possible to measure floc sizes using a micrometre installed in an eyepiece of the microscope, or with computer analysis of microscope photographs. The standard floc size will be unique to different treatment plants, but flocs can be categorised as small (< 150 µm) or large (> 150 µm).

Filaments are long, thin, single celled organisms, which often form the backbone for Flocs to build around. In the wrong conditions filaments can multiply out of control, contributing to poor settleability, bulking or foaming events as their excessive populations begin to degrade floc structures. At higher magnifications, filament abundance can be measured by counting the number present per floc. This 'filament index' can be compared over time to measure whether filament populations are stable or trending out of control.

Higher Organisms affectionately known as 'The Bugs', are a collection of Protozoa and Metazoa that live at the top of the food chain in the Microbial Zoo, and can be seen swimming, eating, and multiplying. Observations include quantity, types of species present, and diversity of species. Whilst the higher organisms do not directly contribute to the biological treatment within the activated sludge, they can be used as an indication of a healthy biomass – these apex predators would not be able to exist without an adequate pyramid of lower organisms to support them. The populations of different species and their average life span can also give an indication of Food: Biomass ratios and Sludge Age, with the balance of organisms shifting with each parameter.

Activated Sludge process data (e.g. effluent temperature, plant flows, sludge age) should also be collected at the time the sludge sample is taken, to allow for a side by side comparison with biomass data and microscope photos across different process conditions. All information should be recorded in a reporting template alongside the microscope observations, saved in a database, and shared with the Operations team to provide additional process information for use in treatment plant control and process optimisation.

Photographs of the microscope imagery also provide a very useful record keeping tool and should be included in every microscope report where possible. Collecting a catalogue of floc structures, filament abundance and types of higher organisms present during standard plant conditions will help to understand what normal, healthy sludge and biomass populations look like, and will therefore help to identify when abnormal conditions start to occur.

2.4 Case study of Microscope Analysis during LMWQCC Wet Weather Event

Microscope Reporting was used to monitor biological health during an August 2020 Wet Weather event at the LMWQCC. By completing daily Microscope reporting, filament interference in the floc structure was closely tracked, providing instantaneous feedback to diagnose and monitor the deteriorating sludge volume index (SVI) in the secondary clarifiers.

Typical influent temperatures in the LMWQCC bioreactors are around 21°C, however following excessive rainfall on August 20, process temperatures began to drop. By August 25 the SVI had peaked at 270, leading to a reduced aerobic fraction as solids began to accumulate in the clarifiers. Anaerobic conditions and cooler temperatures are known to favour filaments, whilst reducing populations of floc forming bacteria. The excessive filament population and impacts on floc structure can be clearly seen in the microscope photography for August 25 below, where flocs are no longer grouping into large, compact structures. These flocs are instead infested with filaments throughout, increasing surface area and reducing settleability.

In response, effluent Nitrates targets were dropped to reduce the nutrients available that favour filament growth. The effects of these actions, along with recovering temperatures promoting growth of floc forming bacteria, can be seen in the September 17 photo, where filaments have begun to retreat inside flocs in search of nutrients. This coincided with recovering SVI data, with the larger and more compact floc structures clearly seen in the microscope photography.

25th August, Temperature 17.8°C, SVI 270

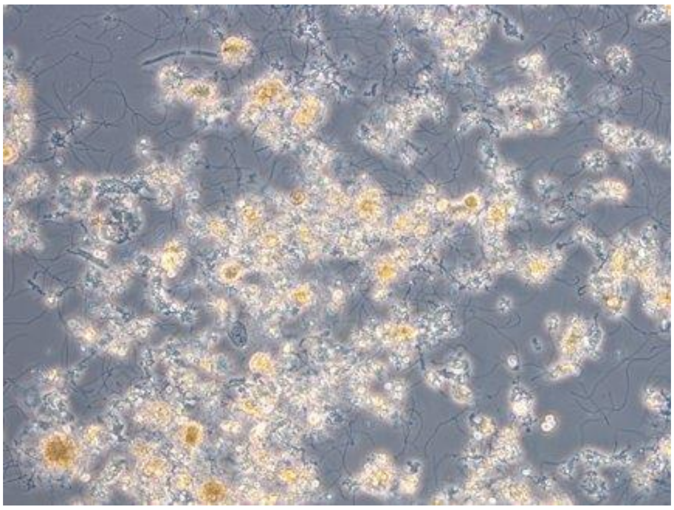

Figure 2: Sustained cool temperatures from dam return contributed to excessive filament growth, causing open floc structures, and bridging throughout – close to peak of poor settleability

17th September, Temperature 20.3°C, SVI 122

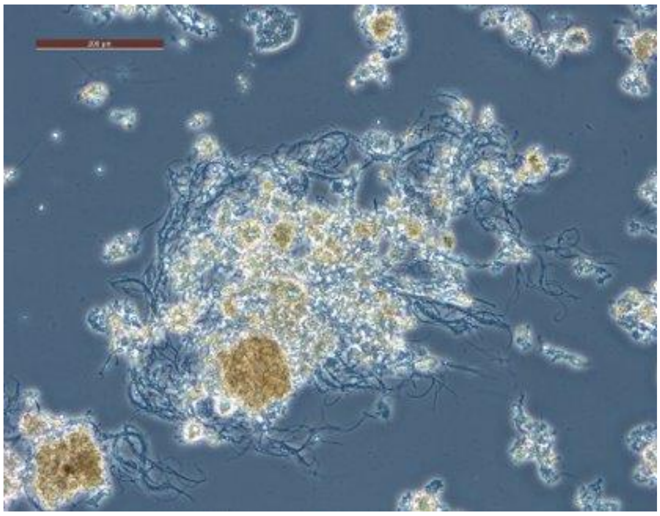

Figure 3: Holding of effluent nitrates at desired position continues to discourage filaments, and as a result they are seen to bunch up and retreat further within flocs, allowing for denser structures and improved settleability

2.5 Opportunities and Challenges of Implementing Microscope Reporting

When implementing a Microscope Reporting process, key opportunities and challenges include:

Opportunities

- Initially, focus on building up as much microscope data as possible, capturing a variety of plant conditions without trying to analyse trends or effects. Insights will come about via comparison of normal vs abnormal sludge/biomass, and this will occur over time.
- A good record keeping system is key – spend some time setting up naming conventions to manage the time, date and location of microscope reports and photographs.
- Camera mounts are available to allow photography using smart phones instead of more expensive specialised microscope cameras, minimising this cost.
- Sharing of microscopy reports and photographs with the whole Operations team can improve interest in biomass health and plant performance via engagement through the fascinating visual medium of biological microscopy.
- Combining microscope analysis reporting with sludge settleability testing (SVI) will allow for direct comparison and cross referencing of causes and outcomes.
- Additional staining and genetic identification techniques can be used to identify filament species, which can allow for comparison to literature to understand specific of formation conditions and troubleshooting options available. Samples can be sent to specialist labs for one-off testing, or additional equipment can be bought to perform staining tests in house.

Challenges

- Justifying the upfront cost of a benchtop microscope can be difficult, with entry level microscopes being in the range of >$1,000. The long-term benefits of the system and upskilling opportunities for Operators should be considered when evaluating this purchase.

- Microscopes must be maintained like all other equipment, and a maintenance plan should be put in place to ensure this. The manufacturer of your microscope may offer technical services for this purpose.
- Familiarity with the microscope and what to look for in the sample slides takes time to develop – it can be tricky to motivate Operators to take time out of their busy schedule to do so. A 'Microscope Champion' within the team may be useful to lead skill uptake and interest in using the microscope.
- It is difficult to get objective measurements through microscope reporting unless consistent observation and counting techniques are implemented. Different observers will inevitably introduce some subjective biases, to counter this, microscope SOPs are key. Photographs are also critical – they provide an objective snapshot of microscope data for later reference.

3.0 CONCLUSION

Microscope analysis of activated sludge provides a unique way to monitor the biological treatment step in a wastewater treatment plant and should be considered by any Operations team as a supplementary monitoring tool to augment understanding of their plant and process control of the relevant systems. By investing in upfront equipment costs, development of sampling and analysis SOPs, and upskilling of Operators, any utility can benefit from the additional insights Microscope reporting can provide about the health and performance of the Microbial Zoo living in their wastewater treatment plant.

4.0 ACKNOWLEDGEMENTS

Thank you to the Icon Water Operations team for their contributions and assistance in developing and testing the microscope analysis procedures.

Special thanks to Laura Fuhrman and Garry Charker for their guidance, mentoring and commitment of time towards my professional development and microscopy skills.

Additional thanks to Peter and Helen Stratton who run a fantastic and highly recommended Activated Sludge course that delves into these microscopy techniques in more detail.

And final thanks to Dick Eikelboom for his publication 'Process Control of Activated Sludge Plants by Microscopic Investigation', of which many of the techniques in this paper are based.

5.0 REFERENCES

Eikelboom D (2000) *Process Control of Activated Sludge Plants by Microscopic Investigation*, IWA Publishing, Alliance House, 12 Caxton Street, London

Fuhrman L (2021) *Using Microscopy to Validate Operational Decision Making During Extreme Events*