Proof of concept: Supernatant treatment after dewatering of faecal sludge using an MBBR configuration at community-scale

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Acknowledgments

Go to Switzerland they said, it will be fun they said!

Oh and indeed it was. For my master's thesis, I had the opportunity to conduct my research at EAWAG, the Swiss Federal Institute for aquatic sciences and technology in Zurich. From March to September 2022, I was part of the MEWS group at the Sandec department. MEWS has a lot of experience in applied faecal sludge treatment, like dewatering, conditioning and overall knowledge about faecal sludge characteristics. During the 5.5 months of my stay, I gained a lot of experience in project management, sampling septic tanks, operating reactors and becoming independent in making decisions about my experimental plan and overall topic. To be able to make use of the labs and experimental hall at EAWAG was a huge opportunity for which I am extremely grateful, as those places are extremely high-tech and convenient.

During this period abroad, I could rely on many people for making this experience so educational and fun. First of all, I want to thank Marij Zwart for introducing me to the group. Thank you to the whole MEWS/ETH group: Stanley, BJ, Kelsey, Nida, Linda, Eberhard, Michi and Nienke. Thank you Michi, for supervising me amazingly every day in the lab, but also for showing me the beautiful country that Switzerland is, and introducing me to traditional Swiss habits. I count the faecal sludge sampling days in Obwalden and in the NEST building as the most fun days of my entire stay! Thank you Nienke, for teaching me so much about faecal sludge treatment plant operation, being my Dutch support system in Zurich, and making me feel at home in the city so quickly! Thanks Linda and Eberhard for all the tips and catch-up meetings! Thank you Sylvia, Marco and Richi for all the support in the lab. Thanks to Merle and Mariska for their feedback and support from the TU Delft side, and the fun discussions. And last but definitely not least, I want to say thanks to my friends and family in the Netherlands and Belgium, for always being eager for a call during this period abroad, all the visits, productive and fun days in the graduation room, and the endless support.

I am forever grateful for all the friendships I could build up in Zurich. For all the outdoor activities during the weekends. I saw all the seasons passing by in Switzerland, allowing me weekends of skiing, hiking, and swimming in remote lakes. For being able to perform research in a research institute like EAWAG. Being able to interact with the endless amounts of interesting people on the Sandec floor. The network I could build up within Sandec and EAWAG.

All the people mentioned above taught me to love the field of non-sewered sanitation and faecal sludge management, making me eager to find a job related to this field.

Merci Vilmal,

Thank you,

Helena Verloo

25th of November 2022

Summary

One-third of the global population relies on non-sewered sanitation. In urban areas of low-and-middle income countries, treatment of faecal sludge is often insufficient. On-site sanitation technologies can provide sustainable and more affordable sanitation solutions for urban areas, but only if functioning faecal sludge management is in place. As a first step for treatment, faecal sludge is dewatered, resulting in a solid stream and liquid stream. There are many existing technologies to treat the solid fraction. However, treatment technologies for the liquid are often insufficient and land-intensive. This liquid after dewatering of faecal sludge is called 'supernatant'. The reason why treatment of this supernatant is difficult, is because the composition is prone to variability.

In conventional sewer-based wastewater treatment, attached growth processes have proven to be robust to influent variability. Those technologies do not take a lot of space, which is an advantage in urban areas. Attached growth processes are aerobic treatment processes in which the biomass responsible for treatment is attached to some type of medium. This research is a proof of concept whether an attached growth system, in this case an MBBR process, could be an alternative for existing supernatant treatment technologies in non-sewered sanitation. A 5000 people urban community-scale scenario is considered, covering variability in influent composition and intermittency in faecal sludge supply.

To prove this concept, there is looked whether COD and N removals can be achieved to certain discharge standards. First there is assessed whether the MBBR is able to run on one type of supernatant. After this, the variability in influent composition regarding COD/N ratio, salt ratio, and pH is tested by spiking the baseline supernatant. Intermittency in influent supply to the reactor is also tested. After these separate experiments, a realistic scenario is tested with supernatants from different types of sources, and intermittent supply over the weekend.

Operating the MBBR reactor on 1 type of supernatant (derived from septic tank faecal sludge from Switzerland) gave COD- and N-removals up until US-EPA standards, with removal efficiencies of 86% for COD and 100% for ammonium. However, alkalinity, COD/N ratio, pH are influent characteristics that need to be monitored for effective reactor operation. The COD/N ratio spikings were based on realistic values, and showed that there is an upper limit of 518/1 for which reactor breakdown occurs by creating a rapid anaerobic environment, killing off the biofilm. A 90/1 COD/N ratio was the highest tested COD/N ratio possible to be treated by MBBR. An organic loading of 7.9 kg COD/m^3/d was feasible for the MBBR. The salt ratio variability did not show any influence on the oxygen uptake rate and COD removal efficiency was still higher than 70%. Adding supernatant with higher pH for a short amount of time also did not show any influence on the MBBR reactor operation. Intermittency tests showed that aeration during intermittency reduces start-up time afterwards. Treatment of supernatant of fresh blackwater is possible without any operational problems and US-EPA discharge standards are met.

The realistic scenario showed that the MBBR is effective in a pH range between 9 and 6, and that variability in influent composition can actually be an asset to keep the reactor operation steady. To be sure of this, a thorough Quantities and Qualities analysis of the faecal sludge of a community needs to be performed to determine trends and the expected variability.

Lastly, the feasibility in the field of an MBBR was assessed by determining loading limits, giving recommendations on pre-treatment and post-treatment, and comparing the MBBR to other existing treatment technologies for supernatant. Factors were determined on which the choice of MBBR should be based. This was done through interviews, literature search and own lab experience. It appeared that availability of electricity, the expertise of operators, land availability, the availability of spare parts, effluent quality and greenhouse gas emissions are the most important trade-off factors to decide upon a MBBR.

As a conclusion, the concept of using an MBBR for treatment of supernatant after dewatering of faecal sludge is proven for a community-scale scenario of 5000 people, and further research has to determine new outcomes like the possibility of pathogen reduction and phosphorous removal. After this, field tests need to determine the actual feasibility full-scale in non-sewered sanitation setting.

List of Abbreviations

AOB	Ammonia Oxidizing Bacteria
bCOD	Biodegradable Chemical Oxygen Demand
BW	Blackwater
Can	Supernatant Canada
CAWST	Centre for Affordable Water and Sanitation Technology
COD	Chemical Oxygen Demand
CSTR	Continuous stirred tank reactors
DO	Dissolved Oxygen
EAWAG	Swiss Federal Institute of Aquatic Science and Technology
EC	Electrical Conductivity
EPS	Extra Polymeric Substances
FS	Faecal Sludge
FSM	Faecal Sludge Management
FSTP	Faecal Sludge Treatment Plant
HL	Hach Lange Test
HRT	Hydraulic Retention time
IC	Ion Chromatography
IFAS	Integrated Fixed Film Activated Sludge Process
Leb 1	Supernatant Lebanon 1
Leb 2	Supernatant Lebanon 2
M/D	Monovalent/Divalent salts ratio
MBBR	Moving Bed Biofilm Reactor
Mix	Supernatant Mix
MO	Microorganisms
nbCOD	Non-Biodegradable Chemical Oxygen Demand
NOB	Nitrite Oxidizing Bacteria
NSS	Non-sewered sanitation
O&M	Operation and Maintenance
OPEX	Operational Expenditures
OSS	On-Site Sanitation
Prim Eff	Conventional Wastewater after primary settling
R1	Reactor 1
R2	Reactor 2
R3	Reactor 3
RBC	Rotating Biological Contactor
sCOD	Soluble Chemical Oxygen Demand
SRT	Solids Retention Time
Supernatant	Liquid stream after dewatering of faecal sludge
tCOD	Total Chemical Oxygen Demand
TSS	Total Suspended Solids
WWTP	Waste Water Treatment Plant

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Introduction

1. Problem Statement

One-third of the world-population relies on non-sewered sanitation for their sanitation needs (Strande et al., 2018). Centralized, sewer-based technologies are well established, with a long record of research, knowledge and implementation (Strande et al., 2014). In comparison, the concept of integrated faecal sludge management (FSM) as part of non-sewered sanitation in urban and peri-urban areas is relatively new research. Appropriate management of faecal sludge (FS) is necessary for achieving Sustainable Development Goal (SDG) 6.2, aiming to safely manage sanitation and hygiene services (United Nations, 2015). A small proportion of human waste is safely treated or disposed of. The vast majority ends up in the surrounding environment, directly impacting public health, especially for the urban poor, who are least able to bear the burden of poor services (Peal et al., 2014). The importance and need for FSM is now being recognized world-wide.

FSM can be considered as an urban sanitation service chain. The sanitation service chain is the predominant sanitation system in the urban areas of low and middle-income countries. The sanitation service chain contains four consecutive steps: Containment - Emptying/Transport – Treatment -Reuse/Disposal (see Figure 1). At this point, semi-centralized treatment in non-sewered areas is mostly used as a way of managing FS (Strande et al., 2014). This means that FS is first contained at the source, is emptied manually or with vacuum trucks and brought to a small faecal sludge treatment plant (FSTP) (Obermann & Sattler, 2013). In this thesis, only step 3, the treatment part of the sanitation service chain, is considered. In many places across the world, treatment FS is insufficient or even non-existent (Taweesan et al., 2015). The treatment technologies that are used are often land-intensive, which is a limited resource, specifically in urban areas (Medland et al., 2016). Low-income countries are undergoing the fastest rates of urbanization in the world, meaning that available space in urban areas for the treatment of FS is becoming more and more a challenge (Gold et al., 2016). Depending on the type of resource recovery or place of discharge, treatment of FS addresses four treatment objectives, namely 'Dewatering', 'Nutrient management', 'Stabilization' and 'Pathogen Reduction' (Strande et al., 2014).

Figure 1 gives an overview of the current possibilities in FSM sanitation service chain. It shows how FS is managed in non-sewered sanitation and shows what type of treatment for liquids can be expected in the non-sewered context. In many cases, the first treatment step (after screening of solid waste) is dewatering . After that, there is a separate solid and liquid treatment. First of all, there are places where there is no treatment for supernatant, and there is direct discharge to the environment. In urban areas where conventional sewered wastewater treatment services are also available, FS is often transported to the WWTP. However, improper co-treatment with FS has been the cause of some failures (Strande et al., 2014). The latter could undesirable shock loads to the system, making treatment not complete. Settling–thickening tanks, drying beds and waste stabilization ponds are the most common treatment technologies for solid–liquid separation, dewatering of FS and liquid treatment, respectively (Strande, 2014). This liquid is called 'supernatant'. However, they are all land-intensive. In addition, FS is typically >90% water, which is prohibitively expensive to transport (Ward et al., 2019). Hence, existing treatment technologies need to be optimized and promising new techniques need to be tested to reduce footprint, increase capacity and make treatment within urban areas feasible. At the moment, there are only a handful of options for supernatant treatment used, i.e. waste stabilization ponds, constructed wetlands, or anaerobic treatment like an anaerobic baffled reactor (ABR). In summary, there is certainly still room for exploring new supernatant treatment technologies. This is where this thesis tries to fill in part of the knowledge gap and the dotted line presents this in Figure 1.

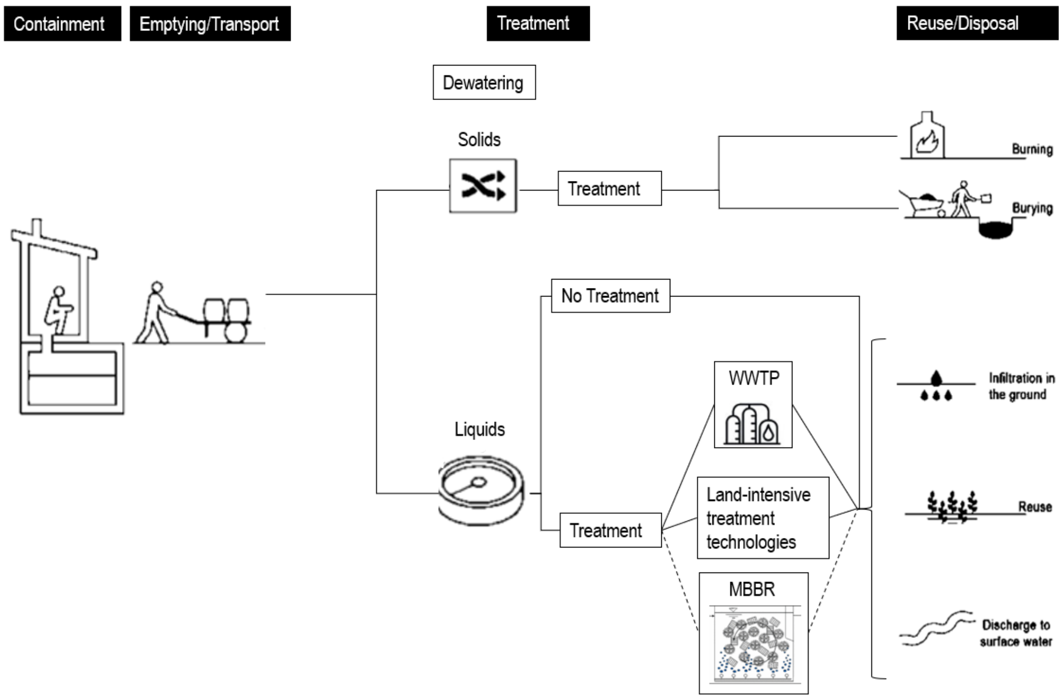


Figure 1: Semi-Centralized Faecal Sludge Management service chain. Adapted from FSM Technical Working Group TWiG (WASH Cluster | Global WASH Cluster, 2022). Full lines represent what is already being implemented in the field, the dotted line represents the knowledge gap being filled in by this thesis.

There is a need for low-cost and robust solutions for supernatant treatment after dewatering of FS. Challenges to handling supernatant are abundant, for multiple reasons. FS is highly variable and therefore composition of the resulting supernatant is highly variable, making treatment to appropriate discharge standards difficult. FS can be comprised of any range of fresh excreta to products of anaerobic digestion from storage in containment, and can include soil, sand, and municipal solid waste (Cofie et al., 2005). The variability in supernatant after dewatering of FS stems from a multitude of factors, including the different types of containments, differences in emptying practices, usage patterns, and the duration of storage in onsite containment. All those factors could affect the level of stabilization (Ward et al., 2019). Furthermore, the dewatering process prior to liquid treatment creates variability in the to be treated supernatant, as the performance of dewatering is sometimes lagging, as it is influenced by factors like pH and the presence of particulate solids. Lastly, processes going on in FS containments are not well understood yet. All of these factors regarding variability contribute to a large knowledge gap in how supernatant can be handled efficiently on-site. Especially when looking at the option of treating supernatant with biological treatment, this variability can cause difficulties.

Most difficulties can be found in the variability in COD/N ratio, salts present in supernatant, and the large range of pH values possible for supernatant (González-Tineo et al., 2022; Semiyaga et al., 2015; Ward et al., 2019):

- Salts can affect biofilms specifically (Xu et al., 2021). Leachate from sludge drying beds and stabilization ponds can be high in salinity. This is of concern if the effluent is to be used for irrigation due to impacts on plant growth, reduced soil permeability, and surface crusting (Strande et al., 2014). Monovalent salts can break up the cation bridges in the biofilm, causing detachment and loss of performance (Xu et al., 2021) .
- For COD/N, the problem often lies in the fact that there is a low fraction of biodegradable organics available compared to the ammonia loading that needs to be removed. Nitrogen is an important factor to consider in supernatant treatment, as the concentrations can be up to 10-100 times higher than conventional wastewater (Strande et al., 2014). Consequently, there is often too little COD to denitrify all the nitrate. At high pH, ammonium can cause inhibition (Fumasoli et al., 2017).

Untreated supernatant has a high oxygen demand. A big fraction of slowly biodegradable COD is present, which first needs to be hydrolyzed. Next to that, there is no standard fractionation of supernatant.. If supernatant (or FS generally) is directly discharged to the environment, eutrophication occurs. Therefore the removal of COD, called 'stabilization', is also an important treatment objective, next to nitrogen removal.

- High variability in pH can affect the optimal functioning of microorganisms (Fumasoli et al., 2015, 2017; Metcalf and Eddy, 2013; Schielke-Jenni et al., 2015). pH fluctuations vastly affect the growth of biofilm as it overpowers several mechanisms and casts detrimental effects on microorganisms (Ells & Hansen, 2006).

In conventional wastewater treatment, attached growth processes are promising when robustness and variability in influent is considered, for the removal of COD and N (Metcalf & Eddy, 2013).Therefore these treatment technologies are considered in this thesis for COD and N removal in supernatant after dewatering of FS. Specifically a moving bed biofilm reactor (MBBR) is useful as it has low energy requirements, can handle shock loadings quite well, takes small space, and is easy in operation and maintenance (Aygun et al., 2008). Therefore, it is important to know what the biodegradable fraction of the organics in supernatant after dewatering is, in order to evaluate to what extent COD can be removed biologically. Furthermore, it is also important to know the fractions of the COD to obtain reliable predictions of COD and N removal for design of the FSTP. Respirometry is a useful analysis technique for this (Mainardis et al., 2021), but it has not yet been applied to analysis of supernatant after dewatering of FS.

This research is a first step in a proof of concept to use an attached growth system (MBBR) that is now only used in centralized waste-water treatment, in a semi-centralized, non-sewered setting for the treatment of supernatant after dewatering of FS. Appropriate COD and N removals are considered, meaning that this research focuses on three of the four research objectives of FSM: 'Dewatering' as a way to obtain the supernatant, 'Nutrient Management' of nitrogen and 'Stabilization', both by MBBR treatment.

A 5000-person community-scale scenario in an urban (Sub-Saharan) setting, with access to semi-centralized treatment is considered. In this scenario, containments are emptied by vacuum trucks or a manual service on an irregular basis, and taken to a (nearby) small semi-centralized faecal sludge treatment plant. An inflow of 10 m^3 FS/day is assumed for this amount of people (2 L per person per day). The composition of the FS is different each time, depending on the containment where it was collected, storage time, inflow and outflow characteristics, type of structure and temperature, etc. For example, there may be unstabilized FS from a busy market place where the containment has to be emptied several times a day. Alternatively, the faecal sludge may come from a remote septic tank, which does not have to be emptied often. These two examples may have different faecal sludge characteristics, as the second might be more stabilized and diluted with greywater. Furthermore, FS supply to the plant can be highly variable, e.g. no truck can arrive for a week, or a day, or no work in the plant is done on weekends.

Figure 2: Solids handling, following dewatering at a community-scale FSTP in Kanyama neighborhood Lusaka, Zambia. (Picture by Nienke Andriessen)

2. Research Questions

Is a moving bed biofilm reactor (MBBR) a viable option for supernatant treatment after dewatering of faecal sludge in non-sewered sanitation, applied to a community-scale scenario of approximately 5000 people (semi-centralized)?

1. Proof of concept: Does an MBBR reactor run on supernatant after dewatering of faecal sludge, meaning that combined C- and N-removal is achieved for different inflows, and a certain effluent standard can be reached?
    a. Does an MBBR run on 1 type of supernatant after dewatering of faecal sludge?
    b. Does it also run on a community-scale scenario regarding inflow variability and intermittency?
2. Is the MBBR process viable in field conditions (loading limits, operation, monitoring, skilled people, risk management).

3. Research method

First a literature review is performed on the concept of FSM and existing treatment technologies for supernatant after dewatering of FS. After that the concept of attached growth and MBBR is explored. To solve research question 1.1 and 1.2, lab work was performed at the Swiss Institute of Aquatic Sciences (EAWAG) in Zurich, Switzerland. First it was tested whether combined C- and N removal is achieved on 1 type of supernatant, obtained from FS sampled in Switzerland. After that, variabilities in COD/N ratio, salts concentrations and pH were tested by spiking that first supernatant. Next, intermittent flows as determined by the community-scale scenario were tested. Next, a realistic scenario was tested with real supernatants from different countries and intermittency. To solve research question 2, the feasibility of the MBRR reactor in the field was examined through literature search and performing interviews of people active in the field.

4. Demarcation

As indicated in the research questions, this research is a proof of concept, a first step to assess whether attached growth processes and more specifically an MBBR could be a supernatant treatment option in non-sewered sanitation setting. When 'supernatant' is mentioned, the liquid fraction after the dewatering of FS is meant. This research only focuses on dewatering, stabilization and nutrient management regarding N removal of supernatant as treatment objectives of FS. 'Pathogen reduction' and 'Nutrient management' regarding phosphorous are points for further research. There is looked whether discharge standards for COD and N are met. An overview and comparison of the considered discharge standards are shown in Appendix 12. Post-treatment steps are necessary for actual discharge. A proposition for that is presented in the feasibility study.

Context

This chapter starts with a summary and explanation of all the terminology that is necessary to understand the topic and research questions. It provides the context of non-sewered sanitation in a semi-centralized approach and explains the concept of faecal sludge management (FSM). After that, more emphasis is put on the variability of supernatant. Next, a literature review is presented of attached growth processes, and more in particular the MBBR process, to provide background knowledge and show for which purposes this technology has already been used.

1. Terminology

According to the International Water Association, **non-sewered sanitation**, also referred to as on-site sanitation, is a sanitation system that is not connected to a networked sewer system but collects at the source in a containment. Then the input is transported to allow for treatment and ideally safe reuse or disposal (IWA,2016). On-site sanitation technologies can provide sustainable and affordable sanitation solutions for dense urban areas, if comprehensive **faecal sludge management (FSM**) of the entire **sanitation service chain** is in place (see Figure 1 in Introduction), including reliable collection, transport, treatment and safe end-use or disposal of faecal sludge (FS) (Dodane et al., 2012).

One way of organizing non-sewered sanitation is by semi-centralized sanitation systems. Obermann and Sattler stated that **semi-centralized sanitation** systems can be categorized by their number of connections of households, or by the outline of the sewer system relative to the central sewerage system. Numbers of connected household vary from 1000 to 10,000 people. Two ways of semi-centralized treatment are constructed wetlands and a small faecal sludge (FSTP) or wastewater treatment plant (WWTP) (Obermann & Sattler, 2013). In this thesis emphasis is put on a FSTP as semi-centralized treatment.

Figure 3 shows the difference between **excreta, faecal sludge and wastewater**. Excreta is urine and faeces. According to Strande et al., FS "comes from onsite sanitation technologies, and has not been transported through a sewer. It is raw or partially digested, a slurry or semi-solid, and results from the collection, storage or treatment of combinations of excreta and blackwater, with or without greywater" (Strande et al., 2018). Wastewater is the term for excreta and additional input, which has been transported through a sewer system. **Blackwater** is the stream coming directly from the user interface (toilet), which has not been stored in any containment, or transported to a sewer yet. This can contain urine and faeces, combined with flush water, and toilet paper.

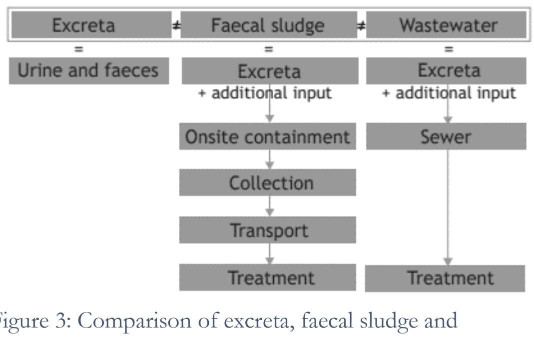


Figure 3: Comparison of excreta, faecal sludge and wastewater according to the Faecal Sludge Methods book (Source: EAWAG) (Velkushanova et al., 2021)

2. The Sanitation Service Chain and Treatment of FS

As this thesis is mostly about the field of non-sewered sanitation, the term FS is used for the to be treated stream. FS needs to be periodically removed from the containment for further treatment and disposal/reuse. Figure 4 shows the urban sanitation service chain. Generally, urban sanitation systems can be broadly categorized as either physically networked (such as conventional sewerage) or as sanitation service networks, known as Faecal Sludge Management (FSM) (Medland et al., 2016). This service chain comprises: excreta capture and storage in a pit latrine or septic tanks; emptying of the pit or the tank; transport of the contents; sludge treatment and end-use or discharge in the environment. FSM is the predominant sanitation system in low- and middle income countries where sewers are lacking. However, due to lack of capital and appropriate technologies, FSM in several cities has been found to be unsatisfactory, causing environmental pollution and health problems (Taweesan et al., 2015).

The last stages of the service chain, comprising of transportation, treatment and disposal or end-use have an environmental focus. A study on FSM by the Water and Sanitation Program in 12 cities in low-income countries highlighted that on average, faecal waste from only 22% of households using on-site systems is safely managed (Peal et al., 2014). In some cases, whilst the excreta might be safely emptied it is then dumped illegally. Any break in the service chain at any stage will cause the FS to be released untreated into the natural environment, endangering the public health of the city and surrounding areas. The apparent simplicity of the sanitation service chain depicted in Figure 4 hides the complexity of the enabling environment within which the activities in the chain occur (Medland et al., 2016).

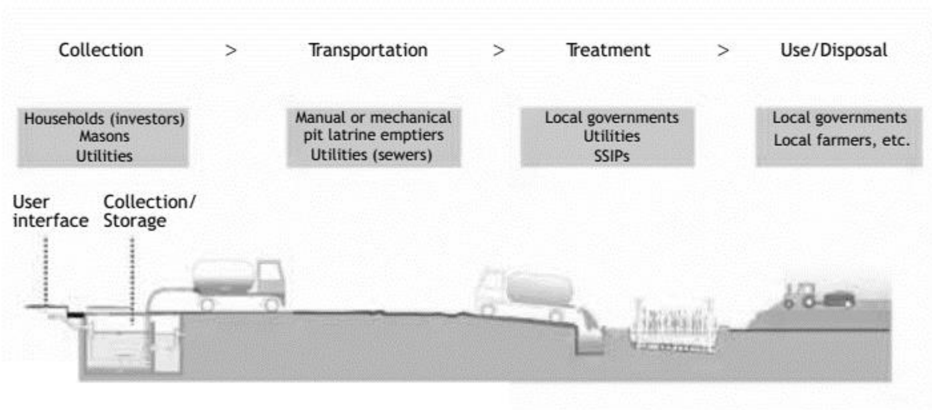


Figure 4: The faecal sludge management sanitation service chain (Strande et al., 2018)

The technologies required to make the service chain function are for the most part known, especially at the beginning of the chain where the challenge is more about encouraging households to build systems that can be emptied easily, than in developing new alternatives (Strande et al., 2014). The key technological challenge remaining is cost-effective, space efficient treatment processes that make the FS safe for disposal or further use.

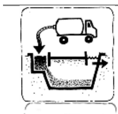
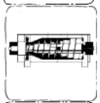
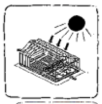
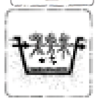
As already mentioned, this thesis focuses on the third step of the service chain, 'treatment'. This step comprises four treatment objectives, regarding dewatering, pathogens, nutrients and stabilization. Dewatering (or "thickening") of FS is an important treatment objective, as FS contains a high proportion of liquid, and the reduction in this volume will greatly reduce the cost of transporting water weight and simplify subsequent treatment steps. Environmental and public health treatment objectives are achieved through pathogen reduction, stabilization of organic matter and nutrients, and the safe end-use or discharge of treatment end-products (Strande et al.,2014).

3. Existing treatment technologies

3.1 Overview

Treatment technologies can be classified according to which treatment objectives they tackle. Table 1 shows an overview of the most important and most frequently used treatment technologies for faecal sludge, ordered according to their treatment objectives. It shows what the treatment end-products are, and how far the technology is already developed. To get to the treatment of supernatant, solid-liquid separation ('Dewatering') must first be done, so this means a treatment technology with dewatering as the treatment objective. Then one considers treatment technologies with the objective of stabilization and nutrient removal. Pathogen reduction is not considered in this thesis and therefore treatments with only this as a treatment objective are excluded from the Table.

Table 1: Overview of treatment technologies in FSM according to their treatment objectives. Table retrieved from CAWST & EAWAG, 2016, and adapted (CAWST & EAWAG, 2016).

	Treatment technology	Treatment objectives	Treatment products	Level of technology development
	Settling-thickening	Dewatering	Liquid sludge Effluent with pathogens	Established
	Mechanical dewatering	Dewatering	Dewatered sludge with pathogens Effluent	Transferring
	Unplanted drying beds	Dewatering	Dewatered or dry sludge with pathogens Effluent	Established
	Planted drying bed	Dewatering Stabilization/Nutrient management	Plants Dry stabilized sludge with pathogens Effluent	Established
	Co-composting	Pathogen inactivation Stabilization/ Nutrient management	Dewatered stabilized sludge with low pathogens Effluent	Established
	Waste stabilization ponds	Pathogen inactivation/ Stabilization/ Nutrient management	Nutrient rich pathogen free effluent	Established
	Deep row entrenchment	Stabilization/Nutrient management	Plants Trees	Established
	Incineration	Pathogen inactivation Dewatering Stabilization/Nutrient management	Ash Biofuel	Transferring
	Anaerobic digestion	Stabilization/Nutrient management	Liquid stabilized sludge with / without pathogens Biogas	Transferring
	Black soldier fly larvae	Stabilization/Nutrient management	Dewatered stabilized sludge with pathogens Black soldier fly larvae	Innovative
	Thermal drying	Dewatering Pathogen inactivation	Dry sludge with pathogens	Transferring
	Co-treatment with wastewater	Depends	Treated effluent	Transferring
	Aquaculture pond	Stabilization/Nutrient management	Fish or aquatic plants Liquid sludge Effluent with pathogens	Innovative / Transferring

In the next two sections, the most important treatment technologies regarding dewatering and nutrient management and stabilization are highlighted.

3.2 Dewatering

Dewatering of FS is a first important objective of the FS treatment process, as FS contains a high fraction of liquids, and further treatment requires separation of the liquid and solid fractions. Common methods for dewatering are gravity settling, filter drying beds and evapo(transpi)ration (Hemkend-Reis et al., 2008; Strande et al., 2014). The dewatering technology used in this thesis is a conditioning step with a mechanical dewatering step . Chemical conditioning is based on the same physical properties as coagulation/flocculation (Metcalf and Eddy, 2013). Common conditioners include ferric chloride, lime, alum and organic polymers (Strande et al., 2014). Important aspects to consider for conditioning are sludge age, pH, containment, solids concentration and alkalinity (Shaw et al., 2022).

3.3 Nutrient Removal and Stabilization

Looking at designs of existing FSTPs and literature, the three most used treatment technologies mentioned in Table 1 are waste stabilization ponds, planted drying beds/constructed wetlands and anaerobic treatment through an anaerobic baffled reactor (ABR).

Waste stabilization ponds are a good option for wastewater treatment in low- and middle-income countries because of the low capital and operation and management (O&M) costs. In general, they consist of a series of ponds named after their function – anaerobic, facultative or maturation ponds – in which water under treatment is allowed to stay for 20 to 180 days, thereby reducing organic, nutrient and pathogen loadings through both sedimentation and biodegradation under anaerobic, anoxic and/or aerobic conditions (Waste Stabilisation Ponds | SSWM, 2022). A picture of waste stabilization ponds in the field is shown in Figure 5.

Figure 5: Waste stabilization ponds from the Imvepi FSTP in Uganda. (Picture by Nienke Andriessen)

The appearance of a **Vertical Flow Constructed Wetland** is similar to **a planted drying bed.** They are both sealed shallow ponds with drainage layers (Planted Drying Beds | SSWM, 2022). Nutrients and organic material are absorbed and degraded by dense microbial populations that grow in the beds. By forcing the organisms into a starvation phase between dosing phases, excessive biomass growth can be decreased and porosity increased. Furthermore there is enhanced treatment due to the plants.

Figure 6: A planted drying bed from a small FSTP in Bangalore. (Picture by Nienke Andriessen)

An Anaerobic Baffled reactor (ABR) is an improved septic tank with a series of baffles under which the supernatant is forced to flow. The increased contact time with the active biomass results in improved treatment. ABRs are robust and can treat a wide range of variability. However, both remaining sludge and effluents still need further treatment to be reused or appropriately discharged (Anaerobic Baffled Reactor (ABR) | SSWM, 2022). An ABR is often followed by an anaerobic filter. An anaerobic filter is constructed like an ABR, but the baffles are partially filled with support media. It allows the fixation of bacteria that organize themselves into well-organized granules, composed of hydrolytic, acidogenic and methanogenic bacteria. (Reysset & Foundation, 2021). Figure 7 shows a sketch of Borda's decentralized wastewater treatment system, which uses an ABR and anaerobic filter.

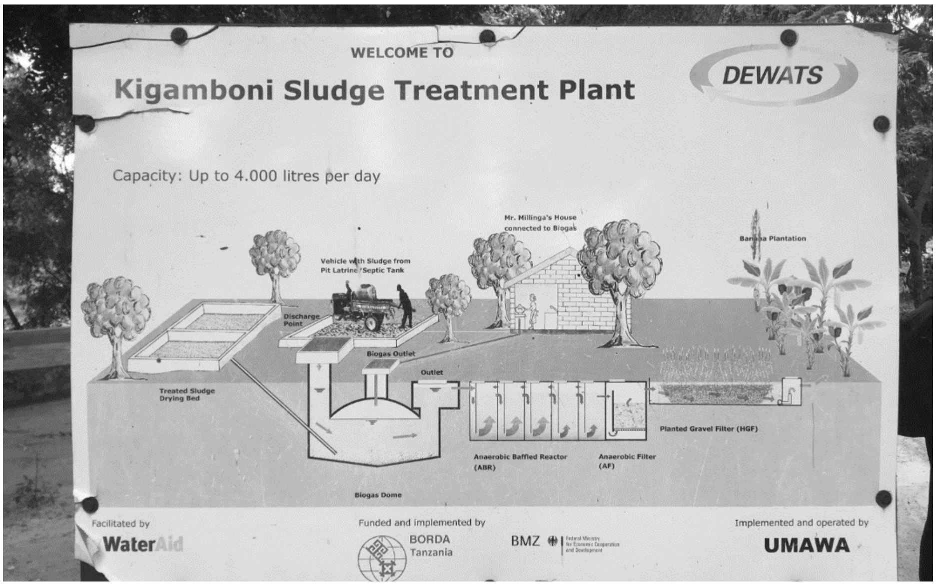


Figure 7: Overview of the BORDA ABR treatment system. Picture taken in Kigamboni treatment plant, Dar Es Salaam, Tanzania, by Nienke Andriessen

Each of these techniques have advantages and disadvantages in comparison to an MBBR. These will be discussed in the 'Feasibility Study' chapter.

4. Variability in supernatant characteristics

Parameters that should be considered for the characterization of supernatant after dewatering of FS include solids concentration, chemical oxygen demand (COD), biochemical oxygen demand (BOD), nutrients, pathogens, salts and metals. This is the same as those considered for domestic wastewater analysis. However, it needs to be highlighted that the characteristics of domestic wastewater and FS are highly different (Strande et al., 2014). As mentioned in the introduction, the biggest challenge in treating supernatant is its composition variability, creating the need for robust and flexible treatment options. It is essential to know the effect of all the preliminary steps in the sanitation service chain on these characteristics (Strande et al., 2014).

First of all, household habits create variability. Household habits associated with toilet usage influence the variability of FS in the onsite containment. The total solids (TS) concentration depends on factors such as dry versus flush toilet, the volume of flush water used, cleansing method (toilet paper and cleansing water) and inclusion or exclusion of grey water. The fat, oil and grease concentration will increase with the inclusion of kitchen wastewater without properly maintained oil and grease traps, and odors will also increase with additional organic waste streams.

Second, the different types of containments create variability in FS. The concentration and volume of FS is also greatly influenced by inflow and infiltration of leachate into the environment from the system and / or ground water into the system. The filling rate of systems will be slower if there is more leaching in the soil, resulting in a thicker and more concentrated FS. The permeability of containment systems is influenced by whether they are unlined, partially lined, wholly lined, connected to drain fields or soak-pits, and the quality of construction. The duration of storage in onsite containment also depends on the type of containment and creates variability. The filling rate and storage duration depend on the type of technology, quality of construction, toilet usage, and inflow and infiltration. The length of time that FS is stored in onsite containment systems before being collected and transported will greatly affect the characteristics due to the stabilization of organic matter that occurs during storage.

Third, emptying practices. The emptying frequency of septic tanks varies greatly based on the volume and number of users and can be anywhere from weeks to years. FS that has been stored in a septic tank for a period of years will have undergone more stabilization than FS from public toilets. During the filling of onsite containment systems, the FS gets denser at the bottom due to compaction. This FS is more difficult to remove by pumping and is therefore frequently not emptied and left at the bottom of the containment system.

By dewatering most of the solids and therefore the particulate COD are removed, resulting that mostly the soluble fraction of the COD remains in supernatant. This will reduce the overall oxygen demand of the system for oxidation of COD. Figure 8 shows the different fractions of COD. By performing respirometry, those different fractions can be determined and it can be examined what the biodegradable fraction is of the COD in the supernatant (Mainardis et al., 2021). This is important to know to verify whether it is at all possible to use biological treatment to remove COD, namely to see if the non-biodegradable COD fraction is not too large. As the ions are dissolved in the liquid, N, P and salts concentrations will remain the same after dewatering.

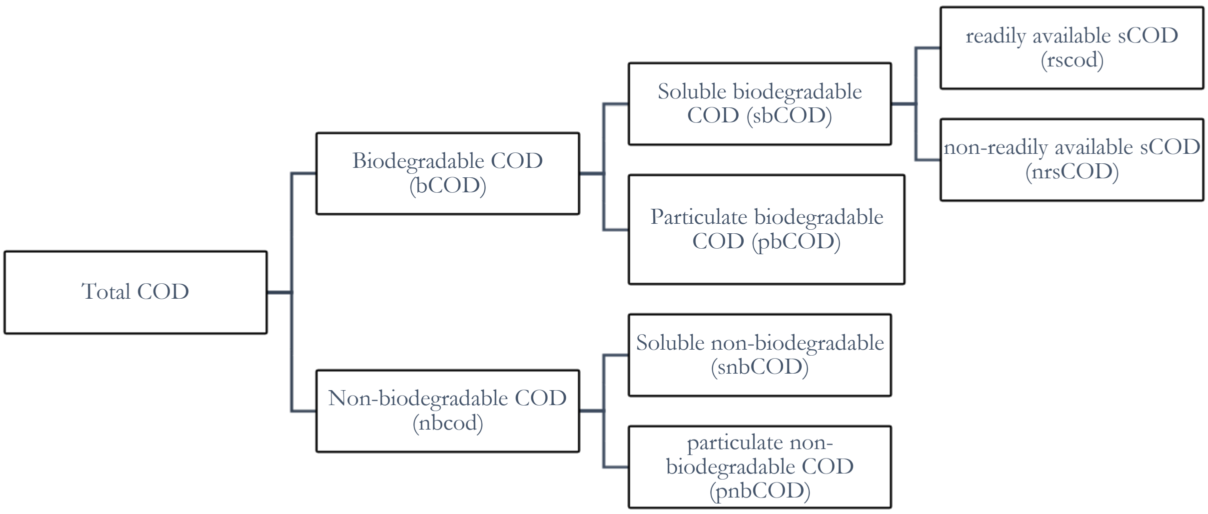


Figure 8: Overview of how COD is fractionated. Adapted from (Ekama et al., 1986) .

In Table 11 in 'Results and Discussion', that demonstrates the variability of the supernatants in the lab for MBBR treatment, the minima and maxima found in literature for the most relevant supernatant characteristics are shown.

5. Attached growth processes

5.1 General

According to Metcalf and Eddy, attached growth processes are 'aerobic treatment processes in which the biomass responsible for treatment is attached to some type of medium (packing material)'. The most important techniques are MBBR, rotating biological contactor (RBC) and trickling filters (Metcalf & Eddy, 2013). An MBBR is under the attached growth category of 'Activated Sludge Processes With Fixed Film Carriers'. This combination of activated sludge process and carriers is referred to as a hybrid process and is also called 'Integrated Fixed-film Activated Sludge process' (IFAS). Other categories of attached growth are: (1) non-submerged attached growth aerobic processes, (2) partially submerged attached growth aerobic processes, (3) sequential non-submerged attached growth-activated sludge process (4) submerged attached growth aerobic processes (Metcalf & Eddy, 2013). Present day designs use more engineered materials and include the use of synthetic media that are suspended in the aeration tank with the mixed liquor, fixed synthetic material placed in portions of the aeration tank, and submerged RBCs.

There are multiple reasons why attached growth might be chosen over conventional biological treatment with activated sludge (Bhattacharya & Mazumder, 2020):

- Greater process stability
- Reduced sludge production
- Enhanced sludge settleability
- Reduced solids loadings on secondary clarifier
- No increase in operation and maintenance costs.

With the rising costs of sludge disposal, there has been a rise in interest into the minimization of sludge production. Excess sludge treatment is 50-60% of the costs of municipal wastewater treatment (Aygun et al., 2008). Overall, attached growth systems are perceived as more robust and better at handling high variability in influent compositions. Removal processes are often diffusion limited (see Figure 9). The removal of substrate in an IFAS system is a complex process involving both substrate uptake by the suspended biomass and diffusion and consumption of substrate in the biofilm. The sloughing of nitrifying bacteria from the attached growth biofilm results in nitrification in the suspended mixed liquor at low SRTs for which nitrification would not normally be maintained (Madan et al., 2022).

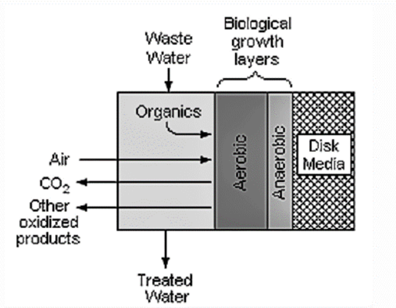


Figure 9: Processes going on in an attached growth biofilm (Aygun et al., 2008)

5.2 MBBR

5.2.1 Overview of the MBBR process

The MBBR process offers an efficient alternative to the conventional biological treatment processes for organic matter removal at high organic loading rates. The MBBR process is a process in which media is suspended and mixed within a reactor, without a return activated sludge (Singh et al., 2018) . Plastic carriers are kept suspended in an aeration tank by an aerator and stirrer for the aerobic processes and by a mechanical stirrer for the anoxic processes (Mazioti et al., 2021).The carrier fill volume is up to 70%, and the suspended solids concentration in the flow to a secondary clarifier may be in the range of 100-250 mg/L. The idea behind developing the MBBR process was to adopt the best features of the activated sludge process as well as those of the attached growth process. Contrary to most biofilm reactors, the MBBR uses the full potential of the reactor volume for the growth of biomass. Contrary to activated sludge processes, it does not need any sludge recycle (Ødegaard, 2006). The carriers move freely in the reactor volume, kept within the reactor volume by a sieve at the effluent outlet. The reactor can be used for both aerobic, anoxic and aerobic processes, however in this thesis it is decided to opt for a SBR configuration, see section 5.2.3.

Advantages of the MBBR process include the small space needed, simplicity of operation with no need for manual sludge wasting and SRT control and the already mentioned sludge recycle. Compared to other attached growth process like the RBC, the MBBR process is much more versatile and adaptable for biological nitrogen removal and phosphorous removal (Bhattacharya & Mazumder, 2020). These are the reasons why it is assumed that an MBBR process is a good candidate for supernatant treatment after dewatering of FS in non-sewered sanitation. The MBBR process can sustain and effectively treat wastewater of varying organic load due to the self-modifying microbial characteristics of the biofilm (Madan et al., 2022). The available surface area for microbial attachment is one of the designing parameters. Lastly it can withstand peak weather flow variations. Disadvantages are sludge bulking and potential heterotrophic overgrowth (Morgenroth, 2008). Though they are more compact, their capital expenditures (CAPEX) are generally higher than that for activated sludge treatment (Mazioti et al., 2021). Compared with fixed-bed biofilm reactors, MBBR systems show lower head losses and no clogging problems. Moreover, existing biological tanks for other processes can be readily modified to the MBBR configuration, instead of constructing new facilities. Last, this process has proved to be highly effective for ammonium removal, even at low temperatures (S. Zhang et al., 2013).

Figure 10 shows a schematic how an MBBR looks like. It shows that as additional treatment step, normally a secondary clarifier is included, as sludge bulking could be an issue. However, in this thesis there is opted for the option to operate the MBBR in an SBR cycle, in which part of the cycle is effluent decant, which removes part of the suspended solids. However, the utility of the secondary clarifier as a post-treatment step, even in supernatant treatment with SBR configuration, will be demonstrated later in this study.

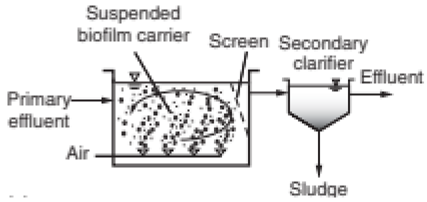


Figure 10: Schematic of an MBBR with secondary clarifier as post-treatment step according to (Metcalf and Eddy, 2013).

5.2.2 Previous research

The last two decades have brought special interest to biofilm processes in wastewater treatment (Madan et al., 2022). Components from both biofilm and activated sludge processes are being used to evolve the moving bed process which is able to remove major pollutants, organic matter and nutrients from municipal as well as industrial wastewaters. According to Aygun et al., MBBRs have already been used for a wide range of industry waters (Aygun et al., 2008). They have already been used in the treatment of dairy wastewater (Boavida-Dias et al., 2022; Rusten et al., 1992b; Santos et al., 2020; Zkeri et al., 2021), forest industry wastewater, pharmaceutical industry water (Bhattacharya & Mazumder, 2020) cheese factory wastewater, newsprint mill wastewater, textile wastewater (Francis & Sosamony, 2016), Italian food industry (Falletti et al., 2015), thermos mechanical pulping whitewater (Patel et al., 2021), municipal wastewater (Wang et al., 2006), and for research of simultaneous nitrification and denitrification (Aygun et al., 2008). Furthermore, an MBBR has extensively been researched for on-site greywater treatment (Masmoudi Jabri et al., 2019; Saidi et al., 2017). MBBR treatment has never been tested on supernatant after dewatering of FS.

A lot of research has been done to gain more knowledge on which factors affect the operational performance of the MBBR. The most important factors affecting this are the biofilm carriers, the filling fraction of those biocarriers, the DO-level maintained in the reactor, and the hydrodynamics and biofilm development (Madan et al., 2022). First, in order to make the MBBR technology more efficient, several studies investigated the optimization of the treatment process by using different types of bed carriers (Chu & Wang, 2011) or filling ratios . As the core technology of the MBBR is the carrier, a lot of research has been performed on the materials, size, surface properties, etc. Xie et al. performed a numerical simulation and experimental investigation on the effect of a new suspended carrier filler on mass transfer in a MBBR reactor. This 3D-printed biocarrier could increase the rotation of the carrier in the reactor, and with that increasing specific surface area of the biofilm. This led to average COD removals of 87.75% and ammonium removals of 94.77% (Xie et al., 2020). Shitu et al. looked a novel sponge biocarriers in aquaculture treatment (Shitu et al., 2020). Deng et al. combined plastic and sponge as carriers (Deng et al., 2016).

Numerous studies have shown that MBBR processes have excellent traits such as a high biomass, COD loading, strong tolerance to loading impact, relatively small reactor requirement and no sludge bulking issues (Leyva-Díaz et al. 2013). Ødegaard (1999) recommended a design value for an MBBR system with 67%

filling ratio using the Kaldnes K1 of 8 kg/m³·d at a high loading rate of bCOD and with a treatment goal that goes up to 75–80%. Comett-Ambriz et al. tested an MBBR configuration to treat anaerobic biowaste effluent with the same SBR cycle as proposed in this thesis and compared to activated sludge process in SBR cycle. The MBBR was shown to be an equivalent treatment to activated sludge, with tCOD removal efficiency of 53%, sCOD removal efficiency of 40% and ammonium concentration removal of 99% (Comett-Ambriz et al., 2003). In a study by Bengtson, alkalinity was added prior to the MBBR treatment step to keep the pH buffered (Bengtson, 2010). Ferrai et al. modelled respirometric tests for the assessment of kinetic and stoichiometric parameters on MBBR films for municipal wastewater treatment, which is also the way of analyzing influent and effluent of the MBBR reactor in this thesis (Ferrai et al., 2010).

Thorough research has also been performed on MBBRs implemented in hybrid systems, as reviewed by Madan et al. (Madan et al., 2022). MBBR systems can be combined with chemical precipitation (Wang et al., 2006) or with chemical coagulation for dyeing wastewater treatment (Shin et al., 2006). However this is often not applicable to non-sewered sanitation purposes.

5.2.3 SBR cycle

In this thesis, the MBBR is operated as a sequencing batch reactor (SBR) system. This configuration is preferred over continuous stirred tank reactors (CSTR) regarding energy requirements and it is easier to adjust loadings (Budiastuti et al., 2021). The SBR is a fill-and draw system typically used in conventional wastewater treatment (Wastewater Technology Fact Sheet Sequencing Batch Reactors, 1999). An explanation on how the SBR cycle time is calculated is mentioned in the 'Materials and Methods' chapter. Applying the SBR cycle concept gives flexibility to the process because it presents the possibility of operating the plant according to a time-oriented sequence of events. In this thesis, a pre-denitrification approach is used, meaning an anoxic phase comes before an aerobic treatment phase. In the anoxic phase, denitrification is performed (and therefore COD removal). In the aerobic phase, there is nitrification and further COD removal by the suspended biomass. The pre-denitrification approach is preferred over pre-nitrification to avoid adding bCOD during a post-anoxic step.

In denitrification, nitrite and/or nitrate are reduced via nitric oxide and nitrous oxide to N2 by heterotrophic bacteria. Contrary to nitrifiers, most denitrifiers are facultative anaerobes; they can utilize oxygen or nitrate/nitrite as electron acceptors, but they prefer to use oxygen. Therefore, bacterial denitrification mainly takes place under anoxic conditions (Reboleiro-Rivas et al., 2015). Therefore it needs bCOD as an electron-donor. As a result, one mole of alkalinity is produced per mol of NO_3^- reduced, and because of the production of OH^- the pH increases.

$$10NO_3^- + 1COD \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^- \ (1)$$

Nitrification is aerobic oxidation of ammonium to nitrite and further to nitrate by obligate aerobic chemo-litho-autotrophic bacteria. Two moles of alkalinity is consumed when nitrifying and overall the pH decreases.

$$NH_4^+ + 2HCO_3^- + 2O_2 \rightarrow NO_3^- + 2CO_2 + 3H_2O \ (2)$$

5.2.4 Problems that variability in influent of supernatant can cause in MBBR operation

Through a literature review of MBBR processes in different contexts, it can be inferred that several factors in the influent characteristics of supernatant can cause problems in attached growth processes, more specifically in MBBRs. This review revealed three main issues: heterotrophic overgrowth, inhibition by sub-optimal pH values and biofilm detachment by high salt concentrations. These are highlighted in this chapter.

Heterotrophic overgrowth

An IFAS process like an MBBR is typically used where fixed or biofilm carrier media is added to an activated sludge system to enable nitrification without constructing additional aeration tanks. The suspended growth SRT is too short to support an adequate ammonia oxidizing bacteria (AOB) population for nitrification in such systems (Metcalf & Eddy, 2013). Nitrifying bacteria can grow on the carrier media to provide nitrification despite the limited SRT in the suspended growth process. However, this creates competition between heterotrophic and autotrophic bacteria on biofilm media. This competition translates directly into a specific design approach. Nitrification and the corresponding ammonia flux depend on the bulk-phase COD and heterotrophic growth.

Limiting amounts of dissolved oxygen (DO) (concentrations below 2 mg/L) inhibit nitrification and cause nitrite accumulation or nitrous and nitric oxide production. Knowledge of the effect of oxygen on nitrification and nitrifying populations has economic importance since aeration of activated sludge is one of the most costly items in the operation of a wastewater treatment plant.

MBBR processes can remove similar bCOD levels and nitrogen as conventional activated sludge processes (Metcalf and Eddy, 2013). A COD/N ratio above 1/1 (Morgenroth, 2008) can decrease the nitrification rate of the biofilm, as heterotrophs will outgrow the autotrophs (Metcalf and Eddy, 2013). This is a problem because then there is no possibility anymore for nitrification, as the ammonium in the bulk solution is not accessible anymore to the autotrophs.

In non-sewered sanitation, single loadings (a filling of one cycle according to the filling ratio) might have unsuitable COD/N, creating a so-called ‘shock-load’. If this often happens over time, heterotrophs will overgrow autotrophs and nitrification will not occur. It needs to be known what the biodegradable fraction of COD is in supernatant, and how the fractionation changes with the same conditioner and dewatering technique, but other sources of FS. If the COD is primarily inert, there is no problem of heterotrophic overgrowth but there might be issues that there is no full denitrification. By adjusting the SBR cycles of the MBBR reactor to allow enough time in the low range COD/N to give the autotrophs enough time to grow, this hypothesis can be rejected.

This will be tested by increasing the concentration of sCOD in the baseline supernatant, up until a ratio of COD/N of 518/1 (Morgenroth, 2020). If the COD and N loadings are too high, the calculated cycle length will be too short and removal will not be sufficient. Too much ammonium and not enough available COD for full denitrifications will lead to no complete treatment. A reason for this can be that there is a lot of inert COD in the supernatant that cannot be removed. If a shock-loading of COD occurs and the operation is not adjusted accordingly, there could be heterotrophic overgrowth (Morgenroth, 2020). In this event, heterotrophs overgrow the autotrophs on the biofilm, preventing the latter from nitrifying optimally.

Effects of pH on MBBR

pH fluctuations vastly affect the growth of biofilm as it overpowers several mechanisms and casts detrimental effects on microorganisms (Ells & Hansen, 2006).During major pH fluctuations, bacteria modify protein activity and synthesis related to various cellular processes. The ideal pH of polysaccharide production differs among a variety of species, but for most bacteria, it is neutral at around 7 (Oliveira et al., 1994). A high variability in pH can cause the microorganisms on the biofilm carriers to operate less efficiently, resulting in lower COD and N removal. As nitrification reduces alkalinity, during nitrification the pH will drop. If the pH of the supernatant is too low (below 6), the biofilm will perform poorly as the pH will drop to destructive levels (pH below 5) during nitrification (Metcalf and Eddy, 2013). A pH higher than 8 will cause the microorganisms to perform at non-ideal (slower) rate. The presence of non-ionized ammonia, the toxic form, increases as pH rises and decreases as pH decreases (Jaroszynski et al., 2011).

Effects of salts on MBBR

Microorganisms in nature do not live as pure cultures or dispersed single cells. Many have a tendency to form polymicrobial aggregates known as biofilm. On this, removal by attached growth in conventional wastewater treatment is based. This phenomenon is very common and is done by a wide range of microorganisms. Microorganisms within the biofilm make up for less than 10% of the biofilm dry weight whereas the matrix itself consists of more than 90% (Flemming and Wingender, 2010). The biofilm matrix is formed of a conglomeration of different bi-polymers and ion bridges produced by the microorganisms, and is a complex, wired system. These 'wires' referred to as extracellular polymeric substances (EPS), enforced by cation bridges (Bales et al., 2013). The biofilm matrix is a three-dimensional structure consisting of different layers in which the microorganisms are embedded. EPS are closely related to the cell since they create the immediate environment in which the microbes exists (Decho, 2000).

FS can be highly variable in salts. If containments are unlined, there is a possibility of salts from the soil increasing the salts concentration in FS (Velkushanova et al., 2021). Furthermore, salts are part of the human diet. According to Rose et al., the high salts content can cause problems for the biofilm (Rose et al., 2015). A high monovalent cation concentration can lead to biofilm disintegration, via disruption of the previously divalent cation bridges (Ward et al., 2019). the monovalent ions then occupy the divalent ions as bridges, and these are less stable. This can cause parts of the biofilm to detach. If that happens, it is likely that biofilm detaches and therefore COD and N removal decreases (Sorensen et al., 2020). This can be monitored by measuring the TSS increase in the effluent and decreasing COD and N removal while increasing the concentration of salts in baseline supernatant. A decrease in dry mass of the biofilm will also show biofilm detachment and therefore process failure.

5.3 Other attached growth processes – Rotating Biological Contactors (RBC)

A second option for attached growth process that could be a candidate for community-scale treatment of supernatant is an RBC. RBC are commonly used to treat domestic black- or greywater and any other low- or high-strength biodegradable wastewater (e.g. industrial wastewater from food processors or paper mills) (Cortez et al., 2008). The RBC concept already originated in 1920 in Germany (Hassard et al., 2015). They have been found to be particularly effective for decentralised applications (on the level of a small to medium community or industry/institution), where electricity and skilled staff are available (Metcalf & Eddy, 2013). Figure 11 shows a schematic overview of an RBC. However, it has a high energy demand, because there are elevated DO concentrations. There is a need to use proprietary media in the form of rotating wheels. Issues such as scale-up remain challenging for the future application of RBC technology in supernatant treatment and topics such as phosphorous removal and denitrification still require further research (Tawfik et al., 2006). Advantages of an RBC process is high volumetric removals, solids retention and a low footprint (Hassard et al., 2015). The RBC therefore looks like an additional candidate to be used for supernatant treatment in non-sewered sanitation.

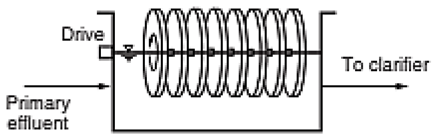


Figure 11: Schematic of an RBC (Metcalf and Eddy, 2013)

Materials and methods

This research took place over a period of 5 months (April – August 2022) at the Swiss Research Institute of Aquatic Sciences (EAWAG) in Zurich, Switzerland.

1. Overview of the experimental plan and timeline

The general research design consists of six big parts. Figure 12 shows an overview of the different steps in the methodology of this thesis. Step 1 is sampling, namely collecting the faecal sludge from which the supernatant will later be collected. Step 2 and 3 are the pre-treatment steps of the faecal sludge, namely adding conditioner to flocculate the solids, and then a physical separation step, namely 'dewatering'. Step 4 is the methodology for solving the first research question, namely whether an MBBR is an option for the treatment of one type of supernatant. Step 5 is then the methodology for the second research question, namely testing various influent compositions and intermittency. Step 4 and 5 both contain an extensive analysis step. In step 6, the applicability of an MBBR in the field will be evaluated. The results from step 4 and 5 are the fundament of this evaluation.

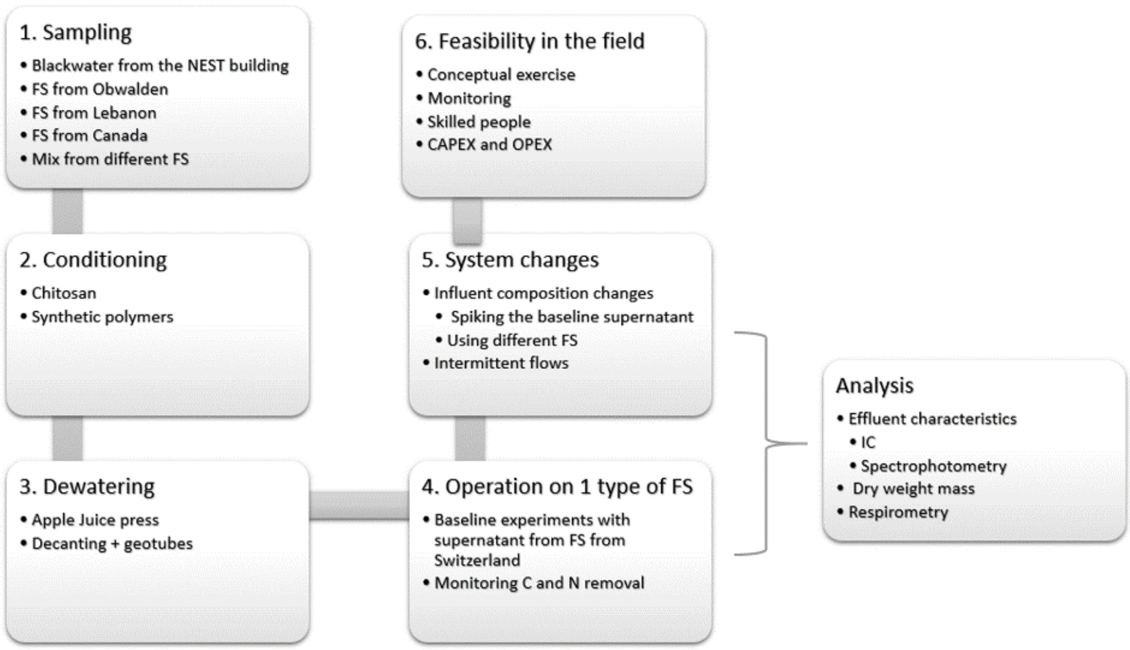


Figure 12: Overview of the methodology steps performed in this thesis. The first 3 steps on the left column show the pre-treatment steps to obtain the supernatants. Column two shows the three steps to solve the research questions. The dark grey line in between the steps presents the timeline.

2. Pre-treatment

a. Sampling – Different influents

Table 2 gives an overview of the different FS that were used to obtain supernatant as influent for the MBBR reactors. Faecal sludge was collected twice in Obwalden, Switzerland (SW1 and SW2), both right before the start of their usage for supernatant for experiments. Three types of sludge (originating from Lebanon, and from Canada) come from septic tanks from households. Furthermore, there was a mix made from smaller samples that were stored from previous projects, coming from FS of Ghana, Guatemala and Uganda. Lastly, there was blackwater sampled at the EAWAG campus from the NEST building (see Appendix 2 for additional explanation on the NEST building). The influent composition and characteristics of each supernatant were determined my measuring pH, EC, sCOD, tCOD, NH_4^+, NO_3^-, TSS, Alkalinity, Ca^{2+}, Mg^{2+}, Cl^-, K^+, Na^+. The methods of these measurements are explained in the 'analysis' part of the 'Materials and Methods' chapter. The samples were stored at 4 degrees.

Table 2: Overview of the influents used in reactor operation, with their origin, type of containment and volume collected.

Influent ID	Location	Containment	Volume collected
SW1	Obwalden, Switzerland	Septic tank/household	500L
SW2	Obwalden, Switzerland	Septic tank/household, sampled from overflow chamber	200L
Le1	Lebanon	Septic tank/household	10L
Le2	Lebanon	Septic tank/household	10L
Can	Canada	Septic tank/household, with kitchen and laundry water	40L
Mix	Ghana, Guatemala, Uganda	Mix from small samples from different countries (Septic tanks and pit latrines)	28L
BW	Zürich, Switzerland	Fresh blackwater from flush toilets from the NEST building of the EAWAG campus	50L

b. Dewatering and conditioning

Before any other pre-treatment step, each type of FS (except for the blackwater), was sieved through a 5 mm sieve.

Figure 13: Picture of the flocculation by the CP314 conditioner on BW

Two dewatering techniques and two conditioners were examined as pre-treatment of the faecal sludge to separate solids from liquid. As for the conditioner, a synthetic (Flonex CP314) and natural flocculant (Chitosan Heppix A) were compared. CP314 contains polyacrylamide and has a cross-linked structure (see Figure 13). It was diluted with tap water to a 0.5% stock solution and mixed for 2 hours. Chitosan was obtained from Biolog Heppe GmgH, Germany in a solid form. According to the manufacturer's directions, chitosan was dissolved in 1% acetic acid and distilled with water to a 0.5% (wt/vol.) stock solution. Shaw et al. (2022) identified optimal dosages for FS conditioning. For chitosan this was calculated to be 22 mL/L FS, and for CP314 37.5 mL/L FS. As this dosing was in the right range for all the used FS, this dosing was used for each FS. Furthermore, dosage was adjusted with visual observations.

Figure 14: Picture of the used fruit press for dewatering

Before the start of the experiments, a fruit press and a geotube were considered as dewatering technique. The fruit press (see Figure 14, from Royal Catering) can contain up to 3 L sludge. The conditioned FS is poured into a nylon mesh that is placed in the fruit press. The supernatant is captured and the solids cake remains in the cloth. The remaining liquid is pressed out by pressing down the handle of the fruit press when the cloth is full. A geotube acts in a similar way, as the conditioned FS is poured into the sack, the supernatant gets through the mesh, and the solids remain inside.

The turbidity and TS of the FS and the supernatant were compared to assess the dewatering efficiency of each pre-treatment combination and with this information, a decision was made which pre-treatment would be used for each FS used as influent for this research.

2. MBBR set-up
    a. General information

For this thesis, three 12 L reactors were available (Figure 15). These were filled up for 33% with biocarriers. Table 3 shows the characteristics of the carriers that were used for attached growth in the MBBR reactors. There was continuous homogenous mixing with a marine impeller. The filling ratio was 0.25 and influent was pumped into the reactor with a vacuum pump. Effluent was directed to the WWTP of Neugut, Dubendorf. O_2 could be added to the reactor by air sparging. The dissolved oxygen (DO) level was kept at 2.0 mg/L with an air flow (Qair) of 5000 mL/min. The temperature of the reactors was kept at a constant 25 degrees with a water jacket. The hydraulic retention time (HRT) = solids retention time (SRT) = 1.33 days.

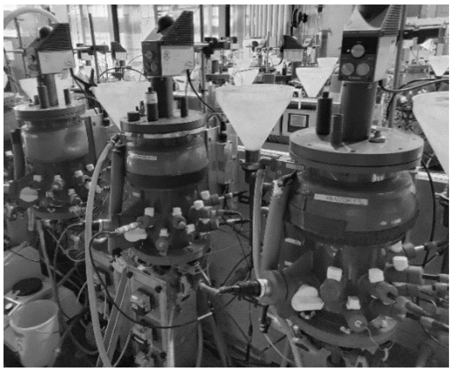


Figure 15: Picture of the 3 reactors in the Experimental Hall at EAWAG

Table 3: Overview information of the used biocarriers.

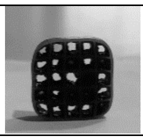
Shape	
Name	BWT15
Manufacturer	Biowater technology
Sampled from	WWTP Wildegg, Aargau, Switzerland
Surface/volume ratio	828 m^2/m^3
Size	15mm*15mm*5 mm
Weight	173 kg/ m^3

Figure 16: Picture of the used carriers in the MBBR reactor

    b. SBR cycle

Three MBBR reactors were operated in an SBR configuration with pre-denitrification, meaning it contains the following steps: Fill – Anoxic phase – Aerobic phase – Decant. A schematic overview of the cycle is given by Figure 17. There is no settling phase considered. First of all, the actual biofilm surface area in the MBBR reactor needs to be calculated. After that the removal rates for each process (nitrification-denitrification- COD removal) can be calculated, making assumptions from wastewater treatment. To calculate the aerobic part of the cycle, the times needed for full nitrification and COD removal were added up, assuming there is no COD removal in the anoxic phase. The assumption with this is that there will be an overestimation in the aerobic part.

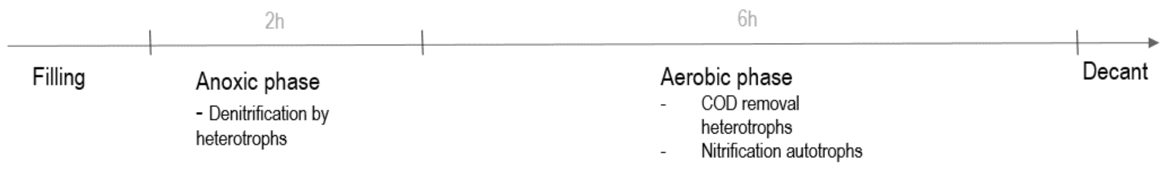


Figure 17: Overview of the different steps of the SBR cycle

Key process design parameters to be determined before are (1) the substrate removal flux, (2) the media specific surface area (m2 /m3), (3) the amount of media added to the activated sludge tank, which is also expressed in terms of the tank media bulk volume fill fraction or percent fill volume, (4) the aerobic tank DO concentration, and (5) the suspended growth MLSS or biomass concentration.

For the biofilm surface area, the surface per volume the reactor was calculated first, and then the correction factor of 0.75 was applied for the actual surface area per volume. According to the carrier manufacturer, the surface/volume ratio is 828 m^2/m^3, so with a 75% correction factor this is 621 m^2/m^3. With the reactor volume being 12 L, the surface area in the reactor is 2.46 m^2 (A in Formula 3).

For the substrate removal fluxes, some assumptions were made regarding supernatant composition and removal fluxes, as this is not known yet for supernatant, but is taken from conventional sewer-based wastewater treatment:

- The BOD/COD ratio is taken the same as for conventional wastewater, namely 0.625. (Metcalf and Eddy, 2013). g BOD * 1.6 to know the amount of COD.
- The removal flux of COD is taken as 6.4 g COD/m^2*d (Metcalf and Eddy, 2013)
- The removal flux of nitrification is taken as 0.6 g N/m^2*d (Metcalf and Eddy, 2013)
- The removal flux of denitrification is taken as 1 g N//m^2*d (Metcalf and Eddy, 2013)

The nitrification rate was calculated with the following formula (see Table 4):

$$A = \frac{Q(N_0 - N_e)}{J_N} \quad (3)$$ (Metcalf and Eddy, 2013)

Where A is the biofilm surface area (m^2) , N_0 the influent NH_4-N concentration going in the MBBR (g/m^3), N_e the desired effluent NH_4-N concentration coming out of the MBBR, taken as 10 mg/L. J_N is the nitrification removal flux, 0.6 g N/m^2*d, like presented in the assumptions. The denitrification rate was calculated with the same formula, but N_0 is the nitrate concentration after nitrification and N_e the desired effluent value for nitrate, taken as 10 mg/L. J_N is the denitrification removal flux, 1 g N/m^2*d, like presented in the assumptions. The COD removal rate is calculated in the same way. Table 4 presents the values used to calculate the different removal rates.

Table 4: Values taken for removal rate calculations.

Influent characteristics	Value
Ammonium (mg N/L)	95.32
Nitrate (after nitrification) (mg N/L)	85.32
COD (mg/L)	350.00
Desired effluent characteristics	
Ammonium (mg N/L)	10
Nitrate (mg N/L)	10
COD (mg/L)	50
Removal fluxes	
Nitrification rate (Ammonium removal rate) (m^3/d)	0.017
Denitrification rate (Nitrate removal rate) (m^3/d)	0.033
COD removal rate (m^3/d)	0.052

With this, the ratio of aerobic to anoxic volume is calculated, as would be calculated for a CSTR system (Metcalf and Eddy, 2013). This resulted in the anoxic phase lasting 2.2h, and the aerobic phase 6 h. the filling and decanting phases both last 10 minutes. This means that a cycle is about 8 h, resulting that 3 cycles can be performed per day.

c. Sensors

There were 5 different sensors used in-line in all three the reactors. They were all placed in the top part of the reactors. The DO sensor is the O2 Oximax from Endress and Hauser (O2 Oximax COS61D Endress Und Hauser, 2022). The temperature, ammonia and nitrate sensors are ion-selective electrodes from Endress and Hauser. (Temperature, Ammonia and Nitrate CAS 41D, 2022) The pH sensors are the type CPS11D from Endress and Hauser as they are suitable for rough applications: process glass for highly alkaline media and pressure-resistant up to 16 bar (PH CPS11D, 2022). All sensors were calibrated every month with a two-point calibration and reference samples were measured using Hach-Lange tests.

d. Start-up of the reactors

The three available MBBR reactors ran for 4 months on primary effluent (Prim Eff) to keep the biocarriers alive in an SBR configuration. This Prim Eff is sewage wastewater after primary settling, coming in the Experimental Hall of EAWAG. The cycle was adapted to the SW1 supernatant cycle calculated in b, when the feeding of the supernatant started. To start the supernatant feeding, a barrel of 60 L supernatant was attached to the influent. In this way, the reactors were gradually filled with supernatant per filling ratio of 25%. After 4 cycles the reactors were entirely filled with SW1 supernatant.

e. Sampling plan

Table 5 shows the sampling plan of the different phases of the reactor operation: start-up with SW1, Spiking experiments and the realistic scenario. Each sampling time a 50 mL sample was taken. 25 mL was filtered through a 0.40 µm filter and stored in a 4°C fridge.

Table 5: Sampling for 1 cycle in the first 10 days of reactor operation

Nr	Description	Start-up	Realistic scenario	Spiking
1	Influent supernatant	COD, NH_4	COD, NH_4	COD, NH_4
2	After filling	COD, NH_4	COD, NH_4	COD, NH_4
3	Anoxic 1 (after 33 minutes)	COD, NH_4		
4	Anoxic 2 (after 66min)	COD, NH_4		
5	Anoxic 3 (after 99min)	COD, NH_4		
6	Start aeration	COD, NH_4	COD, NH_4	COD, NH_4
7	Aerobic 1 (after 82mins)	COD, NH_4		
8	Aerobic 2 (after 165 mins)	COD, NH_4		COD, NH_4
9	Aerobic 3 (after 248mins)	COD, NH_4		
10	End aerobic (after 332 mins)	COD, NH_4		
11	Effluent	COD, NH_4	COD, NH_4	COD, NH_4

A sampling day during reactor operation goes as follows:

- Collection effluent last cycle of previous day
- Sample influent barrel for daily COD and ammonium values
- Sample in reactor after filling
- Sampling throughout the cycle according to Table 5
- Sample three biocarriers per reactor each day

3. Variability of influent tests

a. Kampala data for COD/N spikings in SW1

There was looked at real-life variability in existing COD/N ratios, using a case-study in Kampala, Uganda (Ward et al., 2019). Table 6 gives on overview which situation in Kampala corresponds with which ratio used in this thesis for the spiking experiments. Overall, the COD/N varies from 0.7/1 to 518/1. The different cases were taken randomly, but in view of enough variability in the COD/N ratios and different possible containments and regions. This simulates a quantities and qualities (Q&Q) survey of an urban area.

Table 6: Overview of the different real-life containments in Kampala from the case-study Ward et al., 2019, connected to the different ratios used in the COD/N spikings

	Containment	Source	Place	Solid waste?	COD (mg O_2/L)	NH4 (mg N/L)	COD/N ratio in FS	Glucose dosing (g)	NH_4Cl dosing (g)
3/1	Septic	School	Rubaga	Hygienic products	4451	1555	5/1	0.69	-
90/1	Pit	Multiple household	Nakawa	Food waste	45740	506.00	151/1	25.2	-
8/1	Septic	Commercial	Nakawa	No	4811	554	14/1	6.93	4.72
29/1	Septic	Household	Rubaga	Hygienic products	14470	489.33	50/1	7.49	-
23/1	Pit	Household	Kawempe	Hygienic products	13402	564.00	40/1	12.37	4.18

				and food waste					
518/1	Pit latrine	Multiple household	Rubaga	Hygienic products	57568.00	111.00	829/1	140.00	
4/1	Pit latrine	Multiple household	Nakawa	Hygienic products and food waste	10754.00	1518.00	6.40/1	2.91	
¼	-	-	-	-	-	-	-	-	0.999

The reduction factor for COD removal in supernatant is 60% on average after dewatering, according to Shaw et al., (Shaw et al., 2022). After dewatering, the remaining COD is mostly soluble. Therefore glucose was used as sCOD source for the spiking experiments. Ammonium concentration remains the same after dewatering. The ratio ¼ was not found in the Kampala case study, but this ratio shows the theoretical possibility that there is more urination, and thus the ammonium concentration is higher than the COD concentration. Glucose was used as sCOD source, NH_4Cl as NH_4 spiking. Required dosages were obtained via molecular weight calculations and are presented in Table 6. The glucose and NH_4Cl were added to 50mL lukewarm tap water, and added to the reactor at the start of the first cycle on the day of the specific spiking experiment.. The order of loadings for reactor 1 is chosen randomly and is as follows: (3/1)-(90/1)-(1/4)-(8/1). The order for reactor 2 is (29/1)-(23/1)-(518/1)-(4/1).

b. Lusaka data for salt spikings in SW1

Ward et al. quantified salts with monovalent divalent M/D cation ratio (Ward et al., 2019). In this study cations in different FS found in Lusaka, Zambia, were quantified to look at the performance of dewatering. From the characterization of these samples, the range of cations that is possible in supernatant was derived. As dewatering does not remove salts because they are dissolved in the liquid (Shaw et al., 2022), the concentrations in supernatant are assumed the same. The range that was found for M/D is from 1.9 to 25.3. This was assumed to be the same for the supernatants of those FS.

$$\frac{M}{D} = \frac{([Na^{+}] + [K^{+}])}{([Mg^{2+}] + [Ca^{2+}])} \quad (4)$$

First, the M/D ratio was calculated of the baseline SW1 according to Formula 4, using 14 samples from different batches of IC measurements, assuming salts concentrations do not change during storage or reactor operation. The concentration of monovalent salts in the baseline sludge is. As the ratio appeared to be 1.09/1, which is in the lower range of what is found in Lusaka, only higher ranges were used as spiking experiments. For additions of monovalent salts NaCl and KCl was used, for additions of divalent salts to the baseline concentrations, $MgCl_2$ and $CaCl_2$ was used. Table 7 shows an overview of the different spiking experiments performed regarding different salts concentrations in supernatant of faecal sludge. The ratios were trivially chosen within the range that could be derived from the Lusaka case-study. The amounts of salts mentioned in Table 7 were added to 50mL of 25 degrees deionized water, and this 50mL was added to the reactor when a cycle started. Then for the two other cycles that day regular baseline sludge was added, and the next day a new experiment was started.

Table 7: Overview different salts spikings used for variability testing of different monovalent-divalent cation ratios in supernatant of FS. 'Conc' means concentration.

Nr.	Reactor	Spiking concentration	Ratio	Added NaCl (g)	Added KCl (g)	Added $MgCl_2$ (g)	Added $CaCl_2$ (g)	End conc mono (mol/L)	End conc divalent (mmol/L)
1	2	6x mono	6/1	0.5938	3.7070	-	-	0.020	3.0
2	2	4x divalent	¼	-	-	2.400	0.6544	0.0033	12.0
3	2	11x monovalent	11/1	5.3274	1.3887	-	-	0.03659	3.0
4	1	2x monovalent, 2x divalent	1/1	0.9686	0.2525	0.3839	1.56	0.0066	6.0

The different spikings were prepared in 20 mL lukewarm tap water and added to the reactor at the start of the first cycle at the day of the experiment.

c. pH spikings of SW1

Two pH spikings were performed: one with pH 8.5 and one with pH 10. To obtain this pH in 3 L of influent, a 1M NaOH solution was made and slowly added to 3L of Swiss supernatant until the desired pH was met. The two bottles with different pH were afterwards connected to the influent pump of reactors 1 and 2 for a loading of 1 cycle.

d. Intermittency tests

Two reactors were started up with SW2 on 02/08/2022 at 17h. For 7 consecutive days the reactors ran on this sludge. 9/08/2022 the removal of COD and N was monitored by sampling the influent and the effluent of the last cycle before stopping the cycles of both the reactors. Then the filling of the reactor stopped for 8 days. Reactor 1 still had the SBR cycle going on, with active aeration during the aerobic phase. The cycle of reactor two was entirely stopped. After 8 days of intermittency the reactors were both again fed with SW2 and sampled throughout the cycle. Afterwards the difference in performance between aeration and no aeration was assessed.

4. Realistic scenario

After testing previous spikings and intermittency separately, there were 14 days of a realistic scenario with the existing FS. The FS was dewatered according to the chapter 'Conditioning and Dewatering'. Figure 14 shows the order of supernatants loaded to the MBBR reactor. This order was chosen randomly. The '-' represents 2 days of intermittency. Each day, a barrel of 9 L was attached to the reactor pumps, resulting in influent loadings for the 3 cycles of each day. The way of sampling plan was presented in 3.e.

Figure 18: Overview of which supernatants were added each day from the existing faecal sludges. The blue arrows with '-' show 2 days of intermittency.

5. Respirometry

Table 8 gives an overview of the experiments performed for respirometry. First some preliminary tests were performed to determine the ratio supernatant/activated sludge and appropriate DO-concentrations for the aeration and respiration chamber. For this, the first Swiss supernatant was used. After determining the right ratio activated sludge/supernatant, respirometry tests were performed on the available supernatants and some of their effluents after treatment in the MBBR reactor. R13 was a respirometry experiment to determine the oxygen uptake rate for endogenous respiration, specifically for this type of activated sludge. Due to time limitation, no respirometry experiments were performed of Leb 2 and SW2. The settings and different steps performed during the respirometer operation are shown in Appendix 14 and are based on (J. Zhang et al., 2021). For each experiment, a sample of the influent, after filling of the reactor and the effluent value was taken to determine the tCOD and sCOD values.

Table 8:Overview of the respirometry experiments performed

ID	Date	Experiment	Volume Activated Sludge	Volume supernatant	Nitrification inhibition?
R1	24/05/2022	1 g ammonium	2L	-	No
R2	31/05/2022	Influent SW1	1.5L	0.5L	Yes
R3	01/06/2022	Influent SW1	0.5L	1.5L	Yes
R4	14/06/2022	Influent SW1 1	1L	1L	Yes
R5	15/06/2022	Influent SW1 1	1L	1L	No
R6	20/07/2022	Influent Leb 1	1L	1L	Yes
R7	25/07/2022	Influent Leb 1	1L	1L	Yes
R8	26/07/2022	Effluent Leb 1	1L	1L	Yes
R9	27/07/2022	Influent Mix	1L	1L	Yes
R10	27/07/2022	Influent Mix	1L	1L	Yes

R11	28/07/2022	Influent Can	1L	1L	Yes
R12	28/07/2022	Influent BW	1L	1L	Yes
R13	29/07/2022	-	2L	-	No
R14	02/08/2022	Effluent Mix	1L	1L	Yes
R15	08/08/2022	Effluent BW	1L	1L	Yes

The sludge used for the respirometry tests was from tank 5 of the wastewater treatment plant on site at EAWAG. That reactor is used for conventional wastewater treatment (COD removal and nitrification). A nitrification inhibitor was added to most of the experiments, to inhibit nitrification and therefore only have O_2 usage of COD removal. Allyl-thiourea (ATU) reliably and completely inhibits AOB (Level, 1999). A final concentration was made of 10 mg/L. 1 mL/L in the aeration chamber should be dosed. As for every experiment the reactor was filled up until 2 L, 2 mL was dosed every experiment to the mixed liquid to suppress nitrification activity. Respiration rates were measured in a Plexiglas reactor with working volumes of 3L. During the OUR test of the sludge mixture, when nitrification is inhibited, the ideal OUR curve consists of three stages, namely, the rapidly biodegradable organic matter (rbCOD) degradation stage, slowly biodegradable organic matter (sbCOD) hydrolysis stage and activated sludge endogenous respiration stage, which can be obtained from the OUR experimental curve of R13 (see Table 8) (J. Zhang et al., 2021) .

6. Analysis
    a. Influent and sample characterization

Total COD, soluble COD were measured using commercial Hach Lange Test kits, using the closed reflux colorimetric method. The sCOD was the COD that passed through a 0.45 μm filter. The tCOD samples were homogenized first. Turbidity, pH, EC, TS, VS and TSS were analyzed according to standard methods. sCOD, tCOD, NH4-N were analyzed with Hach vials according to manufacturer's directions and standard methods. tCOD was done with Hach Lange on unfiltered samples, sCOD on the filtered samples. The ions Cl^-, K^+, Ca^{2+}, Mg^{2+}, Na^+ were analyzed for each sample with IC. TSS was determined according to the oven drying method of the Methods for Faecal Sludge Analysis (Velkushanova et al., 2021) . With Formula 5 the alkalinity was measured. V is the volume needed to decrease the pH to a value of 4.3 during titration with a strong acid.

$$Alkalinity\left(mmol\frac{HCO_3^-}{L}\right) = \frac{V * 0.01 * 1000}{volume\ sample\ (mL)}\ (5)$$

    b. Dry weight

The dry-weight mass was determined according to (Fonseca & Bassin, 2019). Three random biocarriers were sampled from reactor, were put in a 105 degree muffle oven and afterwards weighed.

    c. OUR calculations

With the DO sensordata, the OUR was calculated with the negative slope method with the following formula:

$$\frac{d[O_2]}{dt} = OUR\ (6)$$

As the DO-level was monitored to be between 2 and 3 mg/L, the DO-profile is a saw-tooth curve, from which the negative slopes are used to determine the OUR.

    d. Quality Assurance and Quality Control (QA/QC)

For COD, 10% of each batch were analyzed in triplicate, meaning every 10th sample. Triplicate determinations should be within 10% of their average COD value. The pH and EC sensors were calibrated each month. For TSS and turbidity, 10% of the samples was done in duplicate.

7. Feasibility in the field study

Online informative conversations were conducted with:

- Kapanda Kapanda, who operated a small-scale FSTP in Zambia;
- Ronald Sakaya, who is a plant manager of the Lubigi FSTP in Kampala, Uganda;
- Linda Strande and Nienke Andriessen, who are experts on FSM at EAWAG.

Through these informative discussions, the most important factors to consider before deciding to implement an MBBR are considered. In addition, a comparison was made with other techniques already in practice: Waste Stabilization Ponds, Vertical Flow constructed wetlands, and Anaerobic Baffled Reactors. This approach provides an answer to the second research question and is shown in the chapter 'Feasibility Study'.

Results and Discussion

This chapter is divided into four sections. First, the pre-treatment step is discussed, i.e., deciding which conditioning agent and physical dewatering technique to use for this work. Next, the influent characteristics of each supernatant used in this thesis for treatment with MBBR are described. This is followed by the results of the reactor operation. First, the experiment with one type of supernatant is highlighted to answer research question 1.1. This experiment can also be used to determine if adjustments need to be made to the SBR cycle for the next part of the experiments. Then, the community scale scenario will be applied to the MBBR. With this, the variability of the supernatant and the intermittency of the FS input will be tested, which will provide an answer to research question 1.2.

1. Pre-treatment

Table 9 shows the turbidity measurements of BW of the NEST building at EAWAG campus before and after the different conditioners and dewatering combinations were tested. Appendix 1 presents the TS measurements of the experiment, and Appendix 2 provides additional explanation and pictures of the NEST building and how sampling was performed.

Table 9: Turbidity measurements of BW per dewatering and conditioning combination used in this thesis.

Conditioner	Dewatering technique	Turbidity BW (NTU)
-	Before dewatering	174
Chitosan	Fruit press	81.8
Chitosan	Geotube	102.6
CP314	Fruit press	51.6
CP314	Geotube	47.9

Table 9 shows that for chitosan, the fruit press performed better and for CP314, both dewatering techniques performed similarly for BW on that particular day. It was decided to go with the CP314 synthetic conditioner with the fruit press to remove most of the turbidity. Since the fruit press and geotube performed similarly, reducing turbidity by 70% and 72.5% respectively, the technique that was easiest to use and most innovative, the fruit press, was chosen. pH and EC are factors that can influence conditioner efficacy and dosage (Kopp et al., 1998; Turovskiy et al., 2006). High pH (> 7.5) and high EC reduce the effectiveness of conditioners. This may have affected the performance of conditioning, as weaker flocs were produced, causing small particles to flow through the fruit press mesh, resulting in a minimum turbidity value of 47.9. The performance of conditioning and dewatering on the other FS used in this work is discussed in more detail in section 2 on Influent Characteristics.

2. Influent Characteristics

2.1 General

First, the characterisation regarding tCOD, sCOD and turbidity of the raw sludges before dewatering are presented in Table 10. Nitrate, ammonium, pH and EC were not measured for the raw sludges, as they are assumed the same as in the supernatant afterwards because they are dissolved.

Table 10: Raw sludge characteristics for the different FS used to dewater to obtain the supernatant.

Raw sludge	sCOD (mg O_2/L)	tCOD (mg O_2/L)	Turbidity (NTU)
SW1	181	1572	201
SW2	167	176	41
Le1	570	3104	>10000
Le2	270	2802	1645
Can	67	178	40
Mix	756	4522	>10000
BW	262	915	245

Table 11 shows the influent characteristics of all the FS supernatants after dewatering, used as influent for the MBBR reactor. 'Prim Eff' is the wastewater on which the reactors ran first before the actual experiments to grow the biofilm. This is the wastewater of the city of Dubendorf, after a primary settling step. This wastewater was not characterized during thesis, therefore additional information is provided in Appendix 3. SW1 is the supernatant that was used for research question 1.1 and the spiking experiments. SW2 was the supernatant used for intermittency tests, and all the other supernatants were used for the realistic scenario experiment. The COD/N ratio was calculated as the ratio tCOD concentration over ammonium concentration. Furthermore, the minimum and maximum values that are already reported by other literature are presented in Table 11 as well. For some of the supernatants the TSS measurements were not performed and for these supernatants the turbidity is used as a proxy for the solids in the supernatant, and as an indicator of the performance of conditioning and dewatering. The turbidity measurements are after the dewatering step, being carried out with CP314 and the fruit press.

Table 11: Compositions supernatant influents used for MBBR reactor operation. '-' indicates that these measurements were not performed. The '*' indicates that the value was not taken from the study, but from this thesis, as a lower or higher value was found during this research. '1' retrieved from (Shaw et al., 2022). '2' retrieved from (Strande, 2018).

ID	sCOD (mg/L)	tCOD (mg/L)	Turbidity (NTU)	TSS (mg/mL)	pH (-)	EC (ms/cm)	NH4 (mg N/L)	NO3 (mg N/L)	COD/N ratio (-)	Alkalinity (mmol HCO_3^- /L)
Minimum	5	15.6	2.02[1]	0.0035*	5.60[1]	0.11[1]	17.7*	-	1/1*	-
Maximum	-	43950[1]	631[1]	7.76[1]	8.60[1]	13.79[1]	291[1]	-	518/1[2]	-
Prim Eff	277	469	-	-	7.17	-	25.0	0.6	19/1	-
SW1	181	204	102	0.0072	7.29	2.55	95.3	<3.6	4/1	12.40
SW2	95	115	8	-	7.45	1.60	119.0	<3.6	1/1	16.44
Le1	218	409	51	-	7.69	3.71	116.0	<3.6	4/1	31.87
Le2	117	178	5	-	7.36	4.09	261.6	<3.6	1/1.5	32
Can	39	76	22	0.001	7.29	0.81	17.1	<3.6	7/1	7.13
Mix	641	1116	215	0.59	7.54	4.50	59.2	<3.6	19/1	36.85
BW	230	710	221	-	8.50	0.67	105.0	<3.6	9/1	7.4

Table 11 shows that the pH values are quite similar for each influent, except for BW. Other studies have measured that the pH of the supernatant varies between 5.6 and 8.6 (Shaw et al., 2022). A look at the nitrate data shows that the influents are predominantly anaerobic and have undetectable nitrate levels. Mix and BW turbidity data show that still a lot of solids are present in these supernatants. For the Mix, the dosage (based on visual observations) may have been incorrect because many different compositions were thrown together. This Mix also contained FS from a restaurant in Guatemala, which had a high fat content. Fats are difficult to dewater as they are in suspension and difficult to coagulate (Shaw et al., 2022). In addition, visual observations showed a high percentage of solids such as sand in this faecal sludge, which may affect dewatering performance because it is difficult to form flocs. However, looking at the tCOD values before and after dewatering for Mix, a tCOD reduction occurred of 75%, which is higher than the tCOD reduction percentage of 60% proposed by Shaw et al. (2022). Looking at the tCOD reductions in the other supernatants, Le 2 performs 94%, then SW1 and Le 1 both with 87%, all scoring higher than the 60% tCOD reduction proposed in literature (Shaw et al., 2022). High tCOD concentrations enhance flocculation and therefore dewatering. Can, SW2 and BW score lower with 57%, 35% and 22% respectively. Low tCOD concentration and high pH decrease dewatering performance However, the reason why dewatering efficiency is high or low with different supernatants remains a major research gap and beyond the scope of this study. Additionally it is an accurate approximation of reality, in which dewatering is not always performed perfectly.

It is important to note that these characterizations were performed prior to the start of each experiment. Throughout the experiments, the influent values of sCOD and tCOD decreased significantly in the influent due to the storage time. For example, the COD/N ratio of the SW1 supernatant decreased to 1.5/1-1/1. The variability due to storage time is explained in Appendix 8. Relating the COD/N ratios to the range of COD /N ratios expected in practice, the ratios are quite low overall. Can and BW have much lower alkalinity because the blackwater is urine-separated and Can is diluted with greywater. It is assumed that urine contains high alkalinity and high ammonium concentrations, while grey water would have low alkalinity. Using a ratio of the alkalinity and ammonium concentration of each influent, some assumptions can be made about how pH buffering will turn out for each influent (Metcalf and Eddy, 2013). A high alkalinity is favourable because this keeps the pH in the reactor more stable. A ratio of alkalinity to ammonium greater than 2 is required because 2 moles of alkalinity are consumed for each mole of ammonium that is nitrified (see Formula 2 in Context). Conclusions can be drawn by calculating the initial alkalinity to ammonium ratio. The pH is assumed to decrease throughout the cycle for SW1, Le2 and BW as these ratios are less than 2.

2.2 Respirometry for influent tCOD fractions

In addition to knowing the absolute values of tCOD and sCOD as presented in Table 11, it is important to know the biodegradable fraction of COD. For this purpose, respirometry was used as an analysis method. According to Mainardis et al. (2021), this is a useful technique for determining the tCOD fractions in wastewater (Mainardis et al., 2021). The graph in Appendix 4 shows the respirometry performed on 2 L of activated sludge. The OUR remains constant at about 11 mg O_2 l^{-1} h^{-1}. Since this experiment was conducted with 2 L of activated sludge, it can be concluded that 5.5 mg O_2 l^{-1} h^{-1} is required per liter of activated sludge for endogenous respiration. This value is used for all respirometry figures in this report. However, it should be noted that this value is prone to variation with fluctuating temperatures.

Figure 19 shows the respirometry curve of Le1. The different COD fractions calculated are parts of the tCOD. The slowly biodegradable tCOD was calculated to be 101.96 mg O_2/L and the readily biodegradable tCOD was calculated to be 6.77 mg O_2/L. The way the heights of the area for each fraction were chosen is an assumption based on the different slopes of the OUR plot. The transition from the steeper slope to the less steep slope is the height that separates readily biodegradable from slowly biodegradable. The sum, 108.73 mg O_2/L, is the biodegradable fraction. Endogenous respiration does not use organic matter as an electron donor (Moses & Syrett, 1955). However, this value is an underestimate when considering Hach Lange spectrophotometric data for the tCOD values of Le1 at the beginning of the respirometry experiment (161 mg O_2/L). This could be due to the fact that the experiment was not yet over because OUR was not yet at the level of endogenous respiration. Although the absolute data are not quite correct, the respirometry data can be used well to see how the fractions compare. This graph confirms the assumption that Le1 contains a lot of slowly biodegradable COD and little readily biodegradable. This was assumed because Le1 comes from a septic tank that is unlined, is not emptied frequently, and has been stored for a while.

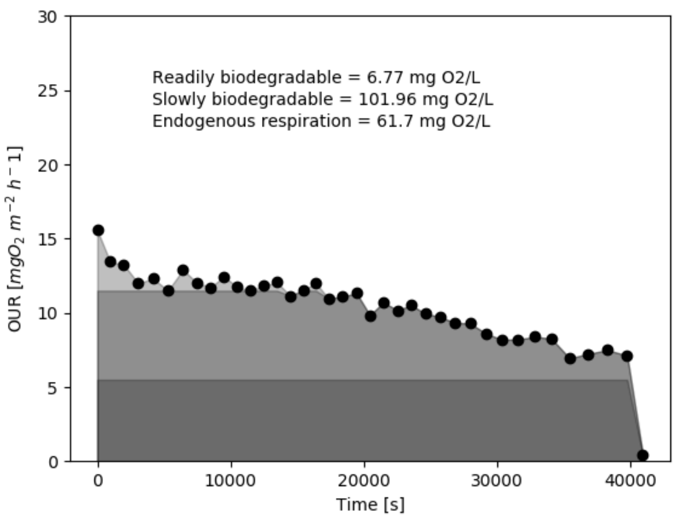


Figure 19: Respirometry graph 1 of Le1. The darkest grey shows endogenous respiration, lighter grey slowly biodegradable, and the lightest shows the readily biodegradable. The last value at zero is an outlier.

Figure 20 shows the respirometry graph of the influent of the Mix. The graph does not end on the endogenous respiration height of 5.5 mg O_2 l^{-1} h^{-1}, which indicates that the experiment was not measured long enough. The figure in Appendix 15 shows that the DO sensordata was not monitored well (seen from the steep decrease in DO during the respirometry experiment). This might again be the reason for the absolute values of tCOD through Hach Lange that are underestimated. However, this graph can again be used to estimate the readily/slowly biodegradable fraction of the tCOD for the Mix. According to Zhang et al. (2021), the accuracy of the respirometric evaluation is strongly dependent on the substrate/biomass ratio, which should therefore be further assessed (J. Zhang et al., 2021). There was assumed that the slowly biodegradable fraction would be high, for the same reasons as for Leb 1. Furthermore, it is assumed that the non-biodegradable fraction in the Mix is high. For this the effluent supernatant after MBBR has to be assessed. From this respirometry graph it would be possible to know the non-biodegradable fraction if full biodegradable COD removal by MBBR is assumed.

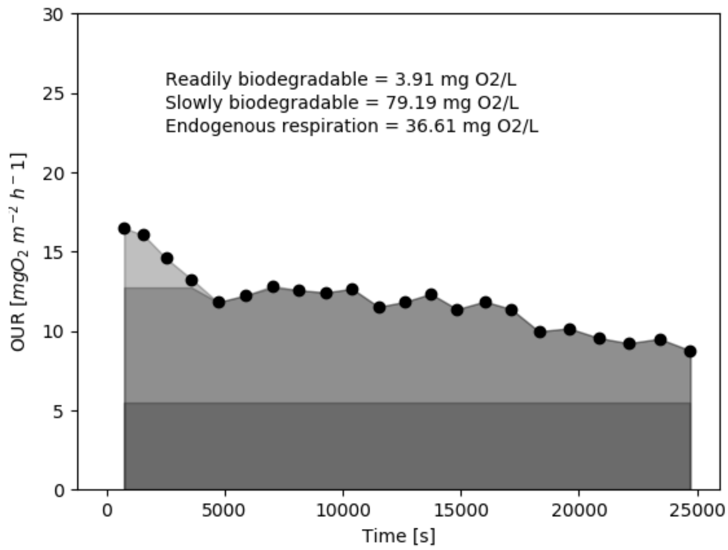


Figure 20: Respirometry graph of the influent of 'Mix'. The darkest grey shows the amount of oxygen used up for endogenous respiration, lighter grey the slowly biodegradable, and the lightest grey readily biodegradable fraction of COD

The respirometry graph of SW1 is shown in Figure 24 as part of the discussion about research question 1.1. It was not possible to plot the influent respirometry graphs of Can, BW and the duplicate of Mix, as the sensor data was disturbed. The registered O_2 concentration of those experiments are depicted in Appendix 15. This could be due to biofilm growth of the sensors during the experiments, or due to the sliminess of the activated sludge during the hot summer days.

As this was the first time that respirometry was performed on supernatant after dewatering of FS, a manual was written during this thesis and is shown in Appendix 14. An assessment on the analysis technique for supernatant COD fractionation and lessons learnt are shown in Appendix 15.

3. Running the MBBR on 1 type of supernatant

3.1 First two weeks of reactor operation on SW1

The reactors ran on Prim Eff for two months before the supernatant experiments began. The change from Prim Eff to SW1 was done from one cycle to the next, without a gradient change. Figure 21 shows the pH, ammonium and nitrate sensor data for the first 14 days of reactor operation. Complete nitrification occurs as ammonium levels drop to 0 after each aerobic cycle. A decrease in pH is observed during the cycles in the first fourteen days. The alkalinity in Prim Eff is more favorable than the alkalinity in the SW1, explaining the initial drop in pH. The alkalinity in SW1 supernatant is 12 mmol HCO_3^-/L, and the amount of ammonium to be removed is 7.14 mmol NH_4^+ , giving a ratio of alkalinity to ammonium of 1.68. The ratio required for complete nitrification without lowering pH in the reactor is 2 (Metcalf and Eddy, 2013). Even if alkalinity used for biomass production is not considered, there is not enough alkalinity to compensate for the pH reduction.

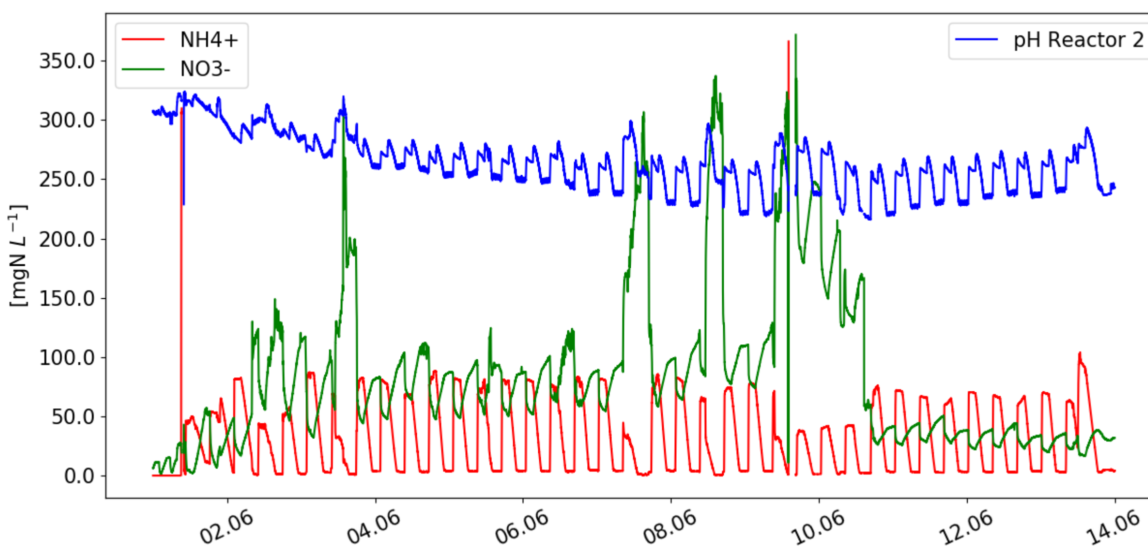


Figure 21: Sensordata for pH, nitrate and ammonium concentrations during the first 13 days of reactor operation, testing the SW1 baseline sludge.

Nitrate concentrations drop around 30 mg/L per cycle, as shown by data from IC in Appendix 8. According to the denitrification reaction (Metcalf & Eddy, 2013), 1 mole of biodegradable chemical oxygen demand ($C_{10}H_{19}O_3N$) is required to denitrify 10 moles of nitrate, which means that 0.048 mmol (97.39 mg/L) of bCOD is consumed per cycle for denitrification .

For SW1, the effluent value of sCOD is always around 25 mg/L (see Appendix 8). Based on this constant final value, it can be assumed that the non-biodegradable fraction of this SW1 supernatant is 25 mg/L, which is approximately 20% of the tCOD fraction sampled as influent from the barrel (also shown in Appendix 8). However, the tCOD levels are still too high to meet discharge standards, implying that a post-treatment step, such as gravity settling, is required. Looking at the IC data of the effluents from the different cycles for ammonium concentration (see Appendix 8), it is safe to assume that the Ugandan discharge standards are easily met as they are mostly below 2 mg/L.

When the non-biodegradable fraction of 25 mg/L and the amount of bCOD used for denitrification are added together, it can be seen that there is not much bCOD left in the aerobic phase for heterotrophic removal or a chance for combined nitrification and denitrification. Looking at the profile of sCOD concentrations in the cycles where the reactor has steady removal in Figure 22, it can be seen that most of the soluble COD is consumed at the end of the anoxic phase. This means that a large portion of the sCOD is readily available as it can be degraded within 2 hours. Only a small decrease in sCOD concentration is observed in the aerobic phase. Indeed, in conventional wastewater treatment as well, special attention has to be brought to the availability and use of the easily biodegradable substate when the COD/N ratio in the wastewater is low, according to a study by Broch in SBR process control (Broch, 2008).

Since the tCOD concentration in the reactor decreases rapidly, there is no chance for heterotrophic overgrowth (Morgenroth, 2008). According to Morgenroth (2008), heterotrophic and autotrophic bacteria can coexist only at COD concentrations lower than 30 mg/L in the bulk phase. Since the tCOD concentration is only higher for a maximum of 2 hours, there is no risk for this. Bulk phase COD concentrations greater than 30 mg/L can be assumed to be oxygen-limited heterotrophic biofilm with no oxygen available for autotrophic growth below the heterotrophic layer. Thus, the coexistence of heterotrophic and autotrophic bacteria is only possible if the oxidation of the organic substrate is COD and not oxygen limited. This means that the bulk oxygen concentration must always be high enough, which is the case in this experiment. Since a large fraction of the tCOD is readily available, this is the case for SW1 (Morgenroth, 2008). The rapid decrease of the sCOD value can also be explained by the respirometry curve of the SW1 supernatant (see Figure 24).

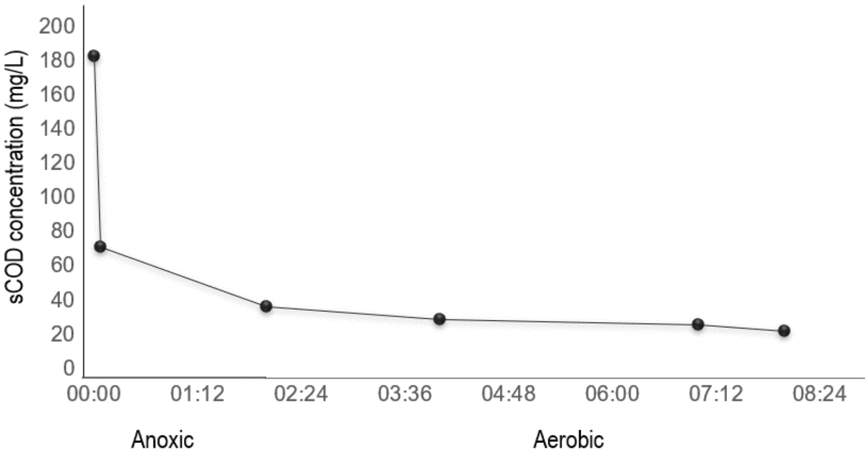


Figure 22: A typical sCOD concentration profile from one SBR cycle while steady removal in an MBBR.

Figure 23 shows the pH profile of 1 cycle after 4 days of MBBR operation on SW1 during steady removal. The pH increases twice because of the filling phase and because of CO_2 stripping when aeration starts (black arrows). This is according to the assumption. The pH decreases twice during the whole cycle. First, a drop in pH is seen in the aerobic phase due to nitrification (see equation 2). Oxidation of ammonia lowers pH in wastewaters where alkalinity is limited relative to total ammonia. A drop in pH is also observed during the anoxic phase (grey arrows), which additionally contributes to the overall drop in pH during each cycle. This is inconsistent with the hypothesis that pH increases during the anoxic phase due to denitrification. This could be because of multiple reasons (or a combination of them):

- Because of an artifact of the sensors since the sensor was placed at the top of the reactor and with the slower mixing in the anoxic phase, there could be an error in the pH measurements due to mixing.
- Another reason for the pH drop in the anoxic phase could be that nitrification was occurring at the surface of the reactor. This can also be inferred from the data from IC (about 5 mg/L ammonia removal per cycle). However, this ammonium decrease could also be due to ammonium adsorption and biofilm growth.
- Due to the fact that there is no active aeration during that phase, CO_2 can dissolve in the bulk liquid. This is according to the following reaction:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \quad (5)$$

  During denitrification, 5 moles of CO_2 are produced during the reduction of 10 moles of nitrate (see reaction 1). This means that 0.24 mmol of CO_2 are produced for SW1. Double the amount of H^+ will cause a decrease in pH (see reaction 5), which cannot be buffered as the alkalinity is not high enough for SW1 and not enough alkalinity is produced during denitrification.

In parallel to the pH drop, the total nitrite (NO_2^- and HNO_2) is assumed to have shown an increase, as NOBs are inhibited at a lower pH (Fumasoli et al., 2015) . However this cannot be seen in IC data, as the nitrite ion is quite unstable and the samples were stored for 24 hours prior to analysis.

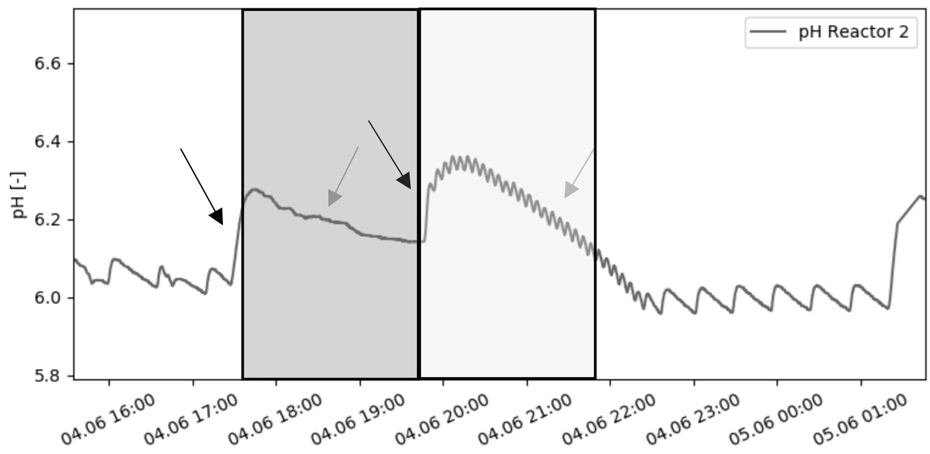


Figure 23: Course of pH in 1 SBR cycle, with SW1 as influent. The black arrows show the increase in pH, the grey arrows show the decreases in pH both in the anoxic (dark grey box) and the aerobic phase (light grey box).

Figure 24 shows the respirometry data for SW1. A distinction was made between readily, and slowly biodegradable COD and the amount of oxygen used for endogenous respiration. Since 0.5 L of activated sludge was added to 1.5 L of SW1 for this experiment, the OUR is 5.5 mg O_2 L-1 h-1. Endogenous respiration does not use organic matter as an electron donor, so it is not included in the calculation of tCOD (Moses & Syrett, 1955). The amount of mg O_2/L per section was calculated by hand (see Appendix 10 for the calculation). The amount of oxygen used for endogenous respiration is 86.6 mg O_2/L of activated sludge. The amount of slowly biodegradable tCOD is 40 mg O_2/L. The amount of readily available tCOD is 22.73 mg O_2/L supernatant. As mentioned earlier, the non-biodegradable fraction varies between 20-30 mg O_2/L. Adding all these values gives a tCOD value of about 93 mg O_2/L, which is an underestimate of the tCOD values measured at the inflow at the beginning of each cycle using Hach Lange spectrophotometry. These values were 192.37 ± 62.87 mg O_2/L. The high variability in this value can be explained by processes going on during storage in the barrels, namely the degradation of organic matter and volatilization.

The ratio of bCOD to tCOD in SW1 is determined in the following calculation. The average of the non-biodegradable tCOD during the different cycles was taken (25 mg/L, see Appendix 8 and Figure 22).

$$\frac{63\frac{mg}{L}}{25\frac{mg}{L}+63\frac{mg}{L}} = 0.72$$

This is higher than the estimated 0.6 ratio taken from Metcalf and Eddy for conventional wastewater.

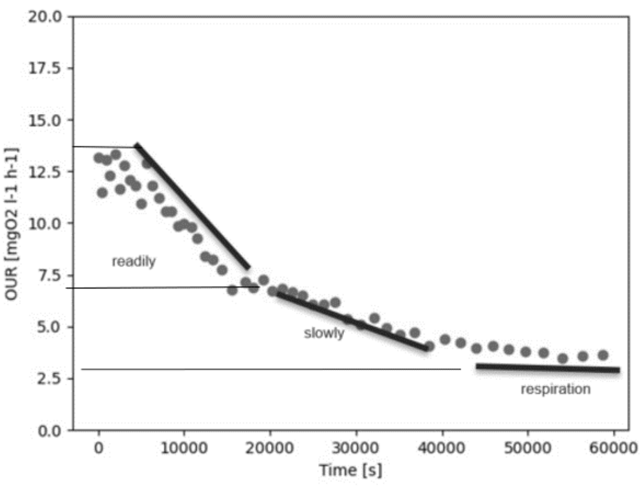


Figure 24: Respirometry graph for the influent of SW1. The horizontal lines show where the cut-off was for the calculation of the different areas for tCOD fractions. The thick black lines show the change in slopes.

TSS measurements taken over 2 cycles while the reactor was running with steady removals show an increase in TSS concentration and an increase in tCOD (see Figure 25). The TSS values shown in Figure 25 can be found in Appendix 7. During the first cycle there is a 0.25 mg/ml increase in TSS and in the second cycle there is a 0.19 mg/ml increase. There is also a steep increase in TSS during the transition from the anoxic phase to the aerobic phase. The most likely reason for this steep increase in TSS is the additional mixing that occurs due to aeration. The tCOD increase suggests that a post-treatment step such as secondary settling is necessary to achieve discharge standards. This tCOD increase may be due in part to biofilm detachment. However, this does not necessarily mean an unhealthy environment, as healthy biofilms also grow and detach, which is known as biofilm sloughing (Sorensen & Morgenroth, 2020). Next to this, no significant change in dry weight mass was seen during the SW1 experiments (see Appendix 5).

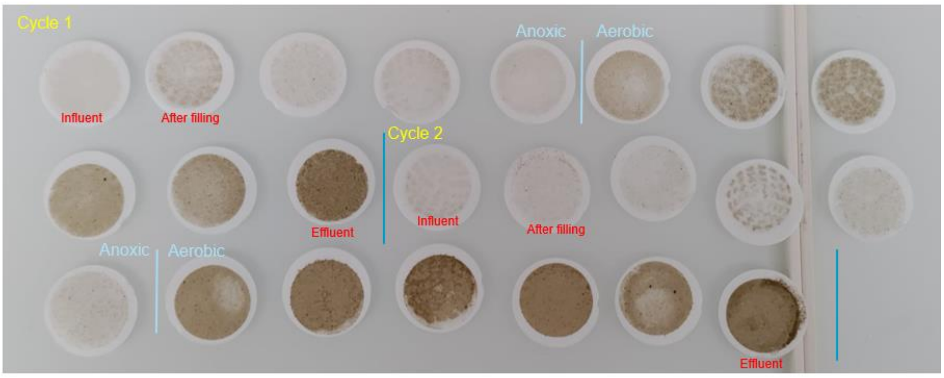


Figure 25: TSS increase in 2 cycles when the reactor shows steady removals. There should be looked at this from left to right, top to bottom.

3.2 Gained knowledge on the SBR cycle

Research question 1.1 was not only about finding out if the process does not fail during the operation of the MBBR when it runs on one type of supernatant, but also about gaining knowledge about the accuracy of the length of the SBR cycle and which removal processes take place in which phase of the cycle. With this information, cycle optimizations can be made. In Materials and Methods it is shown that the SBR cycle was calculated according to conventional wastewater treatment assumptions. As mentioned in 3.1, the bCOD/tCOD ratio for SW1 is different from conventional wastewater treatment, 0.72 instead of 0.6. As a result, the cycle calculations for removal of COD were underestimated, because more COD can be biologically degraded than expected. More COD can be biologically removed and used for denitrification than originally assumed. However, all the bCOD was removed during this experiment, so this did not pose a problem. In the rest of the chapter, each part of the cycle is discussed on the length and assumptions on the processes going on during that part.

Anoxic phase

No complete denitrification was observed in the anoxic phase. This resulted in an overall nitrate increase over the days. To achieve full denitrification, the anoxic phase would have to be prolonged to utilize more slowly biodegradable COD. However, it must then be weighed whether the SBR cycle should be longer and thus less efficient to remove more nitrate and produce more alkalinity. Often there are no discharge requirements for nitrate, as this is the fully oxidized and therefore least environmentally damaging form of nitrogen. Next, as mentioned earlier, there is an assumption that combined surface nitrification and denitrification occurs in the anoxic phase, as oxygen is still dissolved at the surface of the reactor. Nitrification in the anoxic phase would also be limited by diffusion of oxygen in the biofilm (see Figure 9).This could increase the efficiency of the SBR cycle.

Aerobic phase

The data in Figure 26 shows the OUR profile of the aerobic phase during steady removals of SW1. The graph shows that OUR per square meter of biofilm is about 20 mg O_2 m^{-2} h^{-1} for endogenous respiration and about 150 mg O_2 m^{-2} h^{-1} for nitrification. This means that ammonium reaches zero 2 hours before the end of the cycle for each cycle. At the same time, OUR drops rapidly, indicating a change from nitrification to endogenous respiration. This implies that only endogenous respiration occurs in the remaining two OURs, which is not necessary to achieve the treatment goals. Consequently, the cycle length could be shortened by 2 hours to save space and energy. However, it was decided not to shorten the cycle to counteract future high COD loadings from other supernatants.

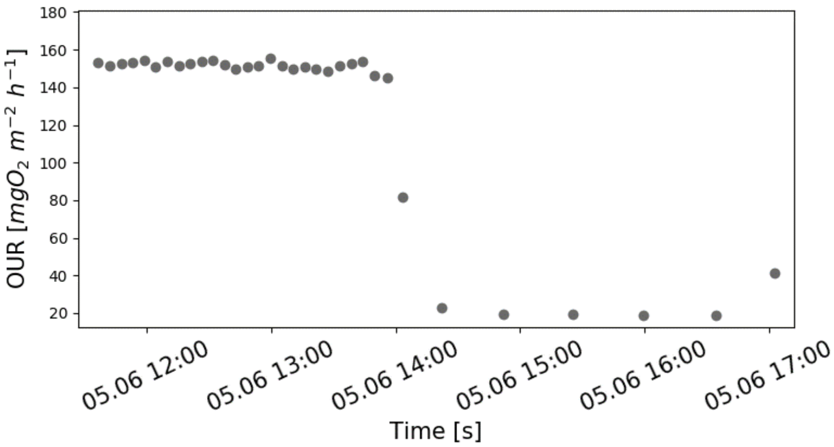


Figure 26: oxygen uptake rate in the aerobic phase of an SBR cycle on 5/06/2022

A second point to check in the cycle changes was whether combined nitrification by the attached growth and denitrification by the suspended biomass occurred in the aerobic phase. This could not be seen. This is because COD was so readily available that there was no bCOD left in the aerobic phase to achieve this combined removal. To achieve further denitrification in the aerobic phase, more readily available bCOD must be available in the aerobic phase (Sin et al., 2020). Further experiments in which the cycle begins directly with an aerobic phase are needed to investigate whether combined nitrification and denitrification could increase the overall performance of the treatment and aerobic denitrification is at all possible for MBBR treatment of supernatant. In literature, oxygen tolerant aerobic denitrification was the key biological mechanisms found for the ammonium removal in MBBR reactors by Janka et al. (Janka et al., 2022).

In addition, the place of nitrification in the MBBR reactor will be described in the next section.

3.3 Place of nitrification in the MBBR reactor

An additional experiment was performed to verify whether the bacteria perform the nitrification on the carriers or in the suspended biomass. For this, the respirometry set-up was used, in which 1 L of supernatant SW1 and 1 L effluent of the MBBR which contained suspended biomass was mixed and aerated for 4 hours in the respirometer reactor. The same aeration settings were used as for the respirometry experiments described in Materials and Methods. Before and after the experiment, the ammonium was measured through IC. There was an ammonium concentration of 48 mg N/L present in the reactor, which remained the same after the aeration time of 4 hours. From this, it can be assumed that no nitrifiers are present in the suspended solids. However, the same experiment should have been performed with 1 L of SW1 and only the biocarriers to positively control the previous experiment. There could also be an issue with the set-up of the respirometry experiments, causing no nitrification. According to Reboleiro-Rivas et al., both suspended biomass and attached biofilm can contribute to the N-removal process. In their experiments on municipal sewage treatment, they combined different operational parameters and assessed the abundance of different bacterial groups. This research showed that under all the operating conditions tested, the abundance of the targeted bacterial groups were fairly similar in both suspended and attached fractions, and the biofilm contributed to more than 20% of the nitrifiers (Reboleiro-Rivas et al., 2015). Next to that, batch tests in Bassin et al. have shown that most of the nitrification of the suspended biomass to the overall biomass was very significant (Bassin et al., 2016). Both studies contradict the findings in this thesis.

However, Reboleiro-Rivas et al. showed the importance of attached growth to enhance the efficiency of N-removal. The biofilm on the carriers is suitable for simultaneous nitrification-denitrification because of oxygen diffusion through the biofilm and can maintain an aerobic environment inside and outside of biofilm and the growth of suspended biomass (Janka et al., 2022) In the attached biofilm phase, the nitrifying organisms are immobilized as part of the biofilm. Thus their growth rates become uncoupled to the SRT in the system. This is a crucial feature, as the SRT required for the optimal development of nitrifying bacteria in suspended biomass is high. In addition, nitrifiers, particularly ammonium oxidizing bacteria (AOB), are highly sensitive to operational parameters. These include low temperature, extreme pH, low DO concentrations and toxic compounds. Attached growth, therefore, increase the robustness of the treatment. Therefore the filling ratio of the carriers substantially influences nitrification performance at a constant organic loading rate. This will be further elaborated in the Chapter 4 on COD/N spikings.

3.4 Performance of the MBBR on COD and N removal in supernatant in comparison to other studies

In summary, the following can be said about the MBBR treatment of SW1: complete bCOD removal; complete nitrification; incomplete denitrification of about 30%. These removal efficiencies can be compared with the literature on MBBR operation for other wastewaters. The organic loading rate calculation of this experiment shows that this reactor was able to treat 0.3 kg COD / m^3/day. However, this could be much higher as can be seen from Figure 22 that most of COD was gone after only two hours. If the entire 8 hours of the cycle were used, 1.2 kg COD /m^3/day could be treated with this MBBR. Ødegaard (2006) reported that the surface organic load should not exceed 65-85 g tCOD/ m^2/d for effluents from high load systems (Ødegaard, 2006). In this thesis the surface organic loading rate is 62.5 g tCOD/m^2/d, which does not exceed this value. Chapter 4 tests whether tCOD levels can be higher than this value. According to Bassin et al, MBBRs can handle high organic loading rates of up to 3.2 kg bCOD / m^3/d, and still achieve complete ammonium removal. The COD was gradually increased, resulting in thicker biofilm and improved surface detachment rates (Bassin et al., 2016). In this study, the amount of suspended solids in the bulk also increased significantly during treatment.

Janka et al. studied the simultaneous treatment of COD and ammonium in an MBBR, but for the treatment of domestic wastewater. The main objective of this study was to use two pilot-scale MBBRs in the main wastewater treatment plant stream for simultaneous removal of C and N. The MBBRs were used in the main wastewater treatment plant stream. The biofilm carriers used had a surface area of 650 m^2/ m^3 with a fill ratio of 60%, which is twice that of the setup in this work, but the surface area-to-volume ratio is 20% smaller. The results show that the combined ammonia removal efficiency in both reactors was 65.9%. (Janka et al., 2022). This aerobic denitrification was not observed in this work because there was not enough COD in the aerobic phase. However, 100% ammonium removal was observed. Lopez-Lopez et al. conducted a study on the effect of fill level and carrier type on COD removal in an MBBR for municipal wastewater. In this study, a comparable fill level (35%) but a quarter of the HRT (7 hours) was used. Only COD removal was targeted and a maximum removal efficiency of 78.4% was achieved (Lopez-Lopez et al., 2012). It can be concluded that the treatment of SW1 for COD and N removal was more efficient than reported in literature. However, the system was underloaded and can presumably handle higher organic loading rates in a more efficient operational way. This will be tested in the next chapter.

4. Influence of variability in supernatant regarding COD/N, salts and pH on MBBR reactor operation

Supernatant after dewatering of FS behaves differently than conventional wastewater, as the FS of which it is derived is so variable. There is not one reference supernatant that is appropriate to serve as a proxy for all supernatants. Hence this emerging research topic cannot be approached by just testing the MBBR out on one supernatant, as the variability needs to be assessed (here SW1, previous chapter).

In the next chapters, emphasis is put on the possible influent variability of the supernatant regarding COD/N ratio, pH and salts concentration on the removal rates of the MBBR reactor, as literature review showed that variability is most risky in these characteristics when treating COD and N biologically. Additionally, the influence of intermittency is discussed. Furthermore, there is a separate part about the treatment of supernatant after dewatering of fresh blackwater. This is because it is assumed that this influent is fresh, more difficult to dewater, and is less stabilized than FS coming from containments. Lastly, two weeks realistic reactor operation was mimicked, approaching the 5000 people community-scale scenario, using supernatants from different FS from different countries, with an intermittency over the weekend.

An overview of all the experiments with their respective removal efficiencies regarding COD and N is shown in the table in Appendix 11. There, a comparison is made between the different experiments performed in this thesis.

4.1 Influence of different COD/N ratios on pH and performance of the MBBR system

In two MBBR reactors, a different COD /N ratio was spiked in SW1 each day for 4 days as mentioned in Materials and Methods. Figure 27 shows the pH sensor data from the start-up of the second reactor with SW1 (from 06/13 to 06/20), followed by 3 experiments in which different COD/N ratios were added to the baseline SW1. The same drop in pH is observed as in the first reactor during start-up (see previous section), with a drop to pH 6. Thereafter, the pH drop was remedied by adding the higher COD/N ratios. With a higher COD /N ratio, denitrification can be performed more thoroughly, causing the pH to rise again (Broch, 2008). During the spiking of 23/1 and 29/1, pH was increased and did not cause a decrease in heterotrophic COD removal and nitrification (see table in Appendix 10). However, effluent standards were not met because the cycle was not adapted to the high COD loadings. However, Rusten et al. installed two MBBR units to treat dairy wastewater. This is similar to the COD/N spiking tests in this thesis, since dairy wastewater has a high COD loading. The two MBBRs were successfully operated at full scale, with 87% tCOD removal in the first MBBR and 95% removal in the second unit. An additional chemical unit resulted in 99% removal of tCOD content from the wastewater (Rusten et al., 1992). A first solution to cope with a high COD /N load is therefore introduced, namely to connect several MBBRs in series when high COD are expected.

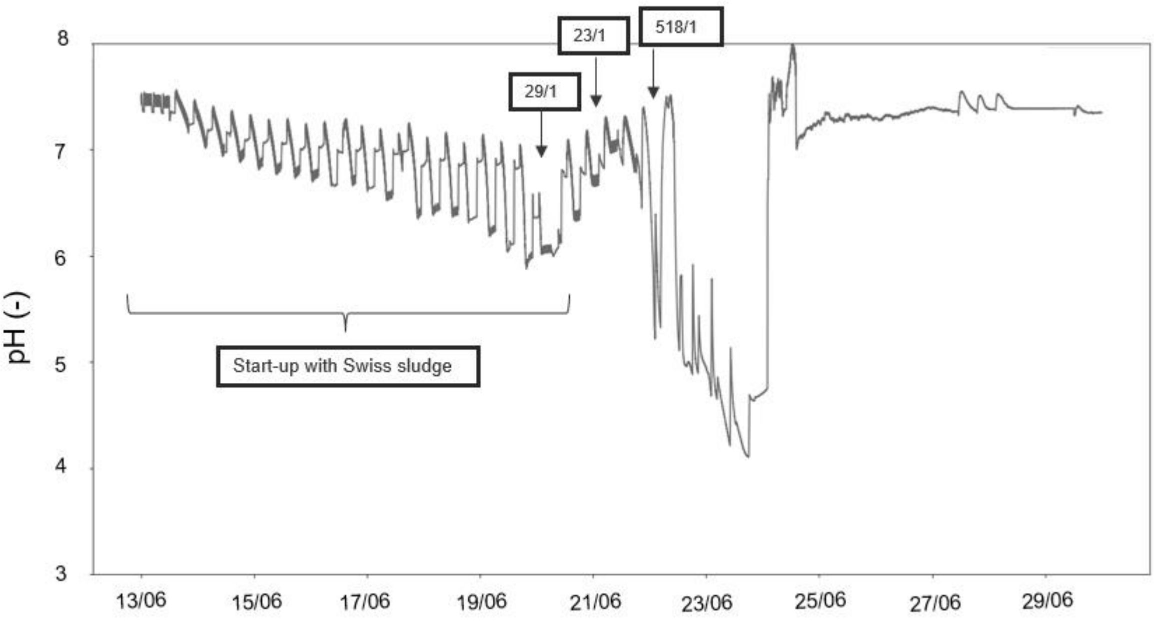


Figure 27: Influence of the different COD/N ratio experiments to the pH in the MBBR reactor.

By adding the extreme value of 518/1, the pH decreased to 4 and the reactor began to foam, as shown in Figure 28 on the right. At this moment, the microbial community in the suspended biomass was considered, as the sudden foaming could have been a consequence of extra polymeric substances (EPS) growth of the microbial community by filamentous bacteria (Campo et al., 2017). However, this is not observed in the microscopic image in Figure 28, as no filamentous bacteria are visible in this image. The addition of this large amount of readily available COD (sCOD in the form of glucose) created an anaerobic environment in the reactor during the anoxic phase and stopped the activity of the microorganisms, resulting in the flat line in the pH graph from 24/06. From this, conclusions can also be drawn regarding a limit to the variability of pH, namely that the reactor performance is irreversibly damaged when the reactor reaches a pH of 4. According to Lund et al., a deviation of pH from the optimal pH reduces bacterial activity according to the mechanism of non-competitive inhibition (Lund et al., 2020). Therefore, it is important to monitor the pH in the MBBR as a control parameter for the reactor performance. and see how different influent parameters affect the pH.

When the pH in the anoxic phase dropped to 4, this was the start pH for the aerobic phase. During the aerobic phase, the activity of ammonia-oxidizing bacteria (AOBs) decreases with pH and often stops altogether in slightly acidic wastewaters (Fumasoli et al., 2017). The nitrification rate stops below a pH of 6, as shown in the graph in Appendix 4. There are several reasons for this: Limitation by free ammonia (NH_3), inhibition by nitrous acid (HNO_2), limitation by inorganic carbon, or direct effect of high proton concentrations (Fumasoli et al., 2015).

Figure 28: Left: Picture of the microscopic view of the microorganisms in suspended biomass during foaming. Right: picture of the foaming reactor after adding the COD/N ratio of 518/1

AOBs release 2 moles of protons per mole of ammonia that is oxidized to nitrite. If the buffer capacity in the bulk solution is low, biological ammonia oxidation causes a substantial pH drop, which in turn affects the rate of ammonia oxidation. In wastewater treatment, ammonia oxidation decreases with pH and usually stops when the pH value drops below pH 6 (Fumasoli et al., 2015). Acidotolerant AOB are AOB that are able to thrive at pH below 5.5 and with low ammonium concentration (Schielke-Jenni et al., 2015). Looking at the graph in Appendix 6, it appears that at this pH, the nitrification is at 10% efficiency. This cannot be seen from the IC data for ammonium in this thesis, as still full removals are observed. This raises the assumption that the nitrifiers in this system have grown acidotolerant. This could mean that low pH supernatant could be treated safely. Nitrite sensors would simplify the observation of the AOB and NOB activity, as nitrite buildup would have been observed. However, reliable nitrite sensors still have to be developed (Britschgi et al., 2020; Fumasoli et al., 2017). Further research should be performed to understand the inhibition of AOB and NOB under low pH and monitoring for NO_2^- by IC analysis during these experiments is proposed.

The COD/N ratios 2.6/1 and 90/1 were both tested as well in reactor 1, before adding the ¼ ratio. The removal efficiencies of the different spiking experiments are shown in the overview table Appendix 11. Both glucose additions did not seem to cause any problems for the microorganisms, however in 1 cycle the discharge standards were not met for the 90/1 addition (effluent values of 423 mg O_2/L sCOD and 635 mg O_2/L tCOD, see Appendix 10), with the least strict discharge standard being 100 mg O_2/L for Lebanon. This created the opportunity to remove the ammonium from the ¼ ammonium addition the next day entirely, as there was still a lot of readily available sCOD (namely the glucose) available in the reactor. In reality, the sCOD will also contain a fraction of slowly biodegradable sCOD. This means that an 'ideal' COD scenario was tested, and in reality COD removal and therefore denitrification will be slower. Earlier Rusten et al. obtained around 85% COD removal from dairy wastewater at a short HRT of 7 h which is only 25% of the HRT used in this thesis. With optimized process design a total of 95% COD removal could be achieved in a pilot plant consisting of two MBBRs in series (Rusten et al., 1992a). Santos et al. treated dairy wastewater to address the influence of organic loading rate, filling ratio and hydraulic retention time. It shows that For high COD loaded wastewater, the MBBR is more stable when a biocarrier filling ratio of 40% is used, which decreases the reduction time, reaching a COD removal of 95%, also allowing a reduction on energy consumption (Santos et al., 2020). Therefore a second solution for high COD loadings is proposed, in places where the organic loading rate expected for treatment is high, the filling ratio should be increased for better removals.

Conversely, there is also the scenario that there is more ammonium than COD present in the supernatant and the COD/N ratio is therefore really low. This occurs when there is urinals connected to a septic tank. In the design of systems where alternating nitrification and denitrification are used, a sudden high load of ammonia in the wastewater can cause a self-destruction of the system, because of the high H^+ concentration developed during nitrification (Metcalf & Eddy, 2013). The denitrification will not occur because of the decreased pH, as the denitrifying organisms cannot denitrify under a low pH condition, as at a pH of 6 the nitrification rate is only at 10% (See Appendix 6). This has been tested once with a ¼ ratio COD/N. A further explanation on this experiment is provided in Appendix 13. There it can be seen that the high ammonium concentration decreases the pH substantially from 6.8 to 6. However if this does not happen for several days in a row, the attached growth system is robust enough to counter for this drop.

As already assumed in the previous chapter, the MBBR can indeed handle higher loadings of COD/N. Bassin et al. proposed 3.2 kg COD/m^3/d as an organic loading limit (Bassin et al., 2016). However, no problems were seen in the operation of the 90/1 COD/N, which translates in a organic loading of 7.9 kg kg COD/m^3/d that is feasible for an MBBR. Adapting operational parameters could even result in reaching discharge standards for these high COD loaded wastewaters.

4.2 Influence of different monovalent/divalent salts ratio on the performance of the MBBR system

The assumption is that more monovalent salts break bridges within the biofilm by replacing divalent salts, and therefore affect biofilm performance, reducing COD and N removal (Ward et al., 2019). To test this assumption, different combinations of higher monovalent and divalent salts were tested (see Materials and Methods). Looking at the removal efficiency data in Table 14 in Appendix 11, it can be seen that the sCOD removal shows a small decrease in efficiency from 85% in the baseline SW1 experiments to 82% for the 6x monovalents salts additions. After that the efficiency remains around 75% for the other experiments. The OUR value in the reactor was calculated during cycles of salt addition to verify biofilm performance. The OUR value indicates how fast oxygen is consumed in the reactor, thus indirectly how fast the COD and N removal proceeds. In the SW1 experiments, the OUR values were determined for nitrification and endogenous respiration without spiking. If the OUR remains at the same value as in the experiments before, performance will not be affected. Figure 29 shows the OUR during the salt addition experiments. The third cycle of the first day of the experiment remains in the lower range because there is no filling. Comparing the profiles of OUR of the cycles in which salts are added to the OUR profile from research question 1.1, it can be seen that the OUR remains in the higher range of 110 mg O_2 m^{-1} h^{-1} for longer.

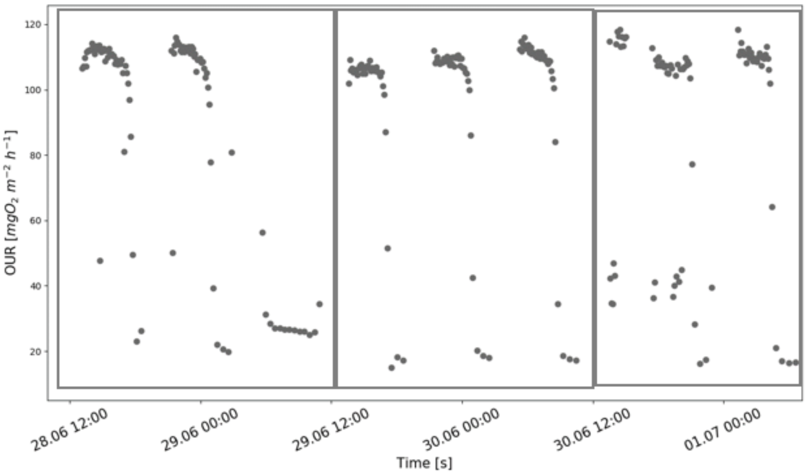


Figure 29: OUR values in reactor 2, during the different salts additions. One rectangle represents 3 a day of 3 cycles. There was no influent loading in the third cycle of day 1 and therefore the OUR stays at endogenous respiration.

Measuring TSS in the effluent and the dry weight of biofilm carriers is a method to determine biofilm detachment. During the experiment with the addition of salts, neither of these indicators changed significantly. Only when 11 times the amount of monovalent salts was added was there an increase from 0.035 mg/mL to 0.045 mg/mL, but this may also be due to growth of the suspended biomass or biofilm sloughing. Therefore, it can be concluded that the addition of salts had no effect on the biofilm.

This is not consistent with the hypothesis, which indicates that the efficiency of attached growth processes decreases when higher salt concentrations are added. Saline wastewaters are commonly encountered in various industries and present challenges for biological treatment. Therefore, extensive research has been conducted on the performance of attached growth systems in high salinity wastewaters. Not much research has been done on MBBR specifically, but there has been done research on the effect of salts on aerobic granular sludge. Aerobic granular sludge is also capable of removing organic carbon and nitrogen in a single process unit. The bacteria are embedded in a matrix of EPS and can therefore be compared to the biofilm on the carriers. According to Sivasubramanian et al. (2021), the efficiency of granular sludge decreases under high salinity conditions, but it can handle it better than conventional activated sludge systems (Sivasubramanian et al., 2021). According to De Graaff et al., aerobic granular sludge can be adapted to high salinity by changing the EPS composition and an increase in hydrophobicity, staying efficient in COD and N removal (Graaff et al., 2020). This robustness was also confirmed by (He et al., 2020). Furthermore, a study by Sadeghi et al. showed that attached growth processes are more efficient than suspended growth systems in treating saline wastewater (Sadeghi et al., 2019). Ghazani (2019) operated a sequencing batch reactor by gradually increasing the salt concentration. The results indicated that the simultaneous use of suspended and attached growth of microorganisms and the gradual increase of salinity in the wastewater could even lead to higher biomass concentration and ultimately improve the degradation of organic matter. In addition, the settling efficiency and settling velocity were noticeably improved by increasing the salinity. Other researches focus on the inoculation of salt-tolerant microorganisms in biofilms. Li et al. (2015) studied the inoculation of salt-tolerant microorganisms in an MBBR to investigate the start of biofilm formation and evaluate the COD removal efficiency in the treatment of high salinity wastewater. After successful inoculation, the MBBR showed high stability and removal efficiency compared with the activated sludge process, and could withstand the effects of variations in high salinity and organic loading (Li et al., 2015).

The monovalent and divalent salts were added to SW1 as chloride salts. Therefore, it should be tested whether Cl^- has a substantial effect on the biofilms and their performance. Various groups of microorganisms are involved in the biological wastewater treatment reactors. Biological nitrogen removal is performed by nitrifying organisms, which are very sensitive to toxic substances and have a low specific growth rate. A low specific growth rate delays the recovery of the nitrogen removal process after inhibition. According to Fonseca et al, only from a level of 40 g Cl^- /L, both AOB and NOB were almost completely inhibited. Concentrations in SW1 and during spikes were much lower. In this work, SW1 without salt spikes had a Cl^- concentration of 89 mg/L and increased up to 201 mg/L during spikes. No decrease in nitrification efficiency was observed as ammonium concentration continued to decrease up to discharge standards.

From this section it can be concluded that single loadings of variable monovalent/divalent salts ratio still result in reaching discharge standards for COD and N and performance is not decreased. From the literature comparison with the experiments in this thesis it can be concluded that long-lasting robustness against high salinity is possible for attached growth, and that a treatment system that combines attached growth and suspended growth can handle salts shock-loads, even in longer periods.

Figure 30 shows the OUR profile of reactor one from 28/06 to 02/07, showing the shorter cycles on Prim Eff first, then starting with SW1, then double the salt concentration, and then the pH 10 experiment. This figure provides the proof that there is no substantial difference in OUR comparing the baseline SW1 and changing the salts ratio. It also already gives a sneak-peak on the fact that a higher pH does not affect the OUR either. This is explained in the next section.

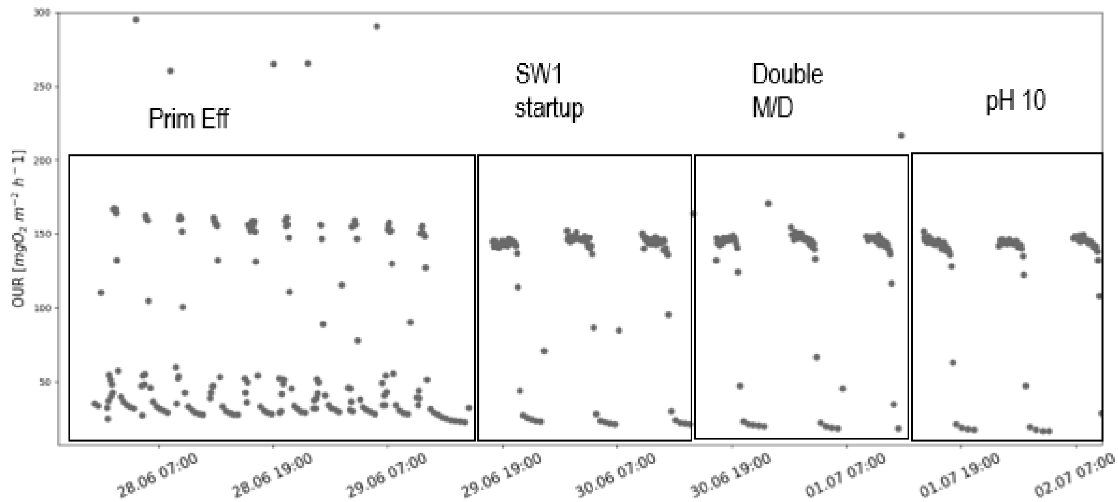


Figure 30: OUR graph of R1 from 28/06 to 02/07. The left rectangle represents the cycles on Prim Eff, the second reactor start-up with SW1, the third rectangle the double salts experiment, and the right rectangle the pH 10 experiment.

4.3 Influence of high pH on the performance of MBBR

A pH of 8.5 and 10 were each tested one day (one 3 L filling) in an MBBR reactor. In Figure 30 no decrease in OUR is seen. The drop in pH that was caused by low alkalinity was temporarily solved by adding a higher pH to the reactor. From this it can be concluded that the variability that is possible in supernatant, could actually buffer the reactor operation. There was looked at literature for the long-term influence of high pH in an MBBR attached growth system. A high pH can cause problems for nitrification, as there is more free ammonia, which inhibits the NOBs (Fumasoli et al., 2017). Lashkarizadeh et al. (2016) researched the influence of 8 days of a pH of 9 on aerobic granular sludge, and saw a decrease in N removal efficiency from 88% to 66% (Lashkarizadeh et al., 2016). Changes in chemical structure and composition of EPS matrix were suggested as the main factors inducing granules instability under high pH. Hence, an MBBR is not a good idea if it is expected that many high pH supernatants need to be treated. If this is an outlier, and is counterbalanced by the variability of pH in supernatant (a lower influent pH the following days), there is no problem as shown in this research.

4.4 Intermittency testing

Intermittency must be considered in non-sewered sanitation because municipal systems do not have a continuous supply of FS as sewered systems do. FS must be delivered by truck, and this may not be the case every day of the week. Intermittent loading of supernatant on weekends is discussed in the next chapter. Aeration causes high electricity costs, which is why 2 types of intermittency tests were performed: in one, aeration is also performed during the aerobic phase, and in one reactor the cycle was completely shut down during the intermittency.

Table 11 shows the sCOD and tCOD values of the effluent before and after the eight-day intermittency experiment. This allows the performance of each reactor to be compared and the effect of aeration to be evaluated. R1 was the reactor without aeration and R2 was the reactor with aeration. Table 11 shows that R2 has similar COD removals after the 8-day intermittency. For R1, the COD removal decreased from 35 mg O_2/L in the effluent to 70 mg O_2/L after eight days of interruption without aeration. The COD removal efficiency decreased from 63% to 26% without aeration. In a study by Falletti et al. (2014), who conducted research on MBBR treatment in a touristic area, forced aeration was proposed as well during periods of intermittency (Falletti et al., 2014). It can be concluded that aeration would help maintain the efficiency of COD removal after eight days of intermittency, decrease start-up time afterwards, and that discharge standards can still be achieved. However, it must be weighed whether the additional power costs offset the need to shorten reactor startup time after an intermittency.

Table 12: tCOD and sCOD values of SW2 after 8 days of intermittency with aeration or no aeration

Sample	Aeration?	sCOD (mg O_2/L)	tCOD (mg O_2/L)
Influent SW2	-	95	115
Effluent SW2 R1 before intermittency	No	35	44
Effluent SW2 R1 after intermittency	No	70	140
Effluent SW2 R2 before intermittency	Yes	42	127
Effluent SW2 R2 after intermittency	Yes	46	144

Figure 31 shows the pH, nitrate, and ammonium profiles during the intermittency tests. The top two graphs are the sensor data when there is no intermittent aeration. The lower graphs show the data when forced aeration is occurring during the intermittency. The yellow boxes show the total period of intermittency, divided into a dark yellow phase and a light yellow phase. The dark yellow phase indicates a period of time when the pH is decreasing. The light yellow phase indicates a period in which the pH value increases again. From the figures on the right, it can be seen that the pH value drops steeply on the first day in both cases. It can be concluded that the first day of intermittency allows time for the remaining ammonium to nitrify, resulting in a decrease in ammonium and the drop in pH. Thereafter, the pH rises again as the remaining, slowly biodegradable COD becomes available and allows denitrification to occur in the following days. In the light yellow phase in the top left of the graph (when there is no aeration), the ammonium sensor data is not considered correct, as an increase in ammonium is not possible during these days, as no new ammonium is loaded. The range over which pH fluctuates is greater when not aerated (between 5.5 and 8.5) than when forced aeration occurs (between 5 and 6). However, this wider range does not appear to be a problem for nitrification, as a decrease in ammonium is observed as a result of nitrification when SW2 is reloaded (indicated by the black arrow). The high ammonium peak after filling in the figure below is considered unreliable.

In conventional wastewater treatment, intermittency has already been investigated in an MBBR during stormwater treatment, as intermittent feed is also present (An et al., 2022). However, this is an intermittency of the hydraulic loading rate rather than the organic loading rate, since the COD is highly diluted in stormwater. However, this study also found a slight decrease in MBBR performance regarding COD removal when the MBBR was kept idle during the intermittency period. Nitrification and denitrification were not discussed.

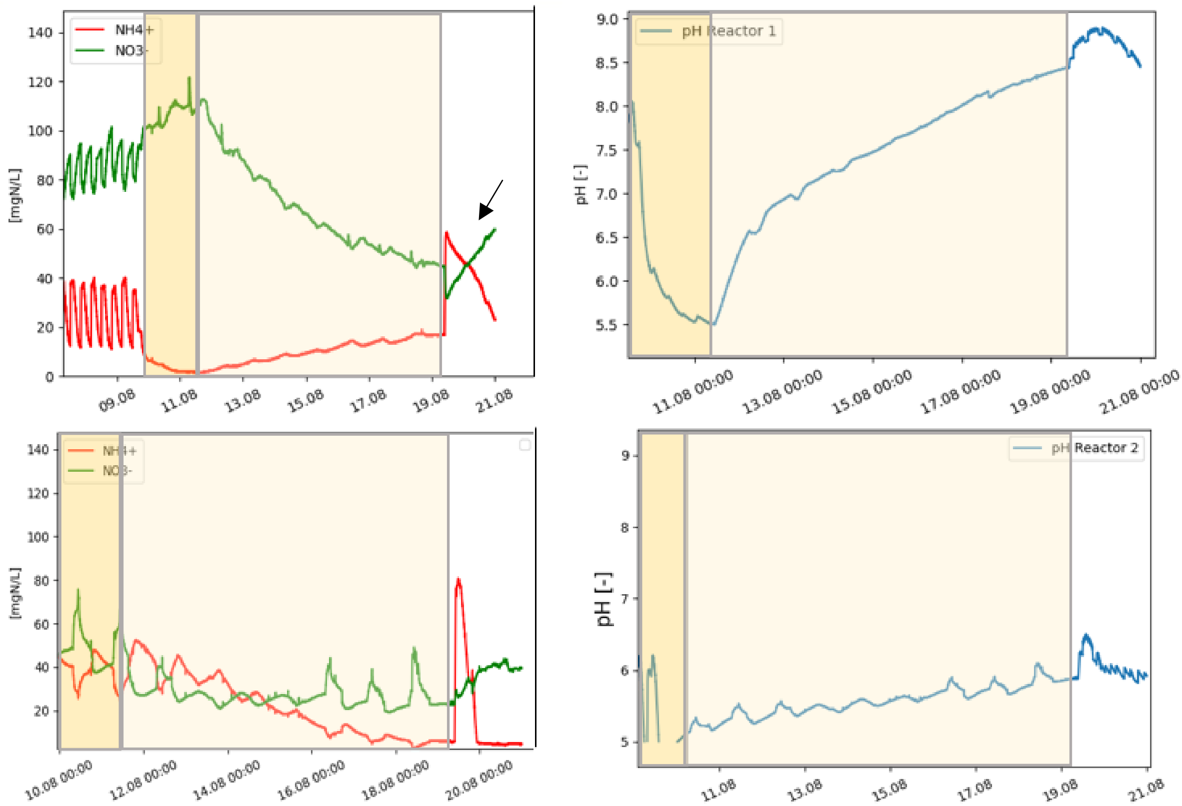


Figure 31: Sensordata for the two intermittency tests. Top: R1 when not aerating. Down: R2 when aerating. Left figures shown ammonium and nitrate sensordata and right figures pH profiles.

5. Realistic scenario

In previous experiments, only the variability of one characteristic, or intermittency was ever tested. In this chapter, the variability of influent composition is tested along with intermittency in a realistic scenario with the supernatants of the existing FS. Figure 32 shows the pH, ammonium, and nitrate sensor data from the realistic scenario period. Before Le1 was loaded, there was intermittency of several days. Prior to starting the realistic scenario, 2 cycles of SW1 were loaded to verify that the nitrifiers and denitrifiers were intact. This showed a slight increase in ammonium and a decrease in nitrate as confirmation of their effect just before Le1 was loaded (see black arrow). As mentioned in the Materials and Methods, the order of supernatants was randomized. The next section of this chapter describes the reasons why pH, nitrate, and ammonium showed this trend for each supernatant. This utilizes the findings from the previous experiments in this study, and the OUR profile of each supernatant was examined (below in Figure 32) and their COD concentrations during the cycles are presented in Table 13.

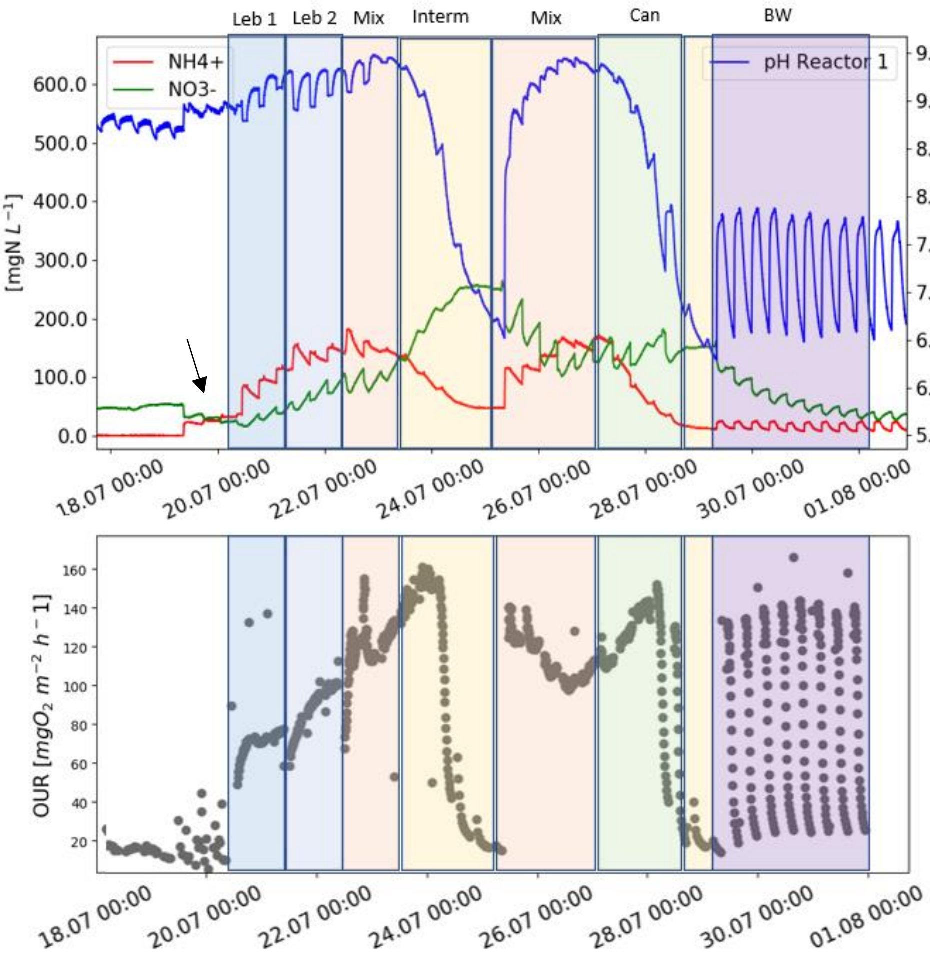


Figure 32 Top: pH, nitrate and ammonium profile of the realistic scenario. Down: OUR profile of the realistic scenario.

At first Le 1 was loaded, which resulted in an increase in ammonium and nitrate throughout the three cycles. There is no noticeable increase or decrease in pH, as the alkalinity of the influent is around 32 mmol HCO_3/L, which suffices to counter nitrification. Looking at the effluent of the Le1 sludge in Figure 50 in Appendix 15, it can be seen that all the bCOD has been removed, as the OUR already starts at the level of endogenous respiration. This means that the SBR cycle took long enough to remove all the biodegradable COD, and all the COD that is left in the effluent is non-biodegradable. This means that the non-biodegradable sCOD is 43.4 mg O_2/L and therefore the COD removal in cycle 3 was not complete (70.4 mg O_2/L). Le2 and Mix show the same increase in ammonium and nitrate. The treatment of the first three supernatants could be compared to pesticide manufacturing industries, especially that of organophosphorus pesticides, where discharge wastewater characterized by very high COD and comparatively low and slowly biodegradable bCOD. Chen et al. investigated the potential of MBBR to treat wastewaters from pesticide manufacturing units. Removal efficiencies in terms of COD and ammonia were achieved as 84% and 97% respectively in an HRT of 48 h (Chen et al., 2007). This shows that discharge standards could be met if cycle times are extended, increasing COD and N removal efficiencies but decreasing the efficiency of the cycle.

For these first 3 supernatants, a gradual increase can be seen in the OUR graph, with the OUR not going back to the OUR for endogenous respiration (previous experiment with SW1 show that this is around 20 mg O_2/L). This also shows the incomplete nitrification and denitrification. For these influents, the cycle should be extended or the aeration adjusted. OUR probably started at a low level because start-up time was necessary after a period of intermittency.

Considering supernatants like Le1, Le2 and Mix, concerns can be raised over the high bulk-phase COD concentration that is present in the MBBR reactor for a while. Performance of an overloaded biofilm reactor can be improved by increasing the bulk-phase oxygen concentrations. During this thesis, the aeration was kept constant at 2 mg O_2/L, but by increasing it to 4-6 mg O_2/L allows the effluent ammonia concentrations to meet the target effluent ammonia concentrations. The advantage of increasing bulk-phase oxygen concentrations is that this change in operation can be made during operation if there is sufficient aeration capacity, whereas reducing surface loading would require making changes in reactor size. Increasing aeration is not only used to improve the performance of overloaded biofilm reactors but also for the operator to respond to variable influent loading. The disadvantage is that this substantially increases the operational costs of MBBR treatment. Other operational parameters can be assessed as well, as those were presented in the COD/N spiking chapter. Many studies have been performed on organic matter removal in MBBRs, studying the change in COD removal efficiencies when changing the degree of filling of the carriers, as already mentioned in the section about COD/N spikings.

After three days of supernatant loading, no new supernatant was added to the MBBR for a weekend, which represents a two-day intermittency. The pH dropped from 9 to 6 as the three-day intermittency gave the system time to complete nitrification of all the ammonium that had accumulated with the Mix, Le2, and Le1 in the previous days. This compares to the yellow boxes of the intermittency experiments in Figure 31. Since no new bCOD is added to the system and the bCOD in the system was limited after Mix treatment, only limited denitrification occurs during the intermittency. This can also be seen in the graph OUR where OUR drops to the level of endogenous respiration in the middle of the weekend. However, even at low pH, the system is still functioning.

When the Mix is added again after the weekend, the pH rises again because the pH of the Mix influent is higher, alkalinity re-enters the system, and denitrification occurs again. Due to incomplete nitrification, the pH decreases slightly. New bCOD is added to the reactor, resulting in a higher OUR. Denitrification is higher than 100% (the nitrate profile shows a general decrease). Nitrification is not complete, resulting in a general decrease of ammonium in the reactor. The latter two factors lead to a general increase in pH.

Table 13: Hach Lange and IC results for the realistic scenario supernatant influents cycles. Effluent was collected of each first and third treatment cycle of the day.

Influent	What	Cycle	Turb (NTU)	sCOD (mg O_2/L)	tCOD (mg O_2/L)	NH_4^+ (mg/L)	NO_3^- (mg/L)	NO_2^- (mg/L)
Leb 1	influent	1	50.6	218.0	409	274	0	0
	after filling	1	166	217.0	362	102	12.4	<3
	effluent	1	212	43.4	952	81	23.8	<3
	effluent	3	137	70.4	332	136	42.9	<3
Leb 2	influent	1	4.55	116.8	178	299	0	0
	after filling	1	76.9	66.9	176	226	32.8	3.8
	effluent	1	154	49.4	809	131	47.6	<3
	effluent	3	64.7	64.6	1340	263	195.0	3.3
Mix	influent	1	893	641.0	1116	347	0	0
	after filling	1	66	155.0	379	219	34.1	<3
	effluent	1	25	52.0	274	<32	113.0	<3
	effluent	3	46	109.8	446	107	164.0	<3
Can	influent	1	30.7	67.2	178	26	0	0
	effluent	1	23.8	51.8	147	114	122	<3
	effluent	3	11	56	60.4	<32	145	<3

Conclusions can be drawn by calculating the initial alkalinity-ammonium ratio. The pH is assumed to decrease throughout the cycle of Le2 and BW, since these ratios were calculated to be less than 2

During Can loading, a steep pH drop is observed, ammonium decreases, a steady nitrate decrease is observed, and OUR drops to endogenous respiration levels toward the end of Can loading. Can has a highly favourable COD /N for COD and N removal, being similar to that of conventional wastewater. The ammonium concentration is only 26 mg N/L, which should counteract the high ammonium concentration present in the reactor at this time. This gives the biomass 'time' to nitrify the ammonium in bulk. Now that a large amount of ammonium is being efficiently nitrified, this explains the steep drop in pH as the alkalinity of Can is low. The sudden drop of OUR shows that at the end of Can loading, all the ammonium has been nitrified and the biomass can proceed to endogenous respiration. The Can supernatant can be compared to greywater treatment for MBBR, as the COD and ammonium concentrations are low. Saidi et al. treated greywater in MBBR and also saw full bCOD removals (Saidi et al., 2017). After Can loading, a small intermittency was introduced, which showed the same trends as the previous longer intermittency period. BW was then loaded as supernatant. This experiment will be explained in the next section.

The realistic scenario demonstrated what this proof-of-concept study was designed to show. 2-week natural variability was introduced. This resulted in a pH fluctuation between 9 and 6, but there was no reactor failure and both the nitrifiers and denitrifiers remained active. This shows that the MBBR is robust enough to withstand the natural fluctuations and intermittency . In fact, variability can be an advantage because it can buffer the system and give it time to adjust. The discharge standards are not met, but adjustments to the operational parameters make this possible.

6. Treatment of supernatant after dewatering of BW

During the MBBR operation, supernatant after dewatering fresh wastewater was also used as influent for 5 days. This fits the community-scale scenario, since in urban areas there are also containments that need to be emptied more than daily. For example, in busy markets. This means that this FS enters the treatment plant very fresh and unstabilized. It is assumed that with the favorable COD /N ratio of 9/1 and the high pH of 8.5, COD and N removal will occur according to the discharge standards.

Figure 33 shows the ammonium and nitrate removal when the fresh black water was added to the MBBR reactor. The nitrate curve starts at about 150 mg/L because complete denitrification did not occur in the previous cycles, as discussed in Chapter 5 (realistic scenario). It appears that loading BW remedies the incomplete denitrification, as the nitrate removal efficiency is greater than 100%. With this, there is also extra alkalinity production. The first steep drop in nitrate in each cycle is due to the filling phase. The less steep decline is due to denitrification and is about 5-10 mg NO_3/L each time. The decrease in ammonium/increase in nitrate in the aerobic phase is about 20 mg/L.

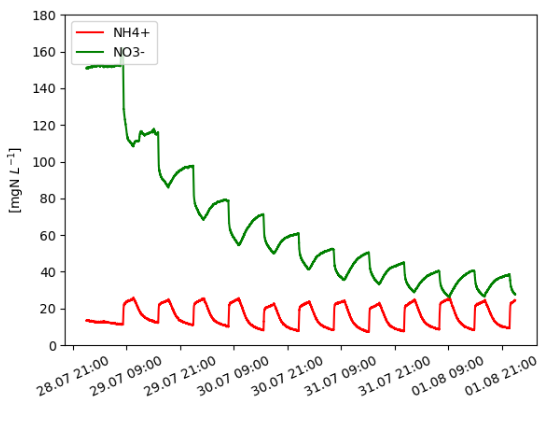


Figure 33: Ammonium and nitrate concentrations during the blackwater additions as influent

Figure 34 shows the OUR profile during the aerobic phase of treatment of BW from the NEST building (see Appendix 2). This figure is an enlarged OUR profile already shown in the purple box in Figure 32. The profile looks different than the OUR profile from SW1 shown in Figure 26. There is no drastic decrease in endogenous respiration at 5 mg O2/L, but a gradual one. This could be because there is still some slowly biodegradable bCOD available for BW before it goes to endogenous respiration.

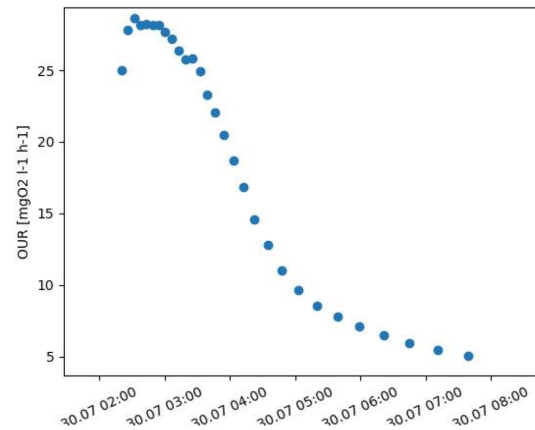


Figure 34: The OUR profile during the aerobic phase of the treatment of fresh blackwater from NEST

Figure 35 shows the respirometry graph of the effluent of BW after treatment with MBBR. The graph shows that there is still some biodegradable COD left (26.43 mg O_2/L), meaning that the COD removal by the MBBR was not complete and the cycle should have been prolonged. However, as explained in Appendix 15 which assesses the respirometry technique, the level of endogenous respiration might have been higher during this experiment due to temperature fluctuations in the lab. This would mean that all the bCOD was removed and 100% COD removal efficiency was achieved.

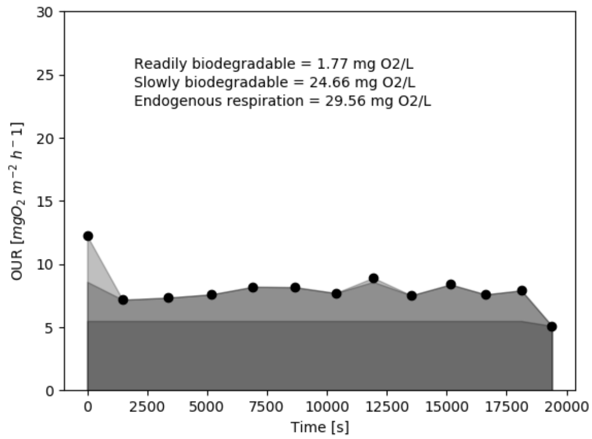


Figure 35: Effluent blackwater respirometry graph

A comparison can be made between the first chapter, which dealt with supernatant from a septic tank, and this chapter, which deals with supernatant from faecal sludge that has never been stored. While there was a drop in pH in the MBBR operation of SW1, for BW there do not appear to be any operational problems and the discharge standards are being met without any problems. Currently, on-site decentralized blackwater treatment is mostly with anaerobic techniques such as a UASB septic tank (Luostarinen & Rintala, 2006). Now an MBBR seems to be an aerobic alternative in that treatment train. However, then a dewatering pre-treatment step needs to be added.

Feasibility study

In this chapter, a general discussion about the usage of an MBBR for supernatant will be made. First of all, with the help of research question 1.1 and 1.2, loading limits are defined and a treatment train that includes an MBBR is proposed. Next, factors that need to be considered for the feasibility for implementation in an FSTP will be assessed and there will be elaborated on whether an MBBR configuration is also suitable for different scenarios than the 5000 people community-scale scenario that is proposed. For this assessment, the MBBR reactor will be compared with existing techniques now (emphasis on space, energy and operation and maintenance). To help with the assessment of feasibility in the field, online expert interviews were conducted with Kapanda Kapanda, who operated a small-scale FSTP in Zambia; Ronald Sakaya, who is a plant manager of the Lubigi FSTP in Kampala, Uganda; and Linda Strande and Nienke Andriessen, who are experts on FSM at EAWAG.

1. Loading Limits

The loading limits for the MBBR in field applications was based on the findings in research question 1. Research question 1.1 shows that the MBBR can be used for one type of supernatant treatment after dewatering of FS, if the alkalinity, COD and N values in the effluent are favourable. As stated in the results and discussion, this means that the alkalinity/ammonium ratio should be higher than 2, and there has to be sufficient readily biodegradable COD available and a favourable COD/N ratio for full denitrification within the proposed cycle length.

Research question 1.2 shows that the variability in the supernatant and intermittency in operation can be an advantage to the MBBR within certain boundaries, and a solution to the problems that occurred in research question 1, which was not the assumption. However, the COD/N ratio cannot approach 518/1, shown in the results and discussion, as this makes the system collapse, as a high amount of COD creates an anaerobic environment and this starts fermentation reactions, which creates foaming and kills off the bacteria (Anaerobic Digester Foaming Control & Prevention - Aquafix, 2022). A high COD/N ratio of 90/1 did not show any problems when this was loaded for 1 day. Consequently, the system boundaries regarding COD/N ratio in the influent of supernatant that can be proposed with information from this thesis, is between ¼ and 90/1. These boundaries should be further examined, as they can probably be more extended, because the reactor operation during these spikings did not give any issues. From the Kampala data of (Strande et al., 2018), it is known that the COD/N ratio of supernatant can be much higher than 90/1, and that the absolute values of COD and ammonium in the supernatants can be much higher than the tested concentrations (see Table 6). Lastly, A long period of high COD concentrations in the reactor might cause heterotrophic overgrowth, so this should also be considered.

Next, it appeared that increased salts concentrations in comparison to the baseline SW1 concentrations did not show any influence in reactor performance in the short term (1 loading). However, it should be verified if longer term high salt concentrations affect reactor performance. Lastly, according to the COD/N spikings of 518/1, it can be seen that a pH of 4 causes reactor breakdown, and the high pH spikings show that a single high pH loading can buffer the decreasing pH caused by unfavorable alkalinity. A longer period of high pH in the reactor can cause toxicity because of the release of free ammonia.

These results show the importance of estimating Quantities and Qualities (Q&Q) of FS at community to city-wide scales. Before designing a treatment plant, expected influent values should be evaluated. It should also be assessed whether the expected variability is appropriate for treatment in an MBBR after dewatering. For example, if after the Q&Q assessment it appears that there is a trend in the 5000 people community of low pH and low COD FS, the MBBR should not be the proposed treatment for COD and N removal. If after this assessment it appears that the average COD/N ratio in the influent is high, the cycle time should be extended and the aeration in the aerobic phase should be increased. Unlined pit latrines will give a problem as the COD content might be high and slowly biodegradable. This is because unlined pit latrines do not have hardened borders, and FS can seep into the soil. This makes the contents of the unlined pit latrine more concentrated. If the dewatering process is then insufficient, this might cause the previously mentioned problems in the MBBR.

2. Pre-treatment and post-treatment steps

In this study, an MBBR was investigated for the treatment of the supernatant, but this technique is part of a whole treatment train, because pre-treatment and post-treatment is required with this technique. Figure 36 shows a proposed supernatant treatment train that includes an MBBR.

Regarding pre-treatment, the question is whether dewatering is beneficial to the operation of the MBBR reactor if the COD value of the supernatant is already estimated to be too low by the characterization of FS. As researched by Shaw et al., a dewatering step can remove 60% of the tCOD (Shaw et al., 2022), significantly lowering the COD /N ratio. However, dewatering removes mainly particulate COD, which is assumed to be slowly biodegradable. Readily biodegradable COD is usually necessary for successful nitrogen removal. Dewatering also reduces the risk of carrying other particulate materials, such as other waste, into the next treatment steps. In addition, dewatering is necessary in non-sewered sanitation for other reasons, namely volume reduction and as a means of resource recovery for solids (Diener et al., 2014). The conclusion, then, is that dewatering is still a useful step. Before dewatering, a screening step is definitely needed to remove the solid wastes, sand, and other things present in FS that could clog the MBBR.

Next, post-treatment steps are required. The results of this work show that TSS and tCOD increase throughout the SBR cycle when the supernatant is treated with MBBR, indicating that a second settling step is required after MBBR before discharge is possible. In conventional centralized wastewater treatment, this increase is also observed, therefore a secondary settling step is added after MBBR (see also Figure 10). Consideration can be given to decanting the treated supernatant from the MBBR, leaving the accumulated solids in the reactor after a settling stage. However, this increases cycle time and therefore decreases treatment efficiency. It would also increase SRT, which would change the composition of the suspended biomass and increase the risk of heterotrophic overgrowth and sludge formation (Morgenroth, 2020). Therefore, a solids removal step was included in the treatment train proposed in Figure 36. The solids stream generated in this step could be recycled to the dewatering step upstream of the MBBR.

Furthermore, this research looked at effluent quality for safe discharge in surface waters regarding COD and N. However, it is not known yet to what extent phoshorous is being removed and whether pathogens or micropollutants are reduced. This would be necessary to discharge the effluent safely in the environment. A MBBR pilot plant in Berlin as described by Saidi et al. (2017) showed pathogen reductions. In this system multiple MBBRs were placed in series. Recent studies have proposed MBBRs as a promising technology with respect to the attenuation of micropollutants via biological treatment (Torresi et al., 2019). Indeed, recently Tang et al. (2017) discovered that an MBBR could also be used for tertiary (polishing) treatment. Ooi et al. describes antibiotics removal with MBBR (Tang & Ooi, 2017). Torresi et al. (2019) proposes that micropollutants can be reduced by aerobic phosphorous accumulating organisms (PAOs). This means that there is potential for micropollutant removal combined with phosphorous removal with an MBBR. However this would require an additional methanogenic MBBR in series that is being operated in anaerobic conditions (Zkeri et al., 2021). Tang et al. (2022) assessed removal of pharmaceuticals through intermittent feeding in an MBBR. A recent study by Pan et al. (2022) proposed a novel synchronous N and P removal pathway at various COD/N ratios in MBBR (Pan et al., 2022).

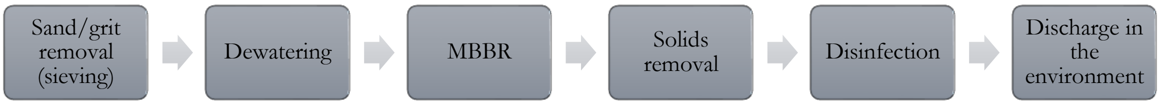


Figure 36: Proposal of a treatment train involving an MBBR.

Finally, a note can be made on the continuity of the proposed treatment train in Figure 36. Both dewatering and MBBR treatment in the SBR configuration occur in batch mode. However, in a 5000 people community-scale urban scenario, FS is also delivered in batches by trucks, allowing time for batch dewatering. In addition, multiple dewatering stations and/or MBBRs could be connected in parallel to provide more continuity.

3. Operation and maintenance requirements

In recent decades, policy has generally moved toward decentralization, and there are now a large number of smaller scale, decentralized wastewater treatment plants. However, challenges for these plants often include irregular monitoring and maintenance, neglect due to a weak regulatory framework, and/or lack of funds for operation, which often results in FSTPs falling into disrepair soon after construction. In order to provide sanitation to as many people as possible, a "good enough" and low-cost sanitation solution is often preferred over more advanced treatment technology (Cid et al., 2022).

FSTPs require ongoing and appropriate operation and maintenance (O&M) activities to ensure long-term functionality, as mentioned in the third part of this chapter. Therefore, these two factors and their costs will strongly influence the decision to install an MBBR. O&M activities are at the intersection of the technical, administrative, and institutional frameworks that enable FSTP to function sustainably (Peal et al., 2014). 'Operation' refers to all activities required to ensure that an FSTP provides treatment services as intended (e.g., opening/closing valves, adding conditioners, regulating aeration cycles), and the term 'maintenance' refers to all activities that ensure the long-term operation of the facilities and infrastructure (e.g., repairs, cleaning, unclogging, weed management) (Strande et al., 2014).

Before choosing MBBR as a treatment option for the FS treatment objectives of nutrient management and stabilization, the risks in the FSTP to MBBR operation and maintenance must be well understood, as outlined in the WHO guidelines for the risk-based approach (Strande, 2022, personal communication). In other words, what situations could lead to failure of the MBBR. These are summed up in the next paragraphs.

First of all, reactor materials and spare parts must be locally available (Sakaya, 2022, personal communication). If something breaks, it should not have to be imported from somewhere else (Bassan et al., 2014), according to Ronald Sakaya. This is an important prerequisite to ensure that maintenance is successful and does not cause the FSTP to fail. The availability and choice of construction material, whether locally produced or imported, matters (Montangero et al., 2002). For the MBBR, this means the availability of the construction parts for building the reactor itself, in-line sensors, biocarriers, stirrers, aerators, feed pumps, and operation software. In this study, reactor operation was started with fully viable biocarriers taken from a healthy wastewater treatment plant in Aarau. If MBBR is the selected treatment option at the plant, the biocarriers must be available and accessible, and there must be a possibility for a healthy biofilm to grow on them. Allowing a biofilm to grow on carriers without conventional wastewater being available, but only the supernatant after dewatering from FS, is a point for further research.

Second, plant operators play a big role in MBBR operation (Sakaya, 2022, personal communication), as a certain expertise is required to handle an MBBR regarding reactor operation. During this thesis, an urban community-scale scenario of 5000 people was considered. In many community-scale FSTP, plant operators are also the containment emptiers. Figure 2 in the introduction of the FSTP in Zambia demonstrates these concerns, as this is an emptier operated treatment facility. This makes them responsible for multiple parts of the sanitation service chain, interrupting its continuity. They spend half their working day emptying the containments manually or with trucks in the city to collect the FS. The other half of the day they operate the FSTP and treat the collected FS This means that half of the day, the plant is not being actively operated. This raises the question whether emptiers and plant operator require two sets of skills. If plant operators are not available for a day, intermittency tests showed that this gives no issue for the biomass, and effluent quality remains according to discharge standards, if aeration in the aerobic phase of the SBR is maintained. If operators do not work on the weekend, two day intermittency tests showed that this could result in a pH decrease. However, the realistic scenario showed that the reactor is highly robust for pH fluctuations.

Third, electricity needs to be affordable and available for aeration in the plant (Kapanda; Sakaya, 2022, personal communication). In a location where electricity is not available, an MBBR is not possible for treatment. Where electricity supply is unreliable, a back-up generator will be needed. If this is not financially feasible, solar energy is another option.

Fourth, another requirement is the operating cost (OPEX) being affordable (Kapanda; Sakaya, 2022, personal communication). There is no information (publicly) available on the capital and operating costs of an MBBR in low- and middle-income settings, but general things can be considered (Strande et al., 2014):

- Economic indicators (land price, labor cost, interest rates, petrol prices).
- Possible income from the sale of treatment products (e.g. hygienized biosolids or compost, biogas).
- Site conditions
- Economy of scale (plant size).
- Legal discharge standards.
- Costs of chemicals

An extensive OPEX and CAPEX calculation for an MBBR is a point for further research.

4. Comparison with existing treatment techniques

Based on the interviews conducted, the literature review, and the review of design plans of existing FSTPs, it can be concluded that there are three major competing technologies for the MBBR reactor: Constructed Wetlands, Waste Stabilization Ponds, and Anaerobic Treatment such as an Anaerobic Baffled Reactor (ABR). These techniques have already been mentioned in the 'Context' chapter. Table 14 shows a decision matrix for an MBBR with the other three treatment techniques considered for the treatment objectives stabilization and nutrient management. For each factor discussed in Part 3 of this chapter, each technique is considered and a decision is made as to how beneficial each technique is for that factor.

Table 14: Comparison of the existing treatment techniques according to the most important factors to be considered according to chapter 3 of the feasibility in the field study. '- ' indicates that the factor will negatively influence the choice for this technique. '+' indicates that the factor will positively influence the choice for this technique.

	MBBR	Waste stabilization ponds	Constructed Wetlands	ABR
Electricity	-	+	+	+
Land	++	--	-	-
Expertise operators	-	++	+	-
Effluent quality	+	-	+	+
Availability materials	-	+	+	-
Temperature control/ weather	+	-	-	-
OPEX	-	+	+	+
Greenhouse gas emissions	+	-	-	-

If the availability of electricity is uncertain, MBBR is never an option because aeration is needed. Then, the other three techniques should be considered. Research question 1.1 introduced the importance of adequate mixing in an MBBR reactor. Kamstra et al (2017) showed the effect of mixing on scaling in an MBBR

(Kamstra et al., 2017). Superficial air velocity is the most important factor when looking at mixing in a full-scale MBBR. This increases energy requirements and underestimates the reactor performance if it is solely based on small scale lab operations. This explains the negative OPEX factor in the table.

A crucial factor in the decision matrix is the available land on site. The most significant barrier for the apt implementation of a supernatant treatment system in urban areas is the necessary space. Therefore, this should be explicitly calculated. Land price is lower for the MBBR than for existing techniques. Labor costs are comparable, because one person can operate all of the aforementioned techniques.

With this laboratory setup, 3 L of supernatant can be treated per cycle, with three cycles per day. This translates to 9 L per day with a laboratory scale 12 L reactor. From own experience, it is possible to run three reactors per operator. This means that with the current SBR cycle, 27 L of supernatant per day can be processed by three 12-liter reactors. However, SBR cycles might need to be extended at higher COD and N levels, which means this is likely an overestimate. Design files of various municipal-scale FSTPs indicate that a community with a population of 5000 can expect 10 m^3 FS /day (2 L/person/day), with days of interruptions. Assuming that FS contains about 10% solids (Shaw et al., 2022), this means that 9 m^3/day of supernatant needs to be treated daily after dewatering.

- According to design documents for constructed wetlands with vertical flow, 40.75 L/m^2 can be treated per 5 days. According to the 5000 people community-scale, 45,000 liters must be treated per 5 days, which results in a required area of 1104.79 m^2 (UN, 2008). The dimensioning should be 2/1, therefore around 55m by 20m.
- The necessary space for an MBBR to treat 9 m^3/day is 1000 times the lab set-up, which treated 9 L per day. Therefore 12 000 L will be required. These would be three 4 m^3 reactors, with the dimensioning of 2 m* 2m* 1m . Therefore the surface area that is taken up by MBBR treatment, is 6 m^2.

When considering the expertise required of operators, the MBBR must compromise because the reactor operation is more difficult than waste stabilization ponds and constructed wetlands, for which no expertise is required and for which people from the community can be trained. ABR is an anaerobic (biological) process which needs equivalent expertise. In terms of materials, the materials for constructed wetlands and waste stabilization ponds are more locally available than those for the MBBR and ABR, as these only require ponds with pumps and filters with natural materials. In terms of effluent quality, waste stabilization ponds are the worst because they do not have an active treatment step. MBBR and ABR treat biologically, and in constructed wetlands the plants provide a treatment step. However, this requires a more in-depth literature review. When considering a factor such as weather, constructed wetlands and waste stabilization ponds are negatively impacted because they are highly dependent on weather. For example, extreme rainfall can result in the need to compromise to install a roof, which then affects UV light. In an MBBR, the temperature can easily be kept constant by installing a thermometer and heating jacket.

According to Cheng et al., the global greenhouse gas emissions for non-sewered sanitation technologies were estimated to be 4.7% of the global anthropogenic methane emissions, the same amount as for wastewater treatment plants (Cheng et al., 2022). After the complete elimination of open defecation, this number will even increase for non-sewered sanitation, as this is the main option for open defecation. This figure gives pause for thought when greenhouse gas emission techniques are also considered. Tanikawa et al. have studied that ABRs produce methane and nitrous oxide (Tanikawa et al., 2019). Since waste stabilization ponds and constructed wetlands also involve anaerobic treatment, it is assumed that these techniques produce the same greenhouse gases. For the MBBR, these would be nitrous oxide and CO2, as there is no anaerobic treatment step which is a less harmful greenhouse gas than methane. Therefore, it is assumed that the MBBR is the least harmful option in terms of emissions. However, further studies should be conducted to quantify the emissions.

Comparing the MBBR with ABR technology, combining all the considered factors, decentralized sanitation systems, such as the ones used by BORDA (*BORDA*, 2022) can be considered (see Figure 7). These are focused on reuse and is a system that is proven to be working (Reuter et al., 2009): the MBBR will only be an advantage over this system if there is really not enough space, or there is big emphasis on achieving discharge standards , or no destination for the biogas that was produced. After the ABR and AF very often a constructed wetland is installed, which means there is no control over the quality of the effluent because it just gets soaked into the ground. The MBBR treatment efficiency regarding should be higher than the constructed wetlands. It should be more robust, takes smaller space (need to look at energy requirements and pH control).

The second technique for which the MBBR could be a substitute is the vertical flow constructed wetland. This technique has comparable disadvantages and advantages to the MBBR. Wetlands do not need aeration. Wetland treatment takes longer than MBBR treatment. In terms of operation and maintenance, it is important to remove weeds that may compete with planted wetland vegetation during the initial growing season. The manifolds should be cleaned once a year to remove sludge and biofilm that may clog the holes. Over time, accumulated solids and bacterial film will clog the gravel. Rest periods can restore the hydraulic conductivity of the bed. If this does not help, the accumulated material must be removed and clogged parts of the filter material replaced. Maintenance activities should focus on ensuring that the primary treatment effectively reduces the solids concentration of the wastewater before it enters the wetland (Vertical Flow Constructed Wetland |SSWM, 2022). Kengne et al. evaluated the performance of vertical flow constructed wetlands for faecal sludge drying bed leachate (type of supernatant). Various hydraulic loadings were investigated and the treatment performance was explored. Vertical flow constructed wetlands removed more than 80% of the pollutants and effluent quality met local guidelines, except for nitrogen and pathogens (Kengne et al., 2014). Wetlands are able to remove nitrogen up to the standard. Due to the mechanical dosing system, this technology is best suited when skilled maintenance personnel, reliable power supply and spare parts are available. This is the same as for MBBR.

A comparison with waste stabilization ponds again shows the trade-off with available land. However, waste stabilization ponds can better handle high loading and are easy to operate (no experts are required, only a trained community) (Waste Stabilization Ponds | SSWM, 2022). In addition, according to Cid et al, a low-cost sanitation solution is often preferred over more advanced treatment technology to provide sanitation to as many people as possible, which would favor the waste stabilization ponds (Cid et al., 2022). Major drawbacks include odours and flies, and the long storage times. The leachate definitely requires an additional treatment step for safe discharge.

5. Different scenarios

During this thesis, only an urban community-scale scenario of 5000 people was considered. Nonetheless, it is interesting to see whether the MBBR would be possible in different scenarios as well.

5.1 >5000 people community-scale scenario

If the scenario is for larger areas with more people and therefore more frequent emptying and deliveries for treatment are at place, there is less chance of intermittency. However, Research Question 1.2 showed that intermittency is not really a problem and discharge standards can even be met if aeration is forced during this intermittency. In this scenario, economies of scale are important, as are the longer transport distances to the treatment plant. In emergency situations, aerobic treatment is considered more robust than anaerobic treatment because it is more difficult to maintain an anaerobic environment (WASH Cluster | Global WASH Cluster, 2022). Here, the unavailability of viable biocarriers will be a problem, as something like this needs to be set up quickly. Also, blackwater is expected to be used rather than old septic sludge, so MBBR treatment is expected to work well (see Part 6 of Results and Discussion). In informal settlements, space is very often limited. Therefore MBBR seems to be a good option. However, electricity and access to carriers should be available. Also, as with emergencies, it takes a while for carriers to grow, so MBBR is not suitable for emergency treatment. Proper maintenance there is at risk as well.

5.2 MBBR as treatment option for one household (completely decentralized)

The decentralized treatment of greywater with MBBR has been extensively researched and applied. This is not the case for blackwater. The treatment of a single household means that the variability in the influent composition of the supernatant is rather limited, as the blackwater comes from the same containment. The blackwater would not be stored long as it would be treated on-site and would be fresh. Treatment of supernatant after dewatering of fresh BW in this thesis showed that treatment with MBBR is possible and even the US-EPA discharge standards can be reached. Mixing the blackwater with greywater would be a possibility and would also decrease the variability, however it is a pity to contaminate the cleaner greywater with the dirtier blackwater. Furthermore, operation and maintenance of the MBBR on household level is a challenge and post-treatment steps need to be implemented. Nonetheless, at this point there are already efficient on-site treatment techniques for blackwater on one household level, more favourable than the MBBR, like composting toilets. Therefore decentralized use for the treatment of BW by MBBR is not proposed.

Conclusion

In this work, it is investigated whether an MBBR is suitable for treating the supernatant after dewatering of FS in an urban community-scale scenario of 5000 people. This proof of concept was divided in two research questions.

Research question 1 investigated whether the MBBR reactor can run on supernatant on lab-scale and whether discharge standards are met. Subquestion 1.1 asked whether it can run one type of supernatant, SW1. Subquestion 1.2 was whether the reactor would still function in a 5000 people community-scale scenario. This means that the composition of the highly variable influent composition was tested and intermittency.

The answer to both subquestions can be 'yes', provided specific changes are made. **Subquestion 1.1** showed that the alkalinity, COD and its fractionation, and ammonium concentration in the influent should be considered in any case. A thorough Q&Q assessment must be performed for this purpose.

For **subquestion 1.2**, a statement can be made within certain limits of variability. At a ratio of 518/1 COD/N derived from a realistic scenario, the reactor cannot cope, as the high COD concentration creates an anaerobic environment in the anoxic phase and the reactor begins to foam and the microorganisms die. The variability of COD /N that the reactor can handle ranges from 1/4 to 90/1. Further expansion of this ratio needs to be investigated. There appeared to be no effect on biofilm performance when spiking different M/D salts ratios. The intermittency tests show that better effluent values are obtained when aeration is forced during the intermittency as usual in SBR configurations. In addition, periods of intermittency do not appear to present any problems as the system has proven to be robust in the realistic scenario. The natural variability that the supernatant may have can be an advantage to regulate the pH in a natural way in the reactor. Treatment of blackwater with MBBR shows that this COD/N ratio is better, meaning it works better if containments are emptied more frequently and fresher FS needs to be treated.

In this work, respirometry was used for the first time as an analytical technique to determine the readily and slowly biodegradable fraction of COD in the supernatant after faecal sludge dewatering. The absolute values were not comparable to the IC values for the different supernatants, suggesting that more flexibility is needed in the supernatant settings and degree of filling. However good estimates could be made on how the fractions compared to each other.

Research question 2 assessed whether the MBBR is viable in the field. The consideration whether to select an MBBR as a treatment option depends on several factors. The most important are availability of electricity, space and materials; greenhouse gas emissions; and operator expertise.

In summary, the concept of using an MBBR to treat the supernatant after faecal sludge dewatering is proven for a 5000 people community-scale scenario, and further research needs to determine new outcomes such as the possibility of pathogen reduction and phosphorus removal. Thereafter, field trials must determine actual feasibility on a large scale.

Recommendations for further research

This work was a first step to find out if a MBBR can be used as an alternative treatment technology for supernatant treatment after dewatering of FS. For this reason, many assumptions were still made and there are still many knowledge gaps remaining to further validate this technology. This chapter provides an overview of the research questions that can be further investigated.

1. Can healthy biofilms be grown on carriers with supernatant as feeding?

Biocarriers were added to the reactor with a healthy biofilm that had grown up in a wastewater treatment plant. In the proposed scenario, there is no such possibility. It should therefore be investigated whether it is possible to grow a healthy biofilm without having a nearby wastewater treatment plant available.

2. How to adapt operational parameters of the MBBR for better COD and N removals?

COD /N ratios were now adjusted based on baseline SW1, which showed low values. For example, the Kampala data showed that there could be up to 100 times more COD in the supernatant, and thus 100 times more ammonium. For this to happen, the reactor operation must be greatly changed, i.e., much longer cycles, larger biocarrier area, larger reactor, increased aeration, increased filling ratio etc. This study has not yet examined the effects of changing the operating parameters, so this could be a good addition. If high COD concentrations are expected, it might be useful to test multiple MBBRs in series. Furthermore, it is necessary to assess which operational changes are most feasible in the field.

3. Can NOBs and AOBs grow acidotolerant in an MBBR when exposed to long periods of low acidity?

When looking for answers as to how it is possible that there were still 100% nitrifications during periods of low pH, a knowledge gap was found on whether it is possible that there is acidotolerance occurring in an MBBR and how long it would take for the MO to become acidotolerant.

4. Can phosphate accumulating and glycogen accumulation organisms handle the variability in supernatant for phosphorous removal in an MBBR? Can other FSM treatment objectives be achieved with MBBR treatment?

This study was a proof of concept and further research needs to be conducted to fully assess whether the MBBR is a good alternative for treating the supernatant after dewatering of FS. At this time, only two treatment objectives for faecal sludge treatment have been investigated, namely stabilization and nutrient management in terms of N removal. Further studies could verify whether pathogen reduction and nutrient management in terms of phosphorus also occur. In addition, the literature review (presented in the feasibility study) shows that even micropollutants can be removed by an MBBR.

5. What are the long term effects of the variability of supernatant on the biofilm?

Next, the longer-term effects on the biofilm should be evaluated. Only the short-term effects on reactor performance and removal efficiency were examined, but no change in biofilm.

6. What are the risks and challenges operating an MBBR full-scale in a community-scale FSTP?

Now that the MBBR has been tested in the lab, the next step is to test it in the field. There are risks associated with scaling up and the operation of a biological treatment system. These need to be identified.

7. Are other attached growth processes, like a RBC, an option for supernatant treatment after dewatering of FS in a 5000 people community-scale scenario (semi-centralized)?

At the beginning of this research, an RBC was also considered for treatment of supernatant. As a start, a small-scale RBC was tested in the experimental hall of EAWAG. The RBC was installed in February 2022. In the beginning, it was fed with primary effluent of the WWTP of EAWAG. The pump was adjusted to low flow with the goal to grow a nitrifying biofilm. After some weeks, a white, slimy biofilm developed (Figure 37 left and middle). It was hypothesized that the white biofilm is coming from anaerobic conditions in the bulk, as it was observed that sludge accumulated at the bottom. As a consequence, the RBC was inclined (Figure 37 on the right). However, these measures did not reduce the white biofilm, additionally nitrification was still not observed. Cortez et al. (2013) reported that white zones of the biofilm might come from filamentous growth (Cortez et al., 2013). As filamentous growth is often induced by a non-optimal COD surface loading, this should be monitored. Additionally, the current design of the RBC has a really low surface area to total volume ratio. That means that the hydraulic retention time in the RBC is really high, if the flow is designed according the surface loadings. Consequently, there is more time for degradation. In a real system, the surface area might be higher. The system should be redesigned before doing further research. Nonetheless, literature suggests other RBC design considerations that could work, such as arranging RBCs in series. Therefore this is a topic for further research.

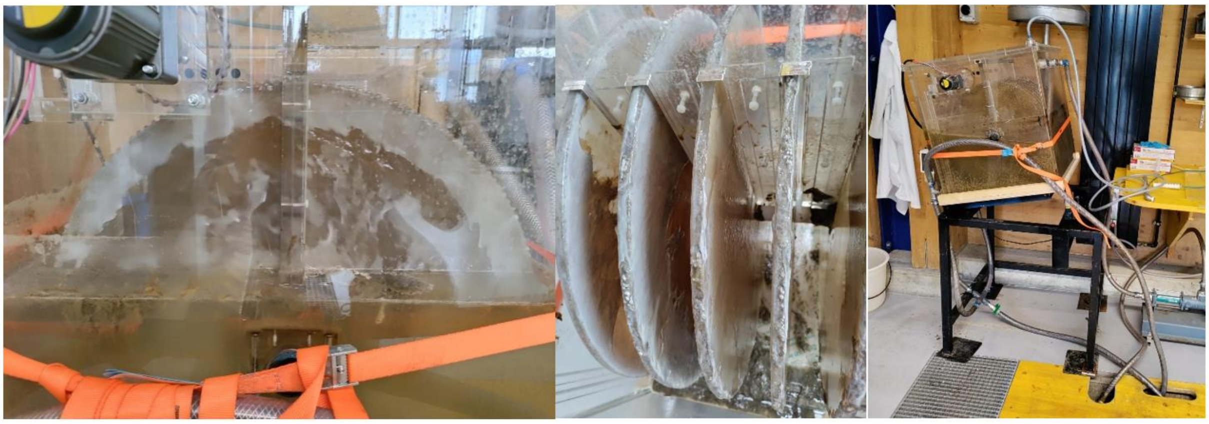


Figure 37: Additional pictures on supernatant treatment with RBC.

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Appendix

1. TS measurements from pre-treatment tests on BW

The following Table 16 presents the TS results of BW sampled on 07/04/2022 at NEST. These values provide a similar conclusion as the turbidity measurements, namely that the combination CP314 and fruit press was most easy to handle, innovative and performed good dewatering. The red value of -0.2 is not possible and is assumed to be an outlier. For this experiment, only the turbidity measurement was used as a proxy.

Table 15: TS measurements from pre-treatment tests on BW to determine the dewatering mode of operation in this thesis. 'geo' means geotube. The influent TS measurement was performed twice.

BW pretreatment test	Before (g)	After (g)	Difference (g)	Vol (mL)	TS (g/L)
TS influent	19.0512	19.0545	0.0033	10	0.33
	19.1482	19.1507	0.0025	10	0.25
TS geo + CP314	19.3957	19.3937	-0.002	10	-0.2
TS geo + chitosan	18.9781	18.9800	0.0019	10	0.19
TS press + CP314	18.8185	18.8207	0.0022	10	0.22

After this first experiment, it appeared better to measure TSS instead of TS. Such measurements are not recommended if the aim is to measure solids. BW has a low concentration of solids of around 200 mg/L while it also contains cations/anions. Thus a filtration step is required to measure “solids” and therefore TSS/VSS method is better.

2. NEST building EAWAG – Sampling point for blackwater (BW)

Nest is the modular research and innovation building at EMPA and EAWAG. At NEST, new technologies, materials and systems are tested, researched, further developed and validated under real conditions. Close cooperation with partners from research, industry and the public sector ensures that innovative construction and energy technologies are put onto the market faster. NEST contributes to making use of resources and energy more sustainable and circular. The fresh blackwater in this thesis was sampled in the NEST building on the EAWAGcampus in Dubendorf (https://www.empa.ch/web/nest). For this, there was a sampling tube installed in the basement of the building. The blackwater only contains faeces, flushwater and toiletpaper, as the user interface in this building is urine-separated toilets (see sketch below).

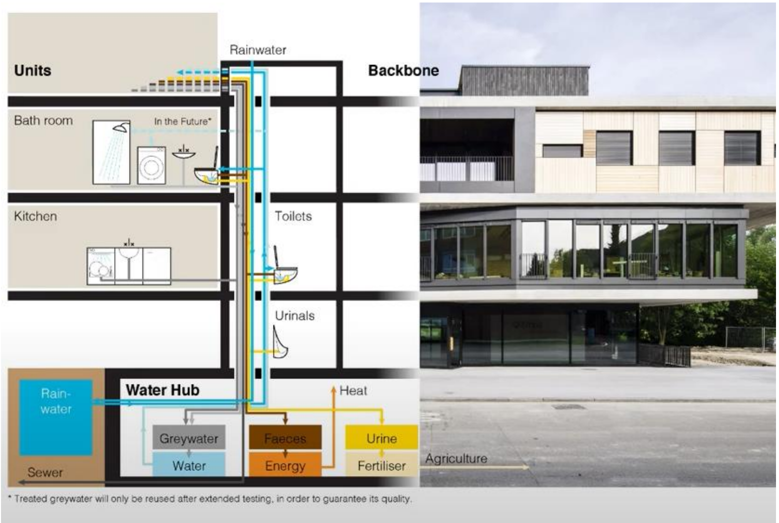

Figure 38: Schematic overview of the waste streams in the NEST building, Dubendorf. (Source: (https://www.empa.ch/web/nest)

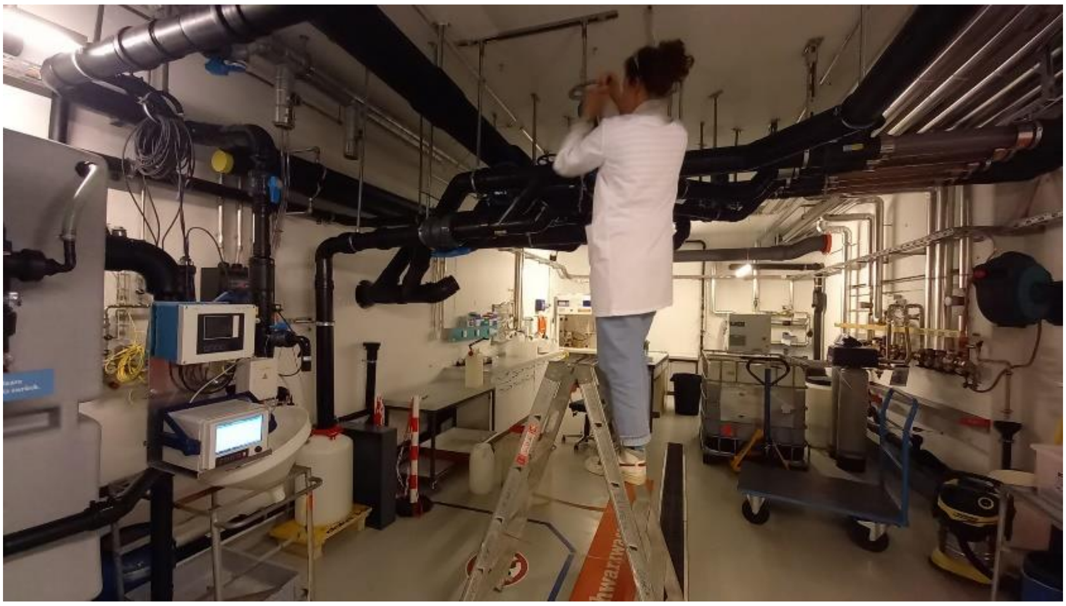

Figure 39: Picture of the BW sampling place in the basement of the NEST building at EAWAG.

Figure 40: Picture of sampled BW on 07/04/2022, on which the dewatering experiments were performed.

3. Composition of the primary effluent (wastewater after primary settling) that enters the Experimental Hall of the center of aquatic sciences in Dubendorf (EAWAG).

During previous theses performed in the Experimental hall (by Damian Hausherr), the composition of the primary effluent (liquid after primary settling) was determined. Additional information on the characterisation of the Primary Effluent of the Experimental Hall of EAWAG is presented in (Layer et al., 2019).

Table 16: Composition of the primary effluent that enters the Experimental Hall in EAWAG

Composition	Value	Unit
COD	469 ± 235	mg/L
sCOD	277 ± 189	mg/L
NO_3^-	0.356 ± 0.03	mg N/L
NH_4	25 ± 7	Mmg N/L
pH	7.17	-
COD/N ratio	24/1	-

4. Respirometry graph of 2 L activated sludge from the WWTP in Experimental Hall of EAWAG

To determine the OUR for endogenous respiration of the activated sludge sampled from the wastewater treatment plant in the Experimental Hall of EAWAG, 2 L of this sludge was added to the respirometer with the same settings as the other respirometry tests. In this way there could also be determined whether there was still some COD left in the sampled activated sludge. The figure below shows that for 2 L the OUR is around 11 mg O_2 L^{-1} h^{-1}. This means that per liter activated sludge, the amount used for the experiments, is 5.5 mg O_2 L^{-1} h^{-1}.

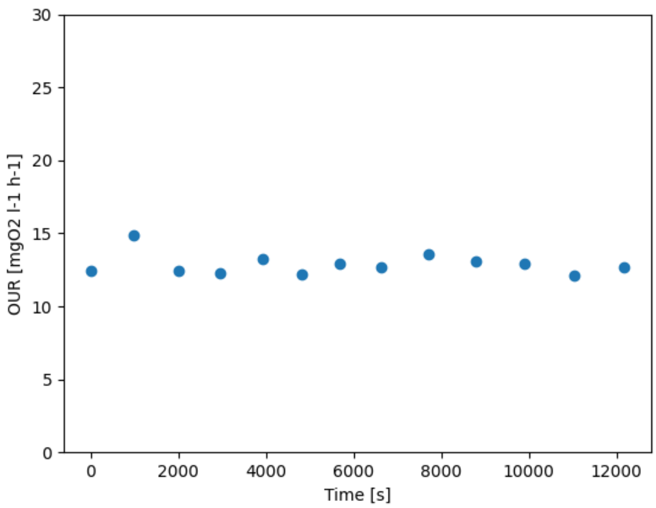

Figure 41: OUR graph of 2 L activated sludge from WWTP in Experimental Hall in Dubendorf.

5. Dry weight mass measurements SW1 experiments

First, the dry and the wet mass of the carrier at the start of the experiments were determined. After that, three dry weight masses were determined in 1 cycle each day of the SW1 experiment.

Table 17: determination of the mass of a carrier prior to the start of the experiments

	Value	Unit
Number of carriers	320,00	carriers
Count 1	248,00	g
Count 2	266,00	g
Count 3	276,00	g
Mean count	263,33	g
Std count	11,59	g
Mass of dry carrier	0.25	g/ dry carrier
Mass of wet carrier	0,82	g/ wet carrier

Cycles	Wet (g)	Dry (g)	Biofilm mass wet (g)	Biofilm mass dry (g)	**Average dry weight mass (g)**
cycle 4	0.8228	0.2845	0.0028	0.0045	0.0056
	0.8805	0.2857	0.0605	0.0057	
	0.8101	0.2866	-0.0099	0.0066	
cycle 7	0.8355	0.2928	0.0155	0.0128	0.0101
	0.7863	0.296	-0.0337	0.016	
	0.765	0.2815	-0.055	0.0015	
cycle 10	0.5194	0.2888	-0.3006	0.0088	0.0119
	0.5604	0.2939	-0.2596	0.0139	
	0.5396	0.293	-0.2804	0.013	
cycle 13	0.5176	0.2846	-0.3024	0.0046	0.006666667
	0.5801	0.2898	-0.2399	0.0098	
	0.5505	0.2856	-0.2695	0.0056	
cycle 16	0.621	0.308	-0.199	0.028	0.0162
	0.6268	0.2876	-0.1932	0.0076	
	0.6257	0.293	-0.1943	0.013	
cycle 19	0.5968	0.292	-0.2232	0.012	0.011433333
	0.5456	0.2955	-0.2744	0.0155	
	0.7215	0.2868	-0.0985	0.0068	
cycle 22	0.7397	0.2919	-0.0803	0.0119	0.011066667
	0.7773	0.2936	-0.0427	0.0136	
	0.5238	0.2877	-0.2962	0.0077	
cycle 25	0.8985	0.2812	0.0785	0.0012	0.008933333
	0.8037	0.3041	-0.0163	0.0241	
	0.8584	0.2815	0.0384	0.0015	

6. Nitrification rate for different pH in room temperature

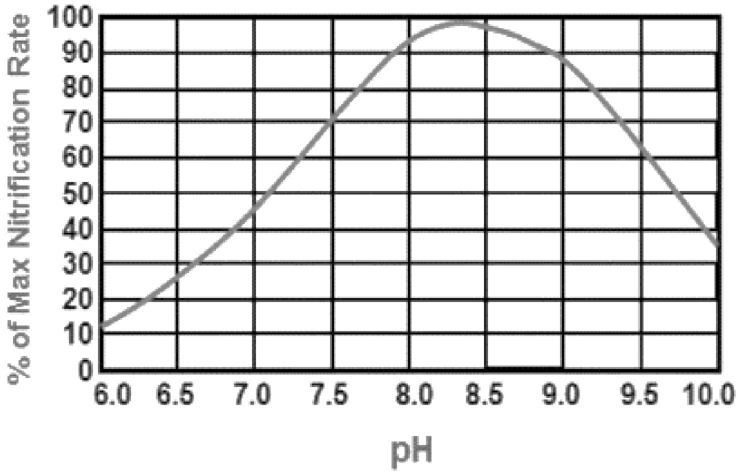

Figure 42: Nitrification rates for different pHs in room temperature. Source: (California Water Environment Association, 2022)

7. TSS measurements during SW1 reactor operation.

Sample	What	TSS (mg/mL)
HV701	influent	0.005
HV702	after filling	0.11
HV703	anoxic	0.085
HV706	end anoxic	0.23
HV708	aerobic	0.185
HV711	effluent	0.255
HV901	effluent	0.175
HV1001	influent	0
HV1002	after filling	0.03
HV1004	aerobic	0.03
HV1005	effluent	0.195
HV1201	effluent	0.17
HV1301	Influent	0.055
HV1303	Effluent	0.49
HV1501	Effluent	0.13
HV1601	Influent	0.12
HV1604	Effluent	0.035

8. IC and Hach Lange results research question 1.1 – running on 1 type of supernatant SW1- Findings of influence of storage and non-biodegradable tCOD fraction

Table 18 shows the IC and Hach Lange data of the first set of experiments on SW1 MBBR reactor operation. As described in the materials and methods. each measured cycle has 11 samples. Then there is a cycle with only 1 sample. being the effluent (e.g. HV301). The values in orange indicate the sCOD effluent values. from which the non-biodegradable fraction of SW1 was determined. The average value of the non-biodegradable fraction is 25.71 ± 5.09 mg O_2/L. The values in light blue indicate the different influent values of tCOD. sCOD and ammonium. The average value for tCOD is 192.37 ± 62.87 mg O_2/L. The average value for sCOD in the influent is 131.80 ± 46.57 mg O_2/L. The average value for ammonium is 85.27 ± 6.37 mg N/L. The high degree of variability in tCOD and sCOD can be dedicated to COD removal due to suspended growth in the barrels during storage. and the high temperature exposed to the supernatant in the lab during summer months. before it was pumped in the reactor.

After a while. no measurements were performed by Hach Lange (HL) analysis technique. as the IC data and HL performed similarly (differences less than 10%. considered values are indicated by the light green data) and IC analysis was easier to handle.

Table 18: Ion Chromatography (IC) and Hach Lange (HL) data from research question 1.1. '-' means that this was not measured.

Name	tCOD	sCOD	NH4	NO3	NH4	NO2	NO3
Unit	mg O_2/L	mg O_2/L	mg N/L	mg N/L	mg N/L	mg N/L	mg N/L
Measuring method	HL	HL	HL	HL	IC	IC	IC
HV101	244	186.8	*	0.006	88.1	<1	<1
HV102	114	74	23.1	0.32	22.1	<1	5.1
HV103	79	56.8	30.2	1.07	28.6	1.4	<1
HV104	80	58.2	28.1	0.506	25.7	<1	<1
HV105	76	17.22	28.1	0.277	25.3	<1	<1
HV106	128.6	47.8	26.9	0.877	25.4	<1	<1
HV107	123	45.2	20.4	4.97	19.2	<1	4.6
HV108	132	44	12.4	10.4	12.1	<1	11.6
HV109	149	45.4	6.26	14.8	6.7	<1	17.5
HV110	146	34.4	5.19	15.3	5.2	<1	17.4
HV111	-	34.8	5.29	15.5	5.5	<1	17
HV301	292	33.6	-	-	<0.5	<1	38
HV401	204	181.8	-	1.45	85.1	<1	1.2
HV402	80.8	70.4	22.9	22.8	23.1	<1	26.2
HV403	60.8	72.6	21.5	18.3	21.8	2.6	21.1
HV404	71.9	71.5	21.7	16.3	21.8	3	19.4
HV405	64.3	35.5	21.2	14.2	21.6	3.3	18.4

HV406	84.6	28.5	21.7	14.8	21.7	3.1	17.1
HV407	174	29.6	13.7	28.7	13.9	<1	28.1
HV408	178	26.1	0	-	1.5	<1	42.6
HV409	178/178/176	25.2	0	-	<0.5	<1	44.1
HV410	176	25.4	0	-	<0.5	<1	42.1
HV411	148	21.6	0.07	-	<0.5	<1	41.8
HV601	132	15	0	-	<0.5	<1	49.9
HV701	113.8	82.5	4	-	93.2	<1	1.4
HV702	79.3	25	24.8	-	24.8	1.2	32.4
HV703	66.5	22.5	21.2	-	21.3	3.1	29.2
HV704	65.3	23.3	21.1	-	22.1	3.4	27.2
HV705	63.5	21.1	20.6	-	20.7	3.5	26.5
HV706	112	18.5	14.7	-	14.7	<1	35.5
HV707	113	16.5	-	-	3.2	<1	37.2
HV708	97.7	16.5	-	-	<0.5	<1	50.8
HV709	116/121/113	19.4/15.3/15.8		-	<0.5	<1	50.4
HV710	-	13.9	-	-	1.4	<1	<1
HV711	138	21.3	-	-	0.5	<1	49.6
HV901	195.4	27.2	-	-	<0.5	<1	53
HV1001	110.8	162.2/155.6/153.8		-	90.3	<1	<1
HV1002	90.8	50.4	-	-	21	<1	39.7
HV1003	-	39	-	-	19	1.7	37.9
HV1004	188.6	32.2	-	-	15.6	0.7	37.8
HV1005	186.6	27.4	-	-	<2	<0.5	52.4
HV1201	352	28.4/26.6/25.6		-	<2	<0.5	53
HV1301	282	129.8	-	-	81	2.32	0.76
HV1302	78.2	40.6	-	-	19.8	0.97	41.2
HV1303	72.2	29.2	-	-	19.5	2.13	36.2
HV1304	-	-	-	-	<2	<0.5	58.3
HV1501	-	-	-	-	<2	<0.5	57.9
HV1601	-	-	-	-	85.4	<0.5	<0.5
HV1602	-	-	-	-	<2	<0.5	<0.5
HV1603	-	-	-	-	20.2	2.07	39.8
HV1604	-	-	-	-	20.5	2.27	37.6

2HV100	216	-	-	-	<2	<0.5	52.2
2HV101	199.6	78.1	-	-	73.9	<0.5	2.6
2HV102	163.8	54.5	-	-	25.3	1.6	32.6
2HV103	135	44	-	-	22.7	3.3	29.8
2HV104	115	45.8	-	-	20.6	3.3	28.4
2HV105	-	-	-	-	11.8	<0.5	41.5
2HV106	-	-	-	-	<2	<0.5	57.7
2HV107	-	-	-	-	79.6	<0.5	10.6
2HV108	-	-	-	-	22.8	1.4	40
2HV109	-	-	-	-	22	2.9	33.2
2HV110	-	-	-	-	19.3	1.1	37.7

9. Calculation COD fractions in SW1

One way to know the COD fractions (readily biodegradable. slowly biodegradable and non-biodegradable) of supernatant is to calculate the area below the respirometry graph by hand. Assumptions need to be made on where the slope change of the OUR graph determines the change to a different fraction of tCOD. The following calculations were made to determine the different fractions in SW1:

- **Endogenous respiration**

$$2 \times 2.6\, mg\ O_2\ h^{-1}L^{-1} \times 60\,000s = 2 \times 2.6\, mg\ O_2\ h^{-1}L^{-1} \times 16.67h = 86.6\ mg\ O_2\ L^{-1}$$

- **Slowly biodegradable tCOD**

$$\frac{(7.4 - 2.6)\ O_2\ h^{-1}L^{-1} \times 16.67h}{2 \times 1.5} = 26.6\ mg\ O_2\ L^{-1}$$

The 2 in the denumerator is for the area. the 1.5 is because of the fact that there was 1.5 L of SW1 in this experiment.

- **Readily biodegradable tCOD**

$$= (13.5 - 7.4) mg\ O_2\ h^{-1}L^{-1} \times 5.5h \times \frac{2}{3} \times \frac{1}{1.5} = 15.15\ mg\ O_2\ L^{-1}$$

10. IC and Hach Lange values of all the spiking experiments

Table 19: Hach Lange and IC values of each spiking experiment on SW1

Ratio	Description sample	Date	TSS (mg/mL)	Turb (NTU)	sCOD (mg O_2/L)	tCOD (mg O_2/L)	NH_4 (mg N/L)	NO_3(mg N/L)	NO_2(mg N/L)
ratio 3/1	r2 + r3 influent spiking 1	20-jun	0.07	40.2	147	210	58.7	<3.6	<3
	r2 after filling	20-jun	-	30.7	79.1	212	<32	56.2	<3
	r2 after filling + spiking	20-jun	-	-	940	1020	<32	28.7	12.8
	r2 anoxic 1	20-jun	-	52.1	3030	38050	<32	41.3	<3
	r2 anoxic 2	20-jun	-	-	334	353	<32	32.1	<3
	r2 end anoxic	20-jun	0.16	53.6	243.5	340	<32	44.5	<3
	r2 aerobic	20-jun	-	-	80	276	<32	55.4	<3
	effluent r2 spiking day 1	20-jun	0.13	98	74.9	208/237/298	<32	25.7	<3
	effluent after 3 cycles salts 1 r2	21-jun	0.28	136	58.4	193	<32	22.6	<3
ratio 29/1	r2 + r3 influent spiking 1	20-jun	0.07	40.2	147	210	58.7	<3.6	<3
	r3 after filling	20-jun	-	-	353.5	467	<32	<3.6	34.2
	r3 after filling + spiking	20-jun	-	-	527	31.8/50.7/56.7	<32	42.5	4.0
	r3 anoxic 1	20-jun	-	67.3	934	1110	<32	38.3	4.9
	r3 anoxic 2	20-jun	0.28	-	0	535	<32	36.8	<3
	r3 end anoxic	20-jun	0.305	71.9	157	179	<32	33.9	<3
	effluent r3 spiking day 1	20-jun	0.34	102	460	786	<32	19.8	<3
	effluent after 3 COD/N salts 1 r3	21-jun	0.135	66.9	276	550	<32	18.0	<3
90/1	influent cod/n spiking	21-jun	0.08	42.5	120	170	41.5	<3.6	<3
	r2 after filling	21-jun	-	16.6	0	0	<32	13.5	<3
	r2 after filling + spiking	21-jun	-	16.8	1290	0	60.3	8.1	<3
	r2 end anoxic	21-jun	0.235	-	117/96.7/102	2500	<80	<9	<7.5
	r2 aerobic	21-jun	-	-	17300	2960	<80	<9	10.0
	r2 effluent	21-jun	0.28	245	2120	2500	<80	<9	<7.5
	effluent 3rd cycle after spiking 2 r2	22-jun	0.28	71.8	423	635	<80	<9	<7.5
ratio 23/1	influent cod/n spiking	21-jun	0.08	42.5	120	170	41.5	<3.6	<3
	r3 after filling	21-jun	-	34.6	79	104	<32	34.5	<3
	r3 after filling + spiking	21-jun	-	17.9	0	0	<32	<3.6	8.1
	r3 anoxic 2	21-jun	0.16	-	3840	3680	100	<9	<7.5
	r3 end anoxic	21-jun	0.125	-	2300	4890	<80	<9	<7.5
	r3 aerobic	21-jun	-	-	1810	2020	<80	<9	<7.5
	r3 effluent	21-jun	0.45	105	2020	2380	<80	<9	<7.5
	effluent 3rd cycle after spiking 2 r3	21-jun	0.165	122	377	618	<80	<9	<7.5
ratio 1/4	influent r2 and r3 spiking 3	21-jun	0.15	-	65.8	230	<80	<9	<7.5
	after filling r2	22-jun	0.035	-	217	694	<80	<9	<7.5
	after spiking r2	22-jun	-	-	190	194	120	<9	<7.5
	r2 anoxic 1	22-jun	-	-	1247	288	<80	<9	<7.5
	anoxic r2	22-jun	0.175	87.2	221	476	297	<9	<7.5

	end anoxic r2	22-jun	0.247	107	358	582	279	<9	<7.5
	aerobic r2	22-jun	0.08	32.1	392	1750	47.5	<3	<3
	#no effluent				0	0	0	0	0
ratio 518/1	influent r2 and r3 spiking 3	22-jun	0.15	-	65.8	230	<80	<9	<7.5
	after filling r3	22-jun	0.05	-	2400	2780	<80	<9	<7.5
	after spiking r3	22-jun	-	-	16900	20100	<80	<9	<7.5
	supernatant wanneer foam r3	22-jun	0.595	454	13500	16400	<80	<9	<7.5
	anoxic r3	22-jun	0.86	377	16700	14300	<80	<9	<7.5
	end anoxic r3	22-jun	0.82	352	16000	15100	<80	<9	<7.5
	aerobic r3	22-jun	0.71	467	0	5630	<32	<3	<3
	# no effluent				0	0	0	0	0
rato 8/1	Influent r2+ r3	23-jun	0.065	60.9	146	195.8	<32	<3	<3
	After filling r2	23-jun	0.11	41.6	0	1740	<32	<3	<3
	Anoxic r2	23-jun	0.135	30	77.7	168	20.6	<3	<3
	effluent r2	23-jun	0.11	28.3	26.3	145	<32	<3	<3
ratio 4/1	Influent r2+ r3	23-jun	0.065	60.9	146	195.8	<32	<3	<3
	After filling r3	23-jun	0.525	458	0	0	52.8	<3	<3
	Anoxic r3	23-jun	0.605	583	0	0	55.7	<3	<3
	effluent r3	23-jun	0.77	735/738/738	0	668	<32	<3	<3
6x mono	r2 salts 1 influent	28-jun	0.06	36.5	1425	178.4	<80	<9	<7.5
	r2 salts 1 after filling	28-jun	0.05	6.84	41.3	75.88	<80	36.7	<7.5
	r2 salts end	28-jun	0.055	2.95	25.2	69/74.9/78.6	<80	<9	<7.5
4x dival	influent r2	29-jun	0.03	11	88.22	91.4	87.1	<9	<7.5
	after filling r2	29-jun	0.03	-	32.6	73.6	<80	35.9	<7.5
	End salts 2 r2	29-jun	0.03	30.4	25.4	81.5	<32	3.0	<3
2xmono. 2xdi	Influent r2 and r1	30-jun	0.035	36	114	135	37.8	<3.6	<3
	r1 after filling salts day 3	30-jun	0.05	64	34.8	132	<32	16.9	<3
	r1 end anoxic	30-jun	0.105	76.7	35.6	116	<32	13.9	<3
	r1 effluent after 3 cycles	30-jun	0.205	120	30.4	174	<32	18.0	<3
	r1 after 3 cycles of salts 3	1-jul	-	91.8	19.8	119	<32	25.0	<3
11x mono	Influent r2 and r1	30-jun	0.035	36	114	135	37.8	<3.6	<3
	r2 after filling salts day 3	30-jun	0.035	40.2	29.8	80.4/79.6/	<32	18.5	<3
	r2 end anoxic	30-jun	0	-	34.9	74.4	<32	15.4	<3
	r2 after 3 cycles of salts 3	1-jul	0.045	36	28.9	113	<32	24.8	<3
ph 8.5	influent pH 8.5 R1	1-jul	-	-	103	147	34.1	<3.6	<3
	r1 after filling pH 8.5	1-jul	-	47.2	33.8	101	<32	17.0	<3
	# no effluent				0	0	0	0	0
pH 10	influent pH 10 R2	1-jul	-	-	106	158	37.7	<3.6	<3
	r2 after filling pH 10	1-jul	-	79.9	32.4	103	<32	15.7	<3
	# no effluent				0	0	0	0	0

11. Comparison of the removal efficiencies of the different experiments

Table 20: Overview of removal efficiencies of each experiment. '-' means the value could not be calculated because of a missing value. A negative tCOD increase means a decrease in tCOD. '>' indicates that the effluent value was below detection limit of the IC

Experiment	COD/N ratio in reactor	Removal efficiency sCOD (%)	Removal efficiency sCOD in reactor (%)	Removal efficiency NH_4 influent (%)	Average denitrification in (difference in mg NO_3/L)	tCOD increase (mg O_2/L)
Baseline SW1	1/1	85.97	63.92	100.00	9.2	97.2
COD/N ratio 3/1	3/1	49.04	92.03	100.00	11.8	-827
COD/N ratio 90/1	42/1	-1666.67	17.83	96.14	9.5	
COD/N ratio 8/1	-	81.99				-1595
COD/N ratio 29/1	-	-212.93	-30.20	100.00	8.6	319
COD/N ratio 23/1	-	-1583.33	47.40	100.00	34.5	2380
COD/N ratio 518/1	-	-	-	-	-	-
COD/N ratio 1/4	1.8/1	-495.74	-80.65	19.08	-	1056
Salts 6x mono	1/1	82.32	38.98	>0.00	>0.00	-0.98
Salts 4x div	1/1	71.44	22.70	>63.00	33.0	7.9
Salts 2x div 2x mono	1/1	73.33	12.64	>15.00	3.0	42
Salts 11x mono	1/1	74.65	3.02	>15.00	3.1	32.6
pH 8.5	1/1	75.44	25.15	100.00	-	24
pH 10	1/1	78.49	29.63	100.0	-	17
Intermittent 1 week no aeration (SW2)	-	63.03	-	-	-	43.6
Intermittent 1 week aeration (SW2)	-	100.00	-	-	-	
Lebanon 1	3.6/1	80.09	80.00	100	2.0	590
Lebanon 2	1/1.5	57.71	26.16	100	-	633
Canadian	-	22.92	-	100	-	
Mix	1.5/1	91.88	66.45			-105
Blackwater	8/1	100.00	-	100		

12. Comparison effluent standards to surface waters

The concept of the MBBR is proven when a certain discharge standards can be met regarding COD and N removal. As discharge standards vary substantially per country. four standards were compared in this thesis. There was looked at standards from Switzerland. Uganda. Lebanon and US-EPA. The standards for Switzerland. Uganda and US-EPA are the ones for discharge in surface waters. For Lebanon this could not be found. therefore standards for Irrigation category 2 were taken. Category 2 means irrigation for fruit trees. lawns and landscape impoundments. such as water bodies where public contact with water is not allowed.

Regarding COD/BOD. it is easiest to reach the Lebanon standards. but there should be aimed for lower concentrations as such COD/BOD concentrations cause eutrophication of the surface waters. As for ammonium. Ugandan standards are least strict.

Effluent guidelines for established biofilm / attached growth media in conventional wastewater in solids fractionation parameters (I.e. Turbidity & TSS) are quite low; indicating that a pre-conditioning step for BW is likely very important. pH. EPS and EC are also listed as important parameters but lacking sufficient data on influent and effluent.

Table 21: Comparison of different effluent standards for discharge to surface waters

Parameter	Switzerland	Uganda	Lebanon (Irrigation category 2)	US-EPA
TSS	20 mg/L	50 mg/L	200 mg/L	50 mg/L
TDS				1500 mg/L
COD	60 mg/L O2	70 mg/L	250 mg/L	250 mg/L
DOC	10 mg/L			
Transparency	30 cm			
Turbidity				75 NTU
NH4+-N and NH3-N	2 mg/L N	10 mg/L		1 mg/L
NO2-N	0.3 mgN/L			
NO3-N		10 mg/L		
TKN		10 mg/L		
BOD	20 mg/L O2	50 mg/L	100 mg/L	50 mg/L
total P	0.8 mg P/L	5 mg/L		2 mg/L
total N	Remove as much as possible (??)	10 mg/L	5-30 mg/L	50 mg/L
pH		5-8.5	6-9	6-9
EC		1000 uS/cm		750 uS/cm
T				<30
Sulphates		500 mg/L		
Chlorides		250 mg/L		
Total Coliforms		400 CFU/100 mL	<1000 CFU/100mL	400 CFU/100mL
E. Coli				10 CFU/100mL
Helminth eggs			<1/L	

Red: more strict

Orange : less strict

Green : least strict

13. COD/N ratio results with higher ammonium concentrations (spiking 1/4)

Figure 44 shows the sensordata of the COD/N spikings from 21/06 to 27/06. The peak in ammonium presents the ¼ COD/N spiking. After that, the pH decreases, as there are high nitrification rates. After two days the ammonium concentration is again at 0 mg/L, reaching the strictest discharge standards. The nitrate sensor data in this graph is not reliable, as the nitrate does not increase as much as the ammonium graph increases. An other assumption is that nitrite levels are high, but this is not likely as this would increase the toxicity in the reactor, and nitrification still went well. If long days of only septic tanks of urinals are expected as loadings, and the alkalinity is not favorable, measures like alkalinity addition need to be considered.

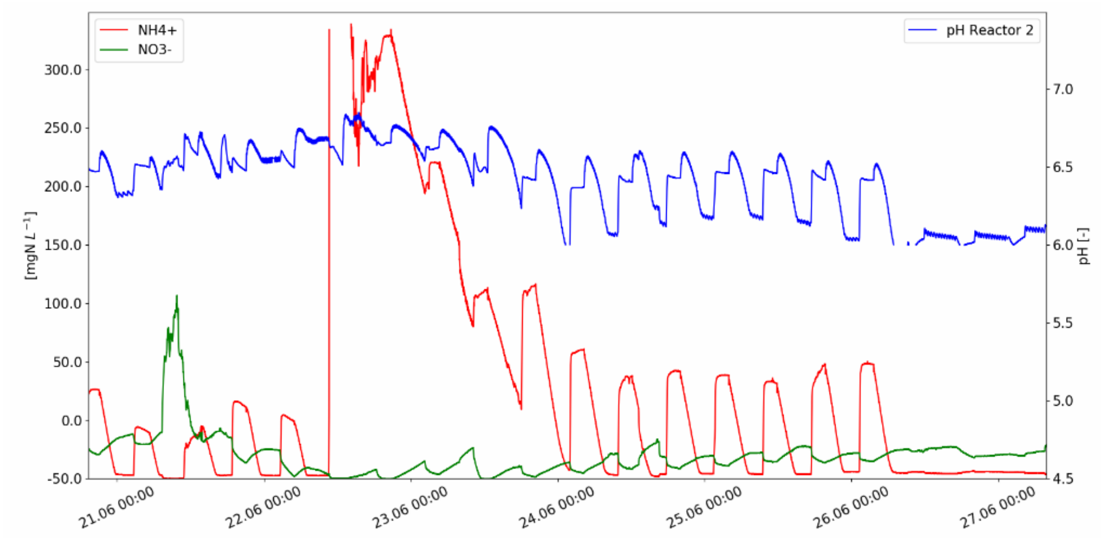

Figure 44: pH, ammonium and nitrate sensordata for the 1/4 COD/N ratio spiking experiment. Nitrate sensordata appear not to be correct.

14. Manual for Respirometry experiments

Respirometry Manual

As the respirometer was never used for supernatant COD fractionation. and never used in the MEWs group. during this thesis a manual was developed as knowledge transfer for future research. This was done through personal experience and with the help of Aurea Heusser and Valentin Faust. Each title shows a different step to be performed with some additional directions and explanations.

1. Set-up

The figure below shows the set-up of the respirometry device in Versuchshalle at Eawag.

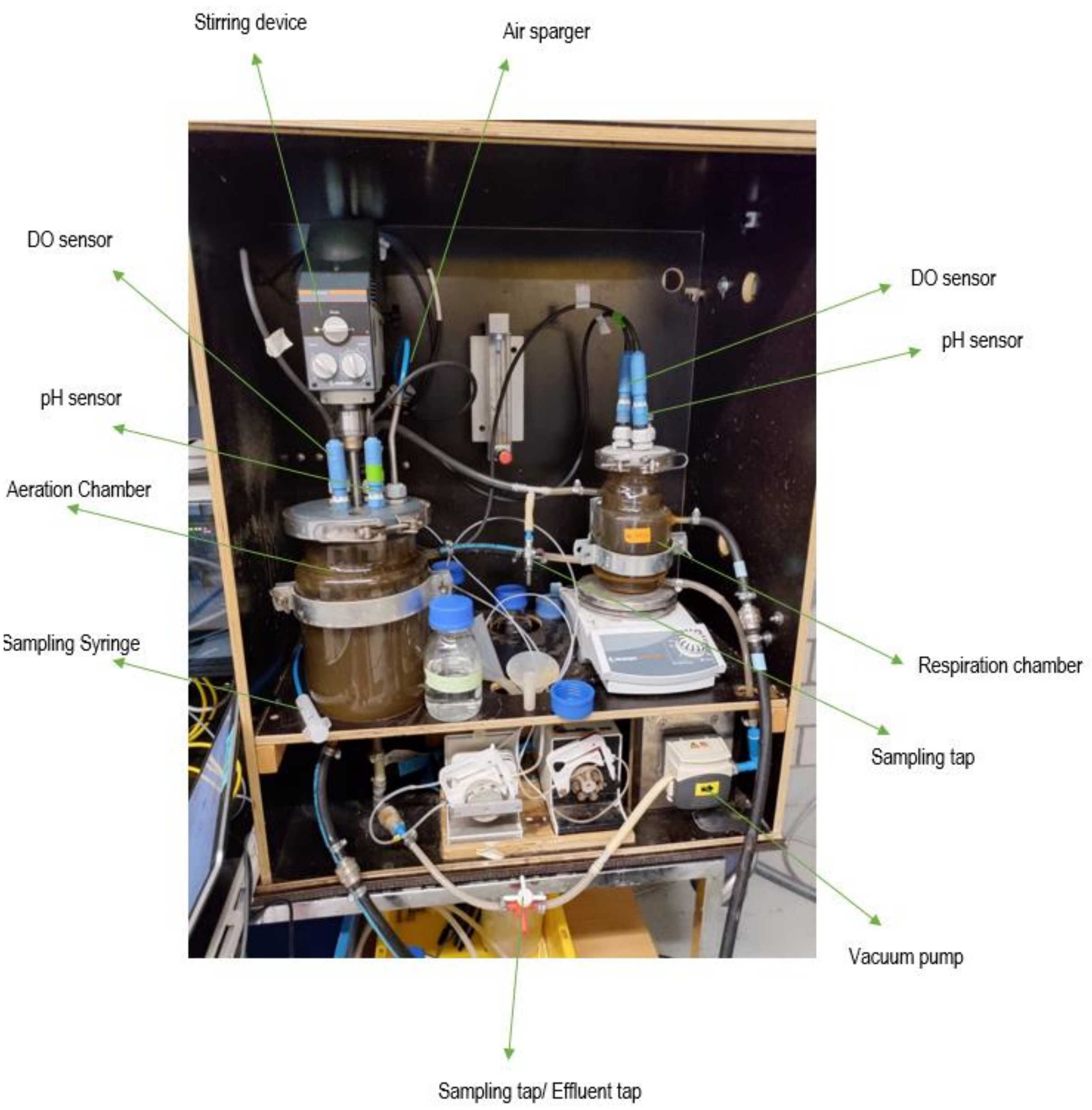

Figure 45: Overview of the respirometry set-up

2. Start-up of the system
- Make sure the computer is connected to the internet and charging
- Start the program ActSys

- Go to the tab 'online' (see Figure 2). and press Login
- Make sure the Endress Hauser sensor monitor is on (Figure below on the right)
- Calibrate all 4 probes

3. Running the experiments

For the respirometry experiments of supernatant. there is made use of single chamber measurements. but with double chamber DO registrations. This means that the aeration chamber will be filled up with activated sludge + supernatant. and mixed there. This mixture will obtain a set DO-level. This mixture will be pumped to the respiration chamber where the actual respirometry experiment will take place. The DO-level in this chamber is set according to your settings. When the DO is decreased to the set lower limit. the mixture from the aeration chamber (with higher DO level) will be pumped to the respiration chamber to increase the DO level again. This creates a DO saw tooth curve that can later be used for OUR calculations.

a. Settings:

General settings which apply for supernatant (and urine) respirometry:

- Wait time ~10s
- Delta DO min ~1mg/L
- Delta DO max~2mg/L
- Set DO max to 20 mg/L
- Set DO min to 0mg/L
- Use single but with both chambers

Settings to be determined through trial and error:

- Aeration control aerated chamber: 6-6.5mg/L (value on- value off)
- Aeration control (pump) Respiration chamber: 4-5mg/L (value on – value off)

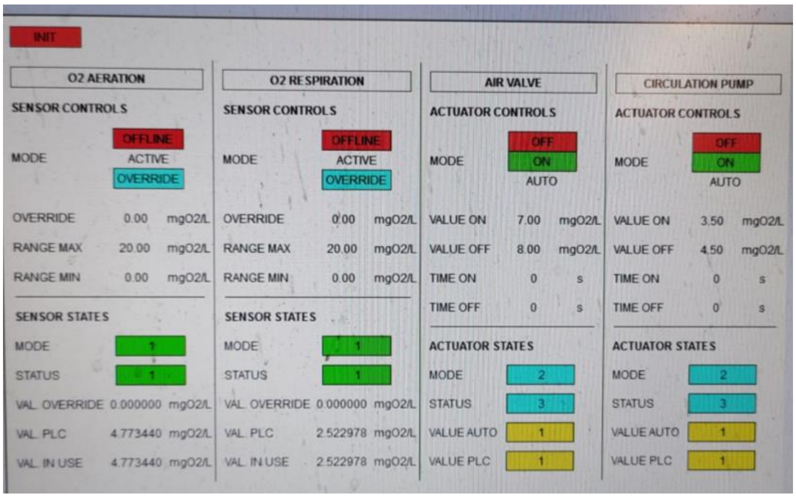

Figure 46: Pumping interface ActSys

It is important to have a big enough difference between the DO concentrations in the aeration chamber and the respiration chamber. to ensure a fast DO increase in the respiration chamber. This makes the respirometry experiment and OUR calculation more accurate. (Because the time the mixture needs to get to the higher set DO-level. COD is also being used up. and this will not be included in the OUR calculations. as only negative DO slopes are used for this).

4. Fill sludge

Through trial and error. the ratio activated sludge / supernatant should be determined. The total volume is 2-3 L. More activated sludge means that the respirometry experiment will go faster (faster COD removal if higher concentration organisms). Adding more supernatant and less activated sludge slows the experiment down. If the bCOD concentrations are expected to be quite low. more supernatant than activated sludge should be added. If a lot of bCOD is expected and a big fraction slowly biodegradable. the amount of activated sludge should be higher.

- Add nitrification inhibitor in the aeration chamber (if you do not want your organisms to use up oxygen for nitrification).
- Start mixing pump in the aeration chamber when sludge + supernatant + nitrification inhibitor is added
- Take 50 mL sample of start mixture and filter half of it and store at 4 degrees for later tCOD and sCOD Hach Lange analysis.

➔ Then go to 'online' and press 'Run'

5. <u>Mix a few minutes to reach wanted DO values in aeration chamber</u>

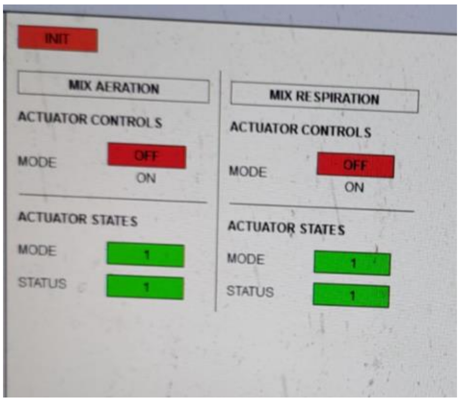

Figure 47: Mixing interface ActSys

6. <u>Start pumping the mixture to the respiration chamber</u>

This is done by putting the circulation pump 'on'.

!!!!!When the respiration chamber is completely filled. turn the circulation pump to 'Auto'. to make sure it only pumps liquid when DO-levels are exceeded !!!! (on the right on the pumping interface)

7. <u>Start registering the OUR</u>

The time needed for an experiment is not exactly known. I most of the time just let it run from the evening until the next morning.

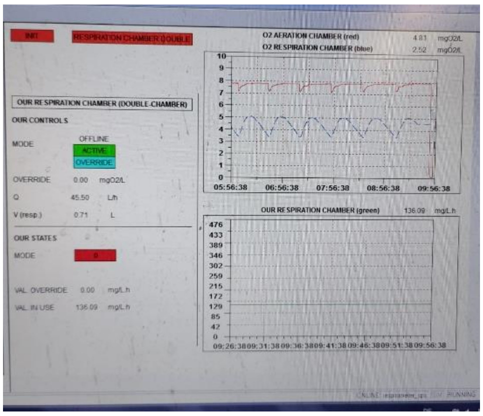

Figure 48: Single chamber OUR registration interface ActSys

8. <u>End of experiment:</u>
    - Online → stop
    - Online → log out
9. <u>Clean all tubes and reactors</u>
10. <u>Plug off</u>
11. <u>Data analysis</u>

The OUR should be plotted with the DO data obtained from the respiration chamber. A supernatant respirometry profile ideally looks like the figure below. Area 1 represents the readily biodegradable COD. Area 2 the more slowly biodegradable and below area 3 the oxygen used up for endogenous respiration.

To determine the baseline oxygen needed for endogenous respiration for that specific activated sludge. it is necessary to perform a respirometry test with 2 L of activated sludge.

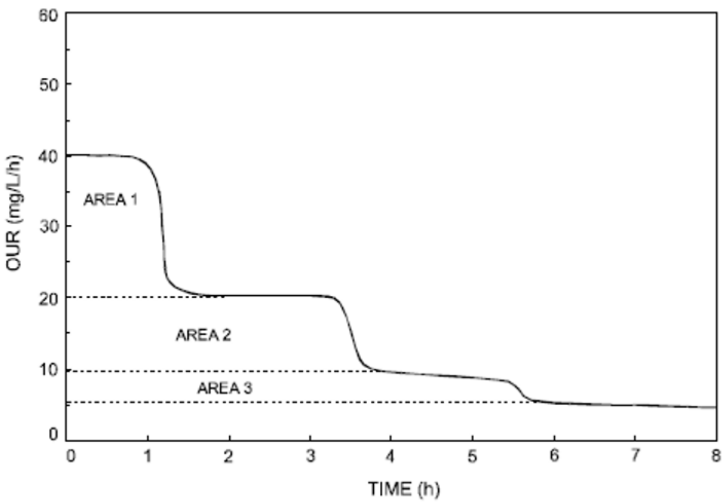

Figure 49: Ideal respirometry graph (Spanjers & Vanrolleghem. 2016).

15. Assessment of respirometry analysis for supernatant COD fractionation

As it was the first time that respirometry was used for the COD fractionation of supernatant, lessons were learnt and recommendations for next users can be made. These are summarized in this Appendix.

1. Respirometry on supernatant effluent after MBBR treatment. First, the respirometry graph of the effluent of Lebanon 1 is shown here. As it was decided that the level for endogenous respiration is 5.5 mg O_2 L^{-1} h^{-1}, it appears that all the biodegradable COD was degraded during the Le1 experiment. With this, it should be noted that a first experiment on only activated sludge is necessary, to assess the endogenous respiration level and to see whether there is bCOD in the activated sludge sample.

   However, already in this first trial of respirometry of supernatant, it has been proven extremely useful to perform the analysis technique on effluent, as this test is a matter of a flat line or a non-flat line, to assess whether the MBBR removed all the possible biodegradable COD in the supernatant.

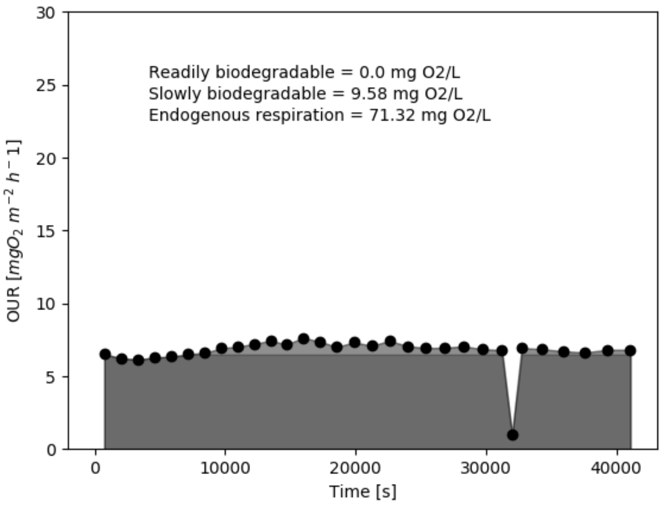

Figure 50: Respirometry graph effluent Lebanon 1. There is an outlier at 32000s.

2. The way the OUR values are calculated is via the negative slopes of the oxygen data. These need to be manually adapted in the python script in such a way that the lowest value and highest value do not exceed the graph. This would give mistakes to the OUR readings. As can be seen in the graph, the DO concentration was set between 3.5 and 5.5 mg O_2 L^{-1} h^{-1} in the respiration chamber. The DO-settings should be substantially higher in the aeration chamber to have an as vertical as possible positive slope.

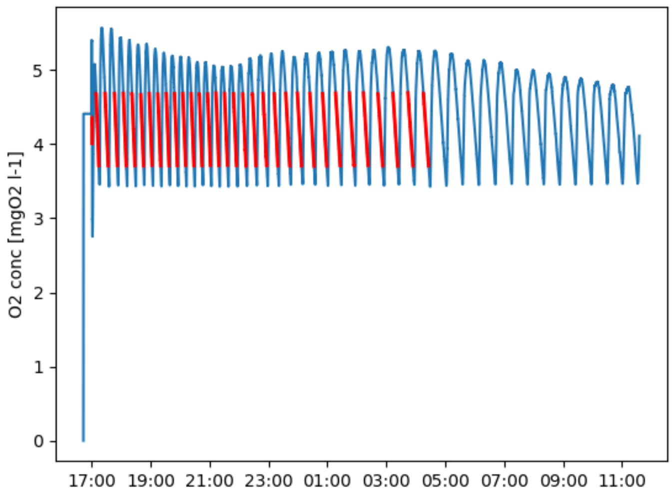

Figure 51: Exemplary DO-profile of a respirometry experiment (Le1). Indicated in red are the negative slopes for OUR calculation.

3. Figure 52 shows the oxygen data over a couple of days, with in red the slopes for the 'Mix' experiment. This figure shows that it is necessary to clean the sensors thoroughly after each experiment. The sliminess of the activated sludge and biofilm growth might jinx the experiments and not register the oxygen data properly. Then the OUR can not be calculated either.

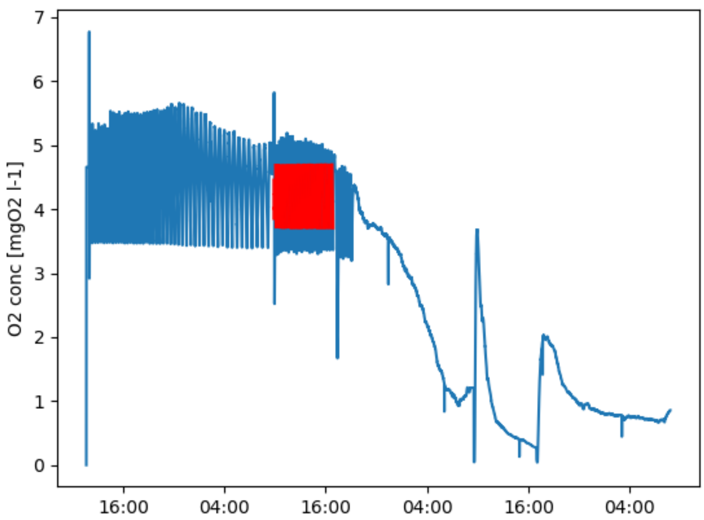

Figure 52: Oxygen sensordata from respirometry experiments. The red part indicates the slopes from the Mix influent experiment.

4. Multiple repetitions need to be done of one experiment to verify the experiments. Figure 53 below shows the duplicate of Le 1, which gives slightly different results than the graph shown in the Results and discussion.

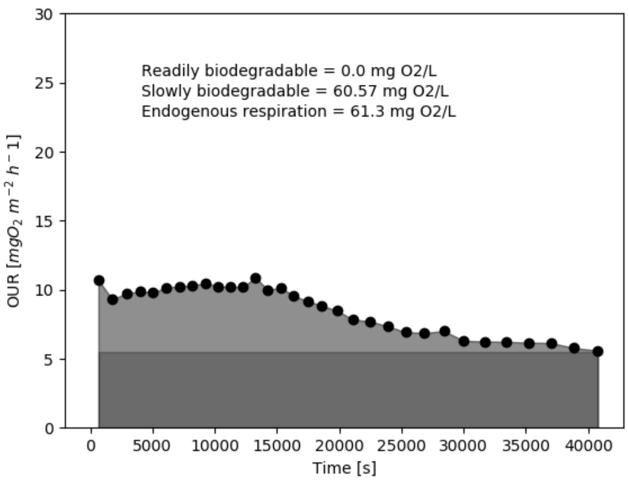

Figure 53: Respirometry duplicate of the Le1 influent

5. Furthermore, the supernatant to activated sludge ratio is an important parameter, and needs to be decided differently for each supernatant. If a big fraction of slowly biodegradable COD is expected, more activated sludge needs to be added, as otherwise the graph will go 'too slow' and a change in slopes will not be seen. If a high readily biodegradable fraction is expected, the portion of activated sludge should be lower, resulting that the experiment does not go too fast.
6. The duration of the experiment can be calculated by measuring the tCOD of the influent and then using the endogenous OUR to calculate how long it takes to use up all the COD. However, this will always be an underestimation. Therefore it is useful to perform the first duplicate experiment of the experiment an entire night.
7. It is important to note that tCOD measurements with Hach Lange are difficult, as the turbidity is high because of the activated sludge in the samples. Therefore the samples need to be diluted 100x and measured with a low COD concentration Hach Lange Test.