Granular Activated Carbon Biofilters for Greywater Treatment

by

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ABSTRACT

The demand for fresh water is steadily increasing as a result of the increasing human populations and activities. On the other hand, water sources are limited, especially as the global warming continues to change the quantities and distribution of water all over the world. Therefore, focusing the research efforts on finding alternate water sources is crucial. Utilizing used waters that were previously considered wastewater has a great potential to reduce the dependency on fresh water sources. Among these used waters is greywater, which is used water generated from domestic activities such as laundry, washing and bathing (excluding toilet and kitchen waste). Due to exclusion of major sources of contaminants, greywater has lower levels of contaminations given its considerably large volumes (50-80% of domestic combined wastewater). Therefore, greywater as a higher potential than domestic wastewater for on-site treatment and reuse. Thus, effective treatment technologies are needed to mitigate the health and environmental risks associated with reclaimed greywater.

In this study, a new design of activated carbon biofilters composed of two zones (unsaturated and saturated) in a single stage was developed for greywater on-site treatment to provide high-quality effluent that is safe for potential domestic uses or safe discharge into the environment. The treatment capacity of the developed technology was tested by evaluating its capability in removing major nutrients under different loading rates where the system achieved an average TCOD removal of 98% and complete nutrients removal throughout its 253 days of operation at highest hydraulic and organic loadings of 1.2 m^3 m^{-2} d^{-1} and 3.5 kg COD m^{-2} d^{-1}, respectively.

The capacity of the system to reduce pathogens was also tested against five pathogen surrogates representing four groups of pathogens (human skin-associated bacteria, human enteric bacteria, viruses, and protozoan cysts and oocysts). The system showed a range of reduction towards the pathogen surrogates ranging from no reduction in viruses to a log reduction of 3.4 in protozoan cysts and oocysts with an intermediate log reduction of 0.26-1.13 in bacteria. The individual capacity of each of the unsaturated and saturated zones was identified for reducing the major nutrients and pathogen surrogates.

Biofilm development and activity was also profiled along the biofilter's depth to show that a well-functioning biofilm developed within the system, and its mass and activity increased over time with the highest values observed at the top layers. The microbial community structure along the depth of the biofilter was analysed and results were reported at class and genus levels where the key microbes were revealed and the bacterial genus *Oleomonas* was found to predominate the system due to its unique and advantageous attributes.

The treatment processes taking place within the system were identified and their kinetics were measured to help understand the behaviour of the biofilter and potentially facilitate its design and operation. Since sorption and biodegradation are the two main treatment processes in biofilters, their individual contribution to the overall treatment was quantified. In a mechanistic study conducted on BAC media collected from the GAC biofilter, biodegradation was found to contribute 26% and 10% after 1 h and 24 h of treatment, respectively, while the rest was attributed to sorption processes. This finding suggested that intermittent dosing of greywater to the

biofilter is preferable due to the difference in removal capacities to allow for bioregeneration of the BAC media by the biodegradation process. A new method was developed to study the adsorption equilibrium and kinetics while completely eliminating the impact of the biofilms surrounding the GAC media. Testing the equilibrium adsorption experimental results against four isotherm models revealed that the Freundlich isotherm was found to best represent the equilibrium adsorption data. A study on the kinetics of isotherm showed that the pseudo-second order and intraparticle diffusion models were found to fit the adsorption kinetics. Intraparticle pore diffusion was found to be the rate limiting step after a few hours of treatment.

PREFACE

The contents of this thesis are my original work under supervision of Dr. Yang Liu, who has made significant contributions to all areas of this research. Several colleagues contributed to this research as listed below.

Ms. Bing Guo contributed to analyzing the microbial community analyses in Chapter 2, 3, 4, and 5. She also contributed to editing relevant sections in these chapters.

Dr. Nicholas Ashbolt and Mr. David C. Shoults (School of Public Health, University of Alberta) contributed to the development and implementation of the pathogen surrogates' methodology described and used in Chapter 2. They also contributed to editing the chapter.

Lastly, a version of Chapter 2 has been submitted for journal publication, a version of Chapter 3 and 5 have been published in journals.

To my beloved parents who gave me strength to pursue my doctoral degree. You were the backbone that kept me upright at the difficult times.

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Chapter 1 - General Introduction and Research Objectives

1.1. Background and Motivation

Although water covers about 70% of our planet's surface, less than 1% is available for human use, with the rest being costly to use or even inaccessible, especially as global warming continues to change the quantities and distribution of water all over the world. While these limited sources of water are constant, the demand for fresh water is steadily increasing as a result of the increasing human populations and/or activities. Therefore, it is crucial to adopt effective strategies to reduce the demand for water by improving water efficiency and conservation.

A significant amount of research has been performed to develop methods that would result in more efficient and conservative water use practices (Van Rossum 2020). This implies maximizing the benefits of used water that were previously considered wastewater. Among these used waters is greywater, which is used water generated from domestic activities such as laundry, washing, and bathing (excluding toilet and kitchen waste). Due to its lower contaminant levels and its considerably large volumes (50-80% of domestic combined wastewater; Z. Chen, Ngo, & Guo, 2013), greywater has a higher potential than domestic wastewater for on-site treatment and reuse. Thus, effective treatment technologies are needed to mitigate the health and environmental risks associated with reclaimed greywater.

The most commonly applied treatment method for greywater is sand filtration, However, many operational problems are associated with this technology such as clogging and poorly graded media (Dalahmeh et al. 2012). Therefore, alternative materials have gained more attention due to their enhanced physiochemical characteristics, such as granular activated carbon (GAC). GAC is well-known for its powerful adsorptive capacity and has been proven to be efficient in removing a wide variety of pollutants from water (Ahmaruzzaman 2008; Snyder et al. 2007; Toles, Marshall, and Johns 1997; Ahmed et al. 2015). In addition, using GAC as filter media allows for biofilm growth, resulting in a synergistic action between the adsorption and biodegradation processes so that the biological activity of biofilms regenerates the adsorptive capacity of the GAC (Aktaş and Çeçen 2007). Filters that combine these two major mechanisms (i.e. adsorption and biodegradation) are known as biofilters (also known as biological filters). Biofilters have been found to effective in treating greywater and can be used on-site on the household or neighbourhood scale (Moges et al. 2017; Jenssen and Vråle 2003). This research investigates and optimises the design and operation of a single-stage unsaturated/saturated granular activated carbon biofilters for greywater treatment.

1.1.1. Greywater characteristics

1.1.1.1. Greywater quantities

Generated quantities of greywater vary largely depending on a variety of factors such as lifestyles, the standard of living, demography, customs and habits, and water installations, and accessibility (Morel and Diener 2006). In general,

amounts of greywater generated in developed countries are 90-120 L p^{-1} d^{-1}. These quantities can remarkably decrease in low-income, water-stressed countries where water consumption can be as low as to 20-30 L p^{-1} d^{-1}. Moreover, smaller amounts can be generated in regions where people perform some water-dependent activities (e.g. showering) directly in the water bodies (e.g. rivers and lakes; Morel & Diener, 2006). For Canada, a recently-published study measured the greywater generation in 22 homes in Southern Ontario and reported its amounts to range between 28 and 124 L p^{-1} d^{-1} (Craig and Richman 2018).

1.1.1.2. Greywater quality

Characteristics of greywater varies largely depending on several demographic and socioeconomical factors such as lifestyle, social and cultural behaviour of the residents, availability of water, and its consumption (Jefferson et al. 2004). In a study that compared greywater characteristics from 18 countries, mean ranges of greywater parameters were as shown in Table 1.1 (Ghaitidak and Yadav 2013).

Table 1.1. Mean ranges for greywater characteristics from 18 countries. Adopted from Ghaitidak & Yadav, (2013)

Parameter	Mean range	
	Value	Unit
pH	6-9	-
Turbidity	12-2,131	NTU
Electrical conductivity (EC)	1.4-703	mS/m
Total solids (TS)	44-2,819	mg L^{-1}
Total suspended solids (TSS)	11-2,180	mg L^{-1}
Biochemical oxygen demand (BOD)	23-942	mg L^{-1}
Chemical oxygen demand (COD)	55-2,000	mg L^{-1}
Surfactants as methylene blue active substances (MBAS)	0.3-118	mg L^{-1}
Oil and grease (O&G)	7-328	mg L^{-1}
Total phosphorus (TP)	0.012-51.58	mg L^{-1}
Total coliforms (TC)	200-2.2E7	MPN
Faecal coliforms (FC)	13-1.9E7	MPN
Escherichia coli	10-3.9E5	MPN

1.1.2. Coupled GAC adsorption and biological degradation

In processes that combine GAC adsorption with biological degradation, such as GAC biofilters, pollutants in greywater (mostly surfactants) are removed from the bulk liquid by a combination of mechanisms including adsorption onto GAC, sorption into biofilms, and biodegradation by microorganisms. Adsorption of surfactants onto GAC has been previously researched (Wu and Pendleton 2001; González-García et al. 2004; Saleh 2006). The GAC was found to be efficient removing surfactants with an efficiency of 98% under optimum conditions.

Biodegradation of surfactants occurs using microorganisms that has the capability (i.e. metabolic pathways) to utilize surfactants as energy or nutrient source (Mungray and Kumar 2008). This process can take place under both aerobic and anaerobic conditions; however, surfactants anaerobic biodegradation is sometimes challenging (Palmer and Hatley 2018).

Aerobic biodegradation of surfactants has been previously investigated (González, Petrovic, and Barceló 2007). Various bacteria species were found to have this capability (Aloui, Kchaou, and Sayadi 2009). The biodegradation efficiency of surfactants using aerobic processes was reported to exceed 90% in most cases, and removal efficiency of 99.9% can be achieved (Mungray and Kumar 2008; González, Petrovic, and Barceló 2007). On the other hand, anaerobic biodegradation of surfactants can achieve lower removal efficiency of 40 to 85% (Haggensen et al. 2002). In addition, surfactants can cause inhibition of the anaerobic treatment process at higher concentrations (Aloui, Kchaou, and Sayadi 2009).

1.1.3. Biofilters for greywater treatment

1.1.3.1. Treatment performance in removing organics and nutrients

Dalahmeh et al. (2012) used unsaturated GAC, sand, bark and polyurethane foam biofilters (depth and diameter of 60 and 20 cm, respectively) to treat synthetic greywater (COD, TN and TP of 890, 75 and 4.2 mg L^{-1}, respectively). The biofilters were operated for 113 days under hydraulic (HLR) and organic (OLR) loading rates of 32 L m^{-2} d^{-1} and 14 g BOD_5 m^{-2} d^{-1}, respectively. Compared to sand, GAC biofilter showed higher efficiency in removing organics, surfactants, TN, TP, and thermotolerant coliforms. For the GAC and sand biofilters, BOD_5 was reduced by 97 and 75%, surfactants by >99 and 73%, TN by 98 and 13%, TP by 91 and 78%, respectively. In addition, the GAC biofilter efficiently removed surfactants during the start-up period prior to the formation of biofilm. The authors concluded that the GAC was one of the

most suitable media for biofilters treating greywater due to the aforementioned high performance. They also recommended challenging this kind of biofilters with higher loading rates and longer operation periods for further research.

The performance of similar GAC and sand biofilters, among a total of four tested media, treating the same greywater was also investigated under a range of HLR and OLR (Dalahmeh, Pell, et al. 2014). Increasing the HLR – but fixing the OLR – from 32 to 128 L m^{-2} d^{-1} caused an increase in the COD reduction of the GAC and sand biofilters from 76 to 90% and 65 to 86%, respectively. Increasing the OLR – at the same HLR – from 13 to 76 g BOD_5 m^{-2} d^{-1} also led to an increase in the reduction efficiency of all pollutants in both biofilters. It was concluded that, from amongst the four tested media, GAC had the highest capacity to operate efficiently under high OLR and HLR.

Niwagaba et al. (2014) used a multi-media filter made of layers of GAC (~60 cm; ~75% of total depth), mulch, geotextile, and gravel to treat real high-strength greywater generated by a household in a slum in Uganda (mean BOD_5, COD, TN and TP of 4,667, 7,307, 69.9, 24.1 mg L^{-1}, respectively). The filter was operated as a batch-type system and. HRT of the filer was controlled at 36 hr using an outlet valve, which caused the bottom portion of the filter to operate saturated during the 36-hr periods. The filter was operated at HLR and OLR of 60 L m^{-2} d^{-1} and 519-1,580 g BOD_5 m^{-2} d^{-1}, respectively. The filer was operated for 3 months and achieved BOD_5, COD, TN and TP removal efficiencies of 96.1, 90.8, 39.0, 30.1%, respectively.

1.1.3.2. Biological activity of biofilm grown on filter media

Dalahmeh et al. (2014) utilized the potential respiration rate (PRR) to assess the biological activity of biomass grown on activated charcoal and in two unsaturated biofilters treating (i.e. removing organic matter and nitrogen) synthetic greywater (COD and total nitrogen of 885 and 75 mg L^{-1}, respectively). They found that, at the top layer (0-2 cm), the biological activity of the activated charcoal was about four times higher than the sand (222 ± 34 and 56 ± 2 mg O_2 L^{-1}, respectively).

1.1.3.3. Microbial community structure

Truu et al. (2019) explored the composition of the bacterial community as well as its activity in vertical- followed by horizontal-flow filters treating municipal greywater. The dominant bacterial phyla were different in the two types of filters. For the vertical filters, the bacterial community was predominated with gammaproteobacteria, Bacteroidetes, and alphaproteobacteria. On the other hand, the bacterial community of the horizontal filters was predominated with firmicutes. The treatment performance was dependant on the diversity of the bacterial community and the abundance of specific genera. They suggested that the nitrogen was removed in the vertical filters by a coupled action of heterotrophic nitrification and denitrification.

Boon, Pycke, Marzorati, & Hammes (2011) investigated the bacterial community composition and dynamics in a full-scale GAC biofilter treating drinking water. They reported that the richness of the bacterial community in the top layer (0-10 cm) was low and did not show noticeable changes over time. However, at greater depths, the richness of the bacterial community increased

with time and depth. The highest removal of organic matter occurred at depth of 45-80 cm, at which the highest biomass concentration was observed. They concluded that the performance of a GAC biofilter does not depend primarily on the quantity of developed biomass, rather it depends on the quality of the biomass functionality resulting from its diversity and dynamics.

Niemi, Heiskanen, Heine, & Rapala (2009) explored the bacterial community composition of a GAC biofilter treating drinking water. They found that the community was predominated with the order Burkholderiales of betaproteobacteria with some abundance of Comamonadaceae.

Dalahmeh et al. (2014) have explored the dynamics and the functionality of the bacterial community in unsaturated activated charcoal and sand biofilters treating (i.e. removing organic matter and nitrogen) synthetic greywater (COD and total nitrogen of 885 and 75 mg L^{-1}, respectively). The depth, HLR, and OLR of the biofilters were 60 cm, 32 L m^{-2} d^{-1}, and 14 g BOD m^{-2} d^{-1}, respectively. Overall, both biofilters developed biofilms gradually with bacterial communities that were highly diverse and dynamic (i.e. changing considerably over time and depth). For the activated charcoal biofilter, the bacterial community was dominated by alphaproteobacteria (Rhizobium genus) and gammaproteobacteria (Pseudomonas and Acinetobacter genera) with the former dominating the top layer and the latter dominating the bottom layer. The dominance of these classes, on the other hand, showed less variation with depths for the sand biofilter.

Liao et al. (2012) studied the profile of the bacteria community along the depth of saturated activated carbon biofilters treating very low-strength (COD of

3-6 mg L^{-1}) lake water. They noticed an obvious decrease in the bacterial community diversity along the flow depth. They also found that alphaproteobacteria, gammaproteobacteria, and *Acidobacteria* predominated the bacterial community in general.

1.1.3.4. Pathogens and indicators in greywater and their fate in biofilters

Pathogenic organisms in greywater originate from three main sources: fecal contamination, opportunistic pathogens (i.e. pathogens associated the skin or respiratory organs), or food handling (Maimon et al. 2010). Many of the potential end uses of treated greywater, such as the residential uses (e.g. toilet flushing and garden watering) and agricultural uses (e.g. crops plantation and animal feeding), can bring it in contact with humans (Z. Chen, Ngo, and Guo 2013). Although the concentration of these pathogenic organisms in greywater is relatively low compared to mixed wastewater (Winward et al. 2008), they are still considered a major hazard that can pose risks to humans (Z. Chen, Ngo, and Guo 2013). Therefore, it is crucial to evaluate the pathogens removal capacity of greywater treatment options in order to ensure mitigating potential risks to the public health and/or the environment.

Biofilters support different types of removal mechanisms for pathogenic organisms (Peng et al. 2016). These types can be divided into two major groups: physical and biological mechanisms. Physical mechanisms include the removal of pathogens by filtration and by attachment. Filtration refers to the entrapment of pathogens by size exclusion, while attachment refers to the capture of pathogens due to attachment to biofilm. The filtration mechanism may occur in two ways:

mechanical filtration (entrapment at the top of the biofilter media) and straining (entrapment at narrow pores). The contribution of each mechanism to the overall removal depends on a variety of factors such as the size of microbes as well as grain and pore size distribution of the biofilter media.

Compared to filtration, the attachment mechanism allows for the capture microbes with smaller sizes by sticking to biofilms. The efficiency of this mechanism depends on the physicochemical properties of the water (e.g., ionic strength, pH, and presence of dissolved organics), the media (e.g., size, chemical composition, electrostatic properties, presence of biofilm, and adsorbed organics), and the microbes (e.g., surface properties, size, and shape; G. Chen & Walker, 2012; Rippy, 2015). Under unsaturated filtration conditions, the attachment mechanism can occur at interfaces between the different phases. For example, microbes can attach to the air-water interface due to strong capillary forces or be attached to the thin water film surrounding the filer media (Shang, Flury, and Deng 2009; Rippy 2015).

Biological pathogen removal mechanisms include the die-off and predation of microbes within the biofilter. The die-off mechanism means the decay of microbes due to a variety of reasons such as their age, competition for nutrients, temperature, and pH. Predation occurs when higher organisms, such as protozoa and bacterial predators, feed on the pathogenic microbes (Zhang et al. 2010; Zhang, Seagren, and Davis 2008).

Hydraulic retention time (HRT), in the context of this study, can be defined as the time greywater spends in both the two zones of the biofilter (i.e.

unsaturated and saturated zones) passing the GAC media. This HRT is directly proportional to the residence time of microbial indicators and/or pathogens in the biofilter, which has a significant impact on the residence-time dependent treatment processes, such as physical filtration of microbes (Peng et al. 2016). Higher HRT would result in longer residence time of microbes in the biofilter, leading to improved removal performance due to longer exposure to the removal processes (Eregno and Heistad 2019).

1.1.4. Aerobic Granular Sludge for greywater treatment

Several studies have concluded that aerobic biological processes, such as rotating biological contactors and membrane bioreactors, can effectively treat GW as compared to other technologies (Li, Wichmann, and Otterpohl 2009). Aerobic granular sludge (AGS) technology is an innovative biotechnological process that has shown high potential to replace conventional treatment technologies such as conventional activated sludge and moving bed bioreactors (Pronk et al. 2015). The advantages of the AGS over conventional activated sludge technologies include simultaneous removal of organics and nutrients, better settling ability, higher biomass concentration, and significantly less footprint and power consumption (Pronk et al. 2015). Many studies have investigated the treatment of domestic and industrial wastewaters using the AGS technology (Sarma, Tay, and Chu 2017). The first AGS full-scale operation started in 2010 in Epe, The Netherlands. Today, more than 30 wastewater treatment plants (WWTP) worldwide are effectively treating different types of wastewater (Pronk et al.

2015). Nonetheless, the treatment of GW using AGS has not been yet investigated.

1.2. Problem statement

Nowadays, the shortage in fresh water has become inevitable and it became a challenge for all governments around the world to improve the water efficiency and conservation in order to face this issue. In Canada, the government has been developing guidelines to encourage the use of reclaimed (i.e. treated) greywater in domestic applications to reduce the demand for fresh water. In Alberta, the government has also established the working groups to develop frameworks to facilitate the safe use of treated greywater for domestic applications such as toilet and urinal flushing, and subsurface irrigation.

However, in order to safely reuse the treated greywater, an efficient treatment system is required to mitigate the health and environmental risks associated with reclaimed greywater. Unfortunately, most of the previous studies have focused on utilizing treatment technologies – that have been commonly used in centralized domestic sewage treatment plants – in the treatment of greywater. This approach ignores a wide range of potential decentralized application of greywater reclamation that will be implemented on, for example, the household or neighbourhood scale and will be probably operated or even maintained by less-trained people. In addition, although greywater might seem better in quality compared to combined sewage, it has special characteristics that make it necessary to develop treatment technologies that are customized to these characteristics.

In response to the aforementioned challenges and to bridge the knowledge and technology gaps, our study developed an on-site greywater treatment system that is to be efficient, low-energy, and easy-to-operate. The design of the treatment system was customized to fit the quality of greywater as well as the operational needs. The performance of the treatment system was monitored to assure achieving a high-quality effluent that is safe for reuse in potential domestic activities or discharge to the environment. Removal mechanisms and kinetics were also investigated to further optimize the design of the system and facilitate its operation and maintenance.

1.3. Study objectives

There are two overall objectives subdivided into one or more specific objectives for the conducted research as per the following:

- **Overall objective 1:** Process evaluation for various technologies.
    - ***Specific objective 1a:*** to evaluate the performance of a GAC biofilter, sand and aerobic granular sludge (AGS) reactor in removing organics and nutrients from greywater.
- **Overall objective 2:** GAC process optimization and treatment mechanism evaluation.
    - ***Specific objective 2a:*** to optimize the treatment performance for COD and nutrients as well as to assess pathogen reduction capacity.

- ***Specific objective 2b:*** to investigate the adsorption characteristics and kinetics of the biofilter media using various equilibrium isotherms and kinetic models.
- ***Specific objective 2c:*** to assess the biofilm development and microbial community structure as well as contribution of biodegradation to the overall treatment process.

1.4. Thesis organization

This thesis consists of six chapters. A general introduction about the research background as well as the research objectives and its significance are presented in Chapter 1. Specifically, it encompasses a brief review of greywater and its treatment using GAC and biofilters and the motivation for the current research, research objectives, and thesis organization. The methodologies and the detailed experimental procedures, results, and discussions are presented separately in each chapter (Chapters 2-5).

Chapter 2 investigates the viability of a single-stage unsaturated-saturated GAC biofilter for greywater on-site treatment. The viability of the GAC biofilter was investigated by evaluating the performance of the system in removing major pollutants from synthetic greywater under different loading rates; assessing the biofilm development and activity; and analysing the microbial community structure within the system. The efficacy of the system in removing major pollutants was tested under a range of loading rates to optimize the operating conditions, with the contribution of each of the unsaturated and saturated zones

assessed separately. This study also included assessing the capacity of the system in reducing five types of pathogen surrogates representing four groups of pathogens, namely human skin-associated bacteria, human enteric bacteria, human enteric viruses, and Cryptosporidium and Giardia cysts and oocysts. The biofilm development and biological activity were quantified over time throughout the depth of the biofilter. Finally, the key microbes within the biofilter were revealed.

Chapter 3 explores the mechanisms and kinetics of biologically active GAC (BGAC) in treating greywater. The individual contribution of each of the sorption and biodegradation mechanisms to the overall treatment process was quantified. A new method was suggested and applied to eliminate the impact of biofilm in BGAC media to study the equilibrium and kinetics of the adsorption mechanism individually. The equilibrium adsorption data were assessed using four commonly used adsorption equilibrium isotherms, namely Freundlich, Langmuir, Temkin, and Dubinin-Radushkevich (D-R) isotherm models. Moreover, the kinetics of adsorption was evaluated using three models, namely, pseudo-first order, pseudo-second order, and intraparticle diffusion models. More insights were provided about the sorption processes and their limiting step. Chapter 4 compares different aspects of the performance of the GAC biofilter to those of a sand biofilter with the same configuration since sand is the most commonly used media in greywater filters. The capacity of the entire system as well as of each of the unsaturated and saturated zones individually in removing major pollutants was assessed and compared for both biofilters at different

loading rates. The development of biofilm on the GAC and sand media was assessed. The microbial community structure was profiled and compared along the depth of the two biofilters.

Chapter 5 presents the results of the preliminary stage of this research project. Our research project had initially proposed the AGS technology for greywater treatment. High strength synthetic wastewater was used to cultivate granules – as suggested by previous studies – prior to gradual shifting to greywater as the feed water. However, moving forward with this technology was hindered by one of the most common operational problems associated with this technology, which is the overgrowth of filamentous organisms and, ultimately, disintegration and washout of the granular biomass. Repetitive failure of the system triggered the shift to another reliable system as presented in Chapters 2-4. This chapter reports the impact of this issue on different aspects including granulation, treatment performance, and microbial community structure. Changes in the granulation process and impacts on its quality were observed and reported. In addition, the impact of filamentous overgrowth on the removal of carbon and nutrients was evaluated and possible reasons were suggested. Analysis of the eukaryotic and prokaryotic communities of the biomass revealed the organisms of concern, leading to further understanding of these operational issues.

Chapter 6 illustrates the main conclusions of the research presented in Chapters 2-5 along with recommendations for further research.

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Chapter 2 - Viability of a Single-Stage Unsaturated-Saturated Granular Activated Carbon Biofilter for Greywater Treatment[1]

2.1. Introduction

Although about 70% of our planet is covered by water, less than 1% is available for human use, with the rest being costly to use or even inaccessible (NASA Goddard Space Flight Center 2016). While these limited sources of water are constant, the demand for fresh water is steadily increasing as a result of increasing human populations and activities. Therefore, it is crucial to adopt effective strategies to reduce the demand for water by improving the ways in which we manage our water resources. A significant amount of research has been conducted to develop methods that would result in more efficient and conservative water use practices, including maximizing the benefits of "used" water, which was previously considered wastewater. Among these alternatives is the on-site treatment and reuse of greywater, which is the domestically-used water from household sources except toilets. Used kitchen water is also excluded in many cases depending on the overall wastewater management scheme (Li, Wichmann, and Otterpohl 2009). Greywater often has lower contaminant levels

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compared to other wastewater streams because most of the contaminants' loadings are excluded (such as faeces and urine); further, greywater typically makes up 50-80% of domestic water consumption (Chen, Ngo, and Guo 2013; Al-Jayyousi 2001). Given the relative lack of contaminants and large volumes, greywater is an attractive source for on-site treatment and reuse. However, risks still remain, thus effective treatment technologies are needed to mitigate both the health and environmental risks associated with the reclaimed greywater for a safe reuse.

Most of the previous greywater studies have focused on utilizing treatment technologies that have been commonly used in centralized domestic sewage treatment plants. However, this approach ignores a wide range of potential decentralized applications of greywater reclamation. For example, greywater treatment systems developed for the household or neighbourhood applications should have low maintenance requirements, as such systems will be probably operated or even maintained by less-trained people. Although greywater is of relatively better quality compared to combined sewage, it has special characteristics that require unique treatment technologies that are customized to these characteristics.

Biofilters are a low-energy technology that have been proven to be efficient for on-site greywater treatment (Jenssen & Vrale, 2003; Moges, Todt, Eregno, & Heistad, 2017). In biofilters, greywater percolates through filter media where various mechanisms take place such as media adsorption, biofilm biosorption, and biological degradation (Aktaş and Çeçen 2007). The most

commonly applied on-site treatment method for greywater is sand filtration. However, many drawbacks are associated with this technology such as clogging and poorly graded media (Dalahmeh et al. 2012). Therefore, alternative materials have gained more attention due to their enhanced physiochemical characteristics, such as granular activated carbon (GAC). GAC is well-known for its powerful adsorptive capacity and has been proven to be efficient in removing a wide variety of pollutants from water (Ahmaruzzaman 2008; Snyder et al. 2007; Toles, Marshall, and Johns 1997; Ahmed et al. 2015). In addition, GAC has been shown to facilitate the growth of biofilm, resulting in a synergistic relationship between the two processes as the biological activity of biofilms regenerates the adsorptive capacity of the GAC (Aktaş and Çeçen 2007). Filters that combine these two mechanisms (*i.e.* adsorption and biodegradation) are known as biologically active filters, or biofilters. Biofilters have been shown to be effective in treating greywater and can be used on-site at the household or neighbourhood scale (Moges et al. 2017; Jenssen and Vråle 2003).

The main objective of this research was to assess the viability of a single-stage unsaturated-saturated GAC biofilter for on-site greywater treatment. This was achieved by evaluating the system's capacity in removing major nutrients and pathogen surrogates from synthetic greywater under different loading rates. Biofilm development and activity was also profiled along the biofilter's depth. Lastly, the key functioning microbes within the biofilter were revealed by analysing the microbial community structure of the biofilter. The results acquired from this study provide insights into the efficacy, functionality, and design of

GAC biofilters as a commercially viable treatment technology that can contribute to maximizing water-use efficiency through on-site greywater treatment and reuse.

2.2. Materials and methods

2.2.1. Greywater formulation

Synthetic greywater was prepared according to the formulation suggested by the NSF/ANSI Standard 350 (NSF International 2012) to mimic combined bathing and laundry greywater. This included mixing a group of commercial cleaning agents, personal care products, and supplementary chemicals in tap water (Table 2.1). Characteristics of the synthetic greywater are summarized in Table 2.2. Synthetic greywater was prepared every two weeks and kept in the fridge at 4 °C until used to feed the biofilter.

Table 2.1. Synthetic greywater formulation adapted from the NSF/ANSI Standard 350 for combined bathing and laundry waters.

Component	Amount per 100 L	Brand
Body wash with moisturizer	15.90 g	Olay®
Toothpaste	1.59 g	Crest®
Deodorant	1.06 g	Gillette®
Shampoo and conditioner	21.20 g	Dove®
Lactic acid	1.59 g	ACROS Organics™
Bath cleaner	5.30 g	Lysol®
Liquid hand soap	12.19 g	Softsoap®
Liquid laundry detergent (2X)	18.80 mL	Tide®
Liquid laundry fabric softener	9.87 mL	Downy®
Na_2SO_4	1.88 g	Fisher Chemical™
$NaHCO_3$	0.94 g	Fisher Chemical™
Na_2PO_4	1.88 g	Fisher Chemical™
Secondary effluent[1]	2 L	-

[1] Obtained from a municipal wastewater treatment plant in Edmonton, AB.

Table 2.2. Characteristics of the synthetic greywater.

Parameter	Unit	Value	Targeted range[1]
TCOD	mg L^{-1}	347 ± 56	250-400
Total organic carbon (TOC; as C)	mg L^{-1}	59 ± 2	50-100
Total Kjeldahl nitrogen (TKN; as N)	mg L^{-1}	3.0 ± 0.2	3.0-5.0
Total phosphorus (TP; as P)	mg L^{-1}	2.5 ± 0.1	1.3-3.0
pH	-	7.2 ± 0.1	6.5-8.0

[1] According to the NSF/ANSI Standard 350 (NSF International 2012)

2.2.2. GAC biofilter setup

The experiments were performed in a laboratory-scale cylindrical column reactor (Figure 2.1) with a diameter of 9 cm and effective height of 60 cm (working volume of 3.8 L). The reactor's effective depth was packed with commercial GAC (MilliporeSigma; ref. 242233), which had a mesh particle size of 4-12 (1.7-4.8 mm). Characterization of the GAC media was previously described by Ahn et al. (2007). Briefly, the BET specific surface area, total pore volume, micropore volume, mesopore volume, and macropore volume were 520 m^2 g^{-1}, 0.543 cm^3 g^{-1}, 0.219 cm^3 g^{-1}, 0.122 cm^3 g^{-1}, 0.202 cm^3 g^{-1}, respectively. The biofilter was composed of two zones: an unsaturated zone at the top (40 cm depth), under-laid by a saturated zone (20 cm depth). The reason for integrating both unsaturated and saturated zones into the design of the biofilter was to combine the advantages associated with each. To clarify, the unsaturated zone supports passive aeration, and was proven to achieve high removal rates for organics (Dalahmeh et al. 2014). Besides, the saturated zone provides longer retention time, which was found to significantly improve the removal performance pathogens (Eregno and Heistad 2019). Previous research showed that organics removal was independent of the depth of the biofilter in the range of 20 to 60 cm, however, deeper filter depths were recommended for enhances pathogens removal (Jenssen and Vråle 2003). Therefore, the biofilter's total depth of the current study was selected to be 60 cm. The term "single stage" implies that the two zones were stacked on top of each other and there were no multiple stages in series or recirculation of water as

to reduce the footprint of the system. Aerobic conditions were maintained in the unsaturated zone through passive aeration, whereas the bottom saturated zone provided anoxic/anaerobic conditions to allow for nitrogen removal in case of increased nitrogen concentration in the influent.

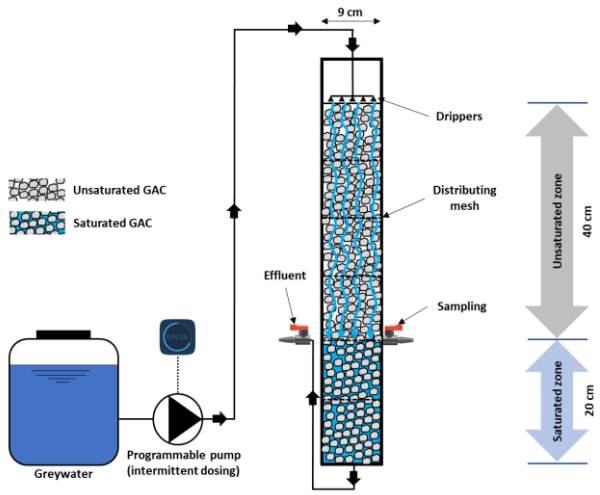

Figure 2.1. Schematic of the laboratory-scale single-stage unsaturated-saturated GAC biofilter for greywater treatment.

Synthetic greywater was pumped from the basin to the inlet at the top of the biofilter using a timer-controlled peristaltic pump (BT100-2 J, LongerPump®, China) and distributed using a network of drippers over a 2-cm-depth layer of epoxy-coated gravel with a particle size of 4-7 mm to ensure an even hydraulic loading of influent synthetic greywater in the active biofilter media underneath. The synthetic greywater then percolated through the GAC media and their covering biofilms. Uniform distribution of the synthetic greywater over the cross-sectional area along the biofilter's depth was maintained by adding polyester distributing-mesh with an opening size of 0.8 mm and open area of 46% every 10 cm along the biofilter depth. Another identical layer of epoxy-coated gravel was also added below the GAC to minimize dead zones within the GAC media.

Treated effluent exited the biofilter at its bottom. The depth of the saturated zone was maintained by connecting the outlet to an elevated port at a height of 20 cm above the biofilter bottom due to the principle of communicating vessels.

2.2.3. Feeding strategy and operation stages

Synthetic greywater was intermittently fed into the biofilter at one-hour interval to allow for effective GAC media bioregeneration to occur (Aktaş and Çeçen 2007). For each cycle, the synthetic greywater was fed over a five-minute interval at the beginning of the cycle, followed by a rest period for the rest of the cycle. The amount of synthetic greywater fed into the biofilter at each cycle was calculated according to the operation stage and the corresponding hydraulic loading rate (HLR). The rest of the cycle. The biofilter was operated under eight operation stages as summarized in Table 2.3.

Table 2.3. Operating conditions of the biofilter during the different operation stages.

Stage	Time (d)			HLR		OLR	HRT
	Start	End	Duration	(L m^{-2} d^{-1})	(cm d^{-1})	(g COD m^{-2} d^{-1})	(h)
I	0	30	30	71	7	22	40.3
II[a]	31	57	26	71	7	24	40.3
III	58	93	35	100	10	35	28.8
IV	94	138	44	150	15	54	19.2
V	139	155	16	250	25	78	11.5
VI	156	180	24	600	60	189	4.8
VII	181	195	14	900	90	333	3.2
VIII	196	253	57	1200	120	454	2.4

[a] Addition of secondary effluent to the synthetic greywater started from stage II as per the NSF/ANSI 350 formulation.

2.2.4. Water sampling and analysis

To evaluate the treatment performance of the system and the two zones individually, water samples were collected from the three sampling points: influent (directly from drippers), the interface between the unsaturated and saturated zones; and the final effluent port. Samples were analysed for total chemical oxygen demand (TCOD) according to the standard methods (APHA 1998) and for ammonia nitrogen (NH_3-N), nitrate (NO_2-N), total nitrogen (TN), and total phosphate (TP) using Hach kits and a spectrophotometer (Hach Co., Loveland, Colorado) as per manufacturer's instructions. The total organic carbon (TOC) was measured using the TOC-L TOC analyser (Shimadzu Corp., Japan) as per manufacturer's instructions. Biofilm mass was quantified as volatile solids (VS) according to the standard methods (APHA 1998).

2.2.5. Biofilm growth and biological activity

To confirm that the filter was biologically active, the biofilm growth and biological activity were assessed along the reactor's depth. The biofilm growth was assessed qualitatively and quantitively on day 218 using two methods: (i) imaging of the biofilm formed and attached to the GAC media using scanning electron microscopy (SEM) of the GAC media and (ii) measuring the mass of the volatile solids attached to the GAC media. SEM was performed on a media sample collected from the top 10 cm of the biofilter. Immediately after collection, the sample was fixed and dehydrated using a series of ethanol and hexamethyldisilazane solutions, followed by drying. Dried samples were then imaged using a Zeiss Sigma 300 VP-FE SEM microscope (Zeiss, Oberkochen,

Germany) after carbon coating. Volatile solids were measured along the GAC biofilter's depth following the standard methods (APHA 1998).

The biological activity of all viable microorganisms was quantitively measured along the depth of the biofilter at 10-cm segments to confirm that the filter was biologically active and to reveal the most active depths. The biological activity was assessed by measuring the adenosine triphosphate (ATP; Velten et al. 2007) concentration in GAC samples in triplicate using the Deposit & Surface Analysis (DSA™) kit (LuminUltra Technologies Ltd.) according to the manufacturer's protocol. GAC sampling was preformed by extracting the media of each 10-cm segment separately, mixing it to ensure uniformity, and collecting approximately 1 g of the media.

2.2.6. Reduction of microbial surrogates

2.2.6.1. Tracer test

A tracer study was conducted during stage VIII on day 208 using bromide (as sodium bromide) as a tracer to verify the HRT within the GAC biofilter and, accordingly, make sure that the pathogen surrogates' measurements were conducted under steady-state conditions. Bromide was selected due to its biological stability and reliability (*i.e.* remains in the phase in which it was injected; Kadlec & Wallace, 2009) to eliminate/minimize the loss of tracer mass due to degradation by microorganisms and/or adsorption on GAC. The tracer solution was prepared by dissolving sodium bromide in ultrapure water at a concentration of 50 g L^{-1} (38.83 g Br^{-} L^{-1}). The tracer solution was then pulse-injected to the biofilter immediately before a feeding cycle started. Since the

feeding strategy of the biofilter was intermittent, composite samples were collected from the effluent every hour before feeding for 10 hours, representing the average bromide concentration during one operating cycle. Bromide was analysed using a Dionex ICS-2100 ion chromatography (IC) system (Thermo Scientific, Waltham MA, USA) equipped with a standard bore separator Dionex IonPac AS18 IC column (2 mm × 250 mm), and the breakthrough curve was developed.

2.2.6.2. Pathogen surrogate study overview

The efficacy of the GAC biofilter in reducing pathogen surrogates was assessed on the last day of stage VII (day 195). A modified feed-water formulation was used during this experiment using a cocktail of pathogen surrogates along with yeast extract in place of the NSF/ANSI Standard 350 formulation to provide the same amount of carbon content as in the (TOC of 58 mg L^{-1}). This adjustment was performed to eliminate antimicrobial effects due the components of the NSF/ANSI Standard 350 formulation and only assess reduction mechanisms related to the system. The modified formulation was used to feed the reactor three days before spiking with the pathogen surrogates to ensure washout of microbes sourced from the feed water. Five pathogen surrogates were used to represent four groups of microbial pathogens as described in Table 2.4. The feed water was spiked with the pathogen surrogates' mixture and the biofilter was operated normally. Growth of some of the surrogates was expected due to the high yeast extract concentration, thus, composite influent grab samples were taken at the beginning (0 h) and end (6 h) of the sampling day in

order to estimate any background changes in surrogate concentrations. Using these two points, influent surrogate concentrations were estimated and adjusted when doing log reduction calculations. Composite grab samples were taken at the saturated/unsaturated interface and the effluent ports at 2, 4, and 6 h, and were combined to estimate log reductions. Table 2.4 outlines the surrogates used along with the pathogens they represent, their source, and enumeration method.

Table 2.4. Pathogen surrogates for the microbial reduction study.

Pathogen surrogate	Pathogen of interest	Source	Enumeration method
Staphylococcus epidermidis	Human skin-associated bacteria	ATCC 12228	Culturing on manitol salt agar (MSA)
Escherichia coli	Human enteric bacteria	ATCC 25922	Colilert™ (IDEXX Canada, ASTM Method #D6503-99)
Enterococcus faecalis	Human enteric bacteria	ATCC 29212	Enterolert™ (IDEXX Canada, ASTM Method #D6503-99)
Bacteriophage MS2	Human enteric viruses	ATCC 15597-B1	Double agar (Method 1601, U.S. Environmental Protection Agency, 2001)
Saccharomyces cerevisiae	Cryptosporidium and Giardia cysts and oocysts	Baker's Yeast (Active Dry, Fleischmann's, DE)	Culturing on malt extract agar (MEA)

All assays were performed at multiple dilutions and in triplicate. Standard errors were calculated to estimate the variance between sampling times and their assays.

2.2.7. DNA extraction and microbial community analysis

Six GAC samples were collected on day 218 at a vertical interval of 10 cm along the biofilter depth to profile the microbial community structure. Prior to collecting the samples, each 10-cm segment of the GAC media was mixed separately to ensure representability of the sample. DNA was extracted from these samples using a DNeasy PowerSoil Kit (Qiagen Inc., Toronto, Canada) according

to the manufacture's protocol. The DNA samples were amplified targeting the V3-V4 region of the 16S rRNA gene sequence by the polymerase chain reaction (PCR) using the primer sets with sequencing adaptors 515F (5'- ACA CTG ACG ACA TGG TTC TAC AGT GYC AGC MGC CGC GGT AA-3') and 806R (5'- TAC GGT AGC AGA GAC TTG GTC TGG ACT CAN VGG GTW TCT AAT -3') (Apprill et al. 2015; Parada, Needham, and Fuhrman 2016). The amplicons were then sent for barcoding and sequencing using an Illumina MiSeq (PE250) platform at McGill University and Génome Québec Innovation Centre (Montréal, Canada). Generated raw data was processed using QIIME 2 (release 2018.8) next-generation microbiome bioinformatics platform (Werner et al. 2012; Callahan et al. 2016). The taxonomy was assigned with 99% similarity using the Greengenes 16S rRNA gene database (release gg_13_5) according to Werner et al. (2012). Beta diversity and principal coordinates analysis (PCoA) of Bray-Curtis distance were performed using the "vegan" package (Oksanen et al. 2018) in RStudio version 3.4.1.

2.3. Results and discussion

2.3.1. Treatment performance

The GAC biofilter operated smoothly for 253 days. The biofilter was operated under eight stages (*i.e.* operating conditions) with incremental increases to the HLR and organic loading rate (OLR) and reducing the HRT, as shown in Table 2.3. Operating conditions of the biofilter during the different operation stages. Figure 2.2 shows the TCOD concentrations in the influent, unsaturated zone effluent, and final effluent as well as its removal efficiencies within the

unsaturated and saturated zones throughout the eight stages of operation. During these stages, the GAC biofilter consistently achieved an average TCOD removal of 98%, ranging between 84% and >99%. Most of the organics in the bathing and laundry synthetic greywater are surfactants sourced from the detergent agents and soaps. The adsorption of surfactants and their aerobic biodegradation were previously researched (González-García et al. 2004; Wu and Pendleton 2001; González, Petrovic, and Barceló 2007). The GAC was efficient in removing surfactants with an efficiency of 98% under optimum conditions. The biodegradation efficiency of surfactants using aerobic processes has been reported to exceed 90% in most cases, and a removal efficiency of 99.9% can be achieved (Mungray and Kumar 2008; González, Petrovic, and Barceló 2007). The effluent TCOD was 5.3 mg L^{-1} on average with a few peaks occurring when the HLR and OLR were increased from one stage to another. After stage VI, no sharp TCOD peaks were observed in the effluent; this can be attributed to the maturation of the biofilm grown on the GAC media. This maturation obviously was more significant in the saturated zone since peaks in the unsaturated zone effluent started to show up starting from stage VI.

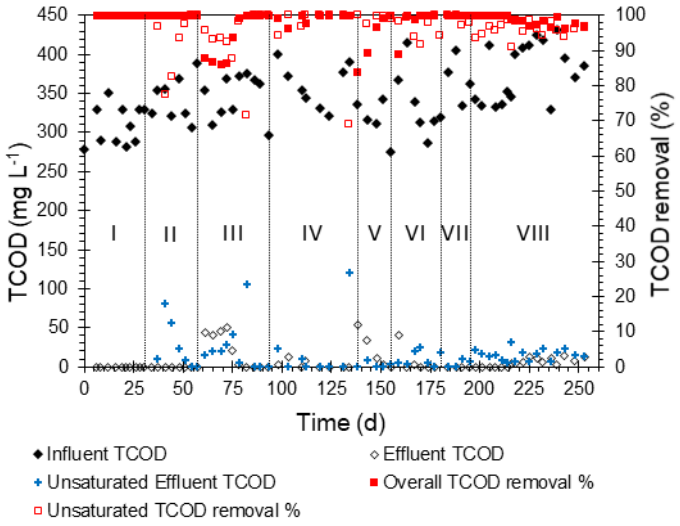

Figure 2.2. Concentration of TCOD (mg L^{-1}) in the influent, unsaturated effluent, and overall effluent as well as the removal percentage in the two effluents as a function of the GAC biofilter's operational time (d). The Latin numerals represent the operation stages.

The lowest HRT achieved was 2.4 hours with most of it occurring in the saturated zone, as the retention time in the unsaturated zone was less than 10 minutes. Despite the shorter HRT in the unsaturated zone, most of the TCOD removal (>95% of the removal in average) in the GAC biofilter occurred in this zone, while <5% was removed in the saturated zones. No clogging issues were observed throughout the operation period in the unsaturated or saturated zones. However, real greywater is expected to include more suspended and floating materials (*e.g.* hair and lint; Health Department of Western Australia, 2002) that might cause clogging to filters, so it is recommended to install an upstream retention tank with an intermediate wall baffle, a tee-connection inlet/outlet, and/or a coarse filter. In terms of nutrients, TN and TP in the NSF/ANSI Standard 350 formulation were originally low (Table 2.1) so they did not impose a

challenge on the biofilter as complete removal was consistently achieved in the unsaturated and final effluents.

2.3.2. Biofilm growth and biological activity

Greywater in general and the formulation used in this study in particular is composed of agents that can inhibit microbial growth and biofilm formation (*e.g.* antimicrobial agents); therefore, it was important to assess the biofilm formation using SEM imaging. The SEM images showed that biofilm developed on the surface of the GAC media, and rod-shaped microorganisms were clearly observed (Figure 2.3). The majority of these microorganisms were identified as the bacterial genus *Oleomonas* and are described later in the microbial community structure analysis section. Microorganisms, in general, tend to attach and form biofilm in areas where substrate is available for their uptake (Ha, Vinitnantharat, and Ozaki 2000). Adsorption of substrate onto the surface of GAC creates an opportunity for the microorganisms to thrive and develop biofilms. Although the SEM imaging only covered the outer surface of the GAC media, microorganisms are also expected to have grown in inner pores of the media and contribute to the treatment mechanisms (Ha, Vinitnantharat, and Ozaki 2000).

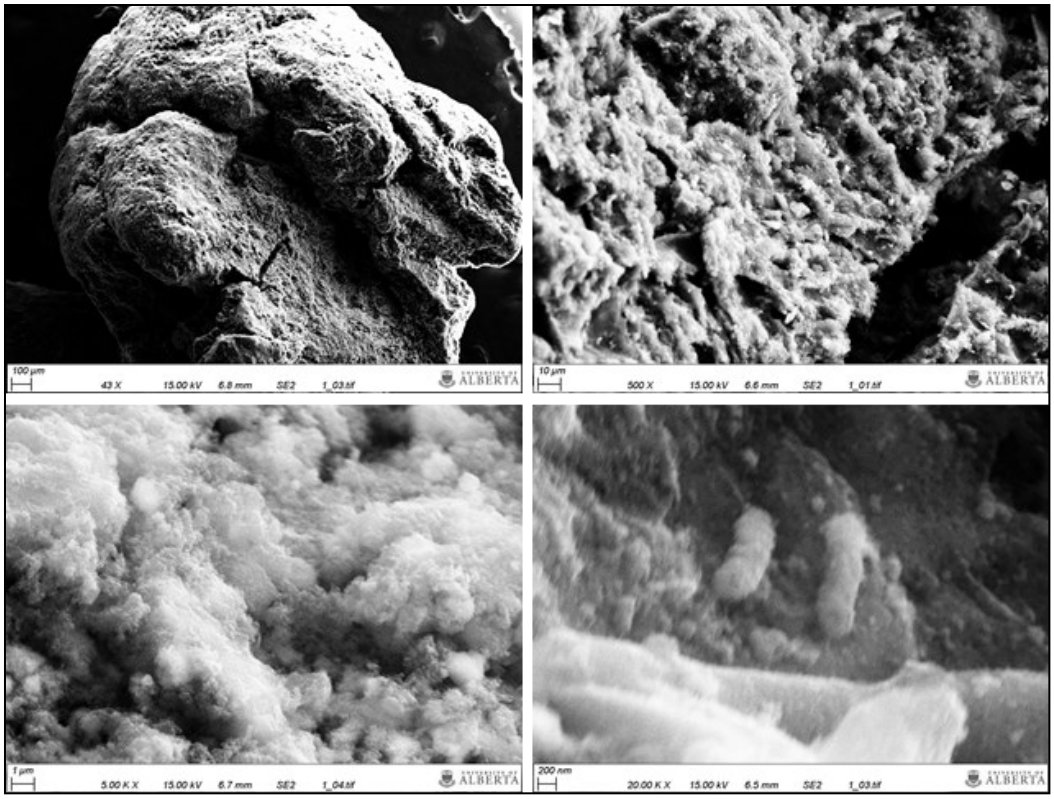

Figure 2.3. Scanning electron microscopy images of the GAC media collected from the GAC biofilter treating greywater.

The mass of the biofilm was also quantified as VS along the GAC biofilter's depth on days 160 and 218 to assess its development (Figure 2.4a). In general, the biomass increased over time and existed in higher amounts at the top layers then decreased into the depth of the biofilter. The VS ranged between 24-140 and 39-179 mg VS g^{-1} GAC in the unsaturated zone on days 160 and 218, respectively. Less biomass was developed in the saturated zone with ranges of 15-17 and 19-24 mg VS g^{-1} GAC on days 160 and 218, respectively. Figure 2.4b demonstrates the biological activity level profile of the developed biomass on days 160 and 218 along the biofilter's depth, measured as concentration of ATP as the primary energy carrier for all microorganisms. Similar to the biofilm mass,

the biological activity was higher at the top layers than the deeper ones and it increased over time, indicating higher levels of biological activity. The ATP in the unsaturated zone ranged between 0.33-1.03 and 0.03-0.63 µg tATP g^{-1} GAC on days 160 and 218, respectively. The saturated zone had less activity levels with an average of 0.01µg tATP g^{-1} GAC on day 160 and a range of 0.02-0.27 µg tATP g^{-1} GAC on day 218.

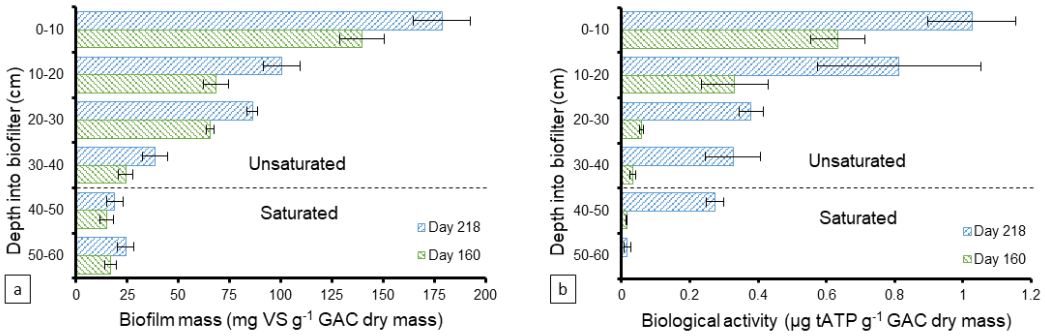

Figure 2.4. Development profile of the (a) attached biofilm mass (mg VS g^{-1} GAC) and (b) biological activity (µg tATP g^{-1} GAC) along the GAB biofilter depth (cm) at 10-centimeter intervals at days 160 and 218. The columns and error bars represent the average and standard deviation values, respectively, for triplicate measurements.

2.3.3. Reduction of pathogen surrogates

Figure 2.5 demonstrates the bromide tracer breakthrough curve. The tracer began to show up in the effluent after 2 h from injecting the tracer with the influent as a slug. This is comparable to the theoretically calculated HRT, which is 2.4 h. Since the samples were composite, the tracer concentration appeared as a sudden, flat peak that was observed for three consecutive samples then the concentration reached near zero. This pattern of breakthrough curve indicates that

the hydraulic behaviour of the GAC biofilter is near the plug flow. The results of this tracer test suggested that microbial concentrations throughout the reactor would likely be stable after 3 h of the initial injection.

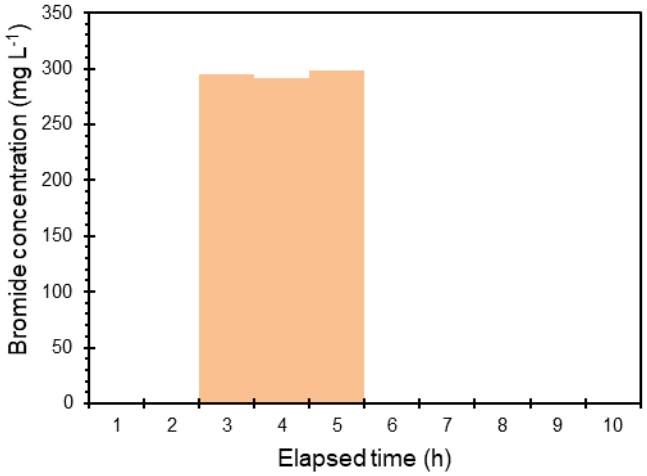

Figure 2.5. Bromide tracer test breakthrough curve.

Figure 2.6 shows the log reduction of the five pathogen surrogates: *S. epidermidis*, *E. coli*, *E. faecalis*, MS2 bacteriophages, and *S cerevisiae* within each of the unsaturated and saturated zones individually and the entire biofilter. The highest reduction occurred with *S. cerevisiae* (surrogates for *Cryptosporidium* and *Giardia* cysts) with an overall log reduction of 3.4 with contributions of 0.7 and 2.7 logs from the unsaturated and saturated zones, respectively. The higher capacity of GAC filters in removing oocysts compared to *E. coli* and MS2 bacteriophages was also reported by Hijnen et al. (2010), which might be contributed to the larger size of these oocysts. Hijnen et al. (2010) achieved *Cryptosporidium* and *Giardia* (oo)cysts log reductions of 2.7 and 2.1, respectively, from river water using a saturated GAC filter with a depth of 1.35 m operated under an HLR of 5,000 L m^{-2} d^{-1}.

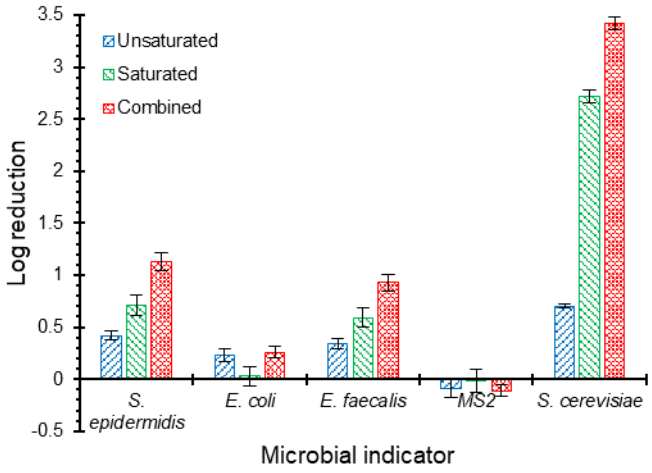

Figure 2.6. Log reductions of five pathogen surrogates: *S. epidermidis*, *E. coli*, *E. faecalis*, MS2 bacteriophage, and *S. cerevisiae* within each of the unsaturated and saturated zones individually and the entire biofilter. The columns and error bars represent the average and standard deviation values, respectively, for triplicate measurements.

In our study, the removals of *S. epidermidis* (log reduction of 1.1) and *E. faecalis* (log reduction of 0.9) were both lower than those for oocysts. It was observed that the unsaturated zone contributed to the log reduction by 0.4 and 0.3, while the saturated zone contributed by 0.7 and 0.6 for *S. epidermidis* and *E. faecalis*, respectively. Further, the biofilter had lower reduction towards *E. coli* with and overall value of 0.3, most of which occurred in the unsaturated zone. The limited *E. coli* removal in carbon-based filters was reported in previous studies (Hijnen et al. 2010; Afrooz and Boehm 2016). Afrooz and Boehm (2016) reported that the presence of biofilm on carbon-based media in stormwater biofilters significantly reduced *E. coli* removal, as the case in the current study. This reduction in *E. coli* log removal can be attributed to the altered surface properties such as roughness and hydrophobicity (Afrooz and Boehm 2016). Biofilms

significantly reduce the surface roughness of GAC media and create smoother deposition surfaces, which makes it less likely for bacteria (*i.e. E. coli*) to attach onto those smooth surfaces (Afrooz and Boehm 2016; Díaz et al. 2007; Arnold and Bailey 2000). In addition, biofilm formation results in a reduced hydrophobic interaction bacterial cells and biofilm surfaces (Afrooz and Boehm 2016), leading to a decreased *E. coli* log reduction.

No log reduction was observed for MS2 bacteriophages, which is consistent with previous studies that reported no reduction (Hijnen et al. 2010) or low reduction (Guy, McIver, and Lewis 1977; Scott et al. 2002; Persson et al. 2005) of viruses and bacteriophages using granular activated carbon filters. Many studies suggested that the deposition of microbial particles, such as viruses, is inconsistent with the classical colloid filtration theory (CFT) due to the presence of Derjaguin-Landau-Verwey-Overbeek (DLVO) repulsive interactions (Tufenkji and Elimelech 2005, 2004; Molnar et al. 2015), causing different colloidal behaviour and unfavourable conditions for deposition, as the case herein. Mechanisms controlling this behaviour have not been fully understood (Tufenkji and Elimelech 2005; Molnar et al. 2015). Nonetheless, experimental evidence suggested that anionic surfactants, which forms the majority of surfactants in greywater, are capable of masking uncharged or slightly positively charged regions, providing favourable conditions for deposition (Tufenkji and Elimelech 2005). This case was not available in this part of the study since greywater was replaced by a modified formulation that does not contain anionic surfactants as described earlier.

2.3.4. Microbial community structure

The 16S rRNA gene amplicons of the GAC biofilm were sequenced to identify the most abundant members of the bacterial community along the biofilter depth. At the class level (Figure 2.7), a clear pattern of niche segregation was observed between the unsaturated and saturated zones. The bacterial communities of the unsaturated zone were predominated by *α-Proteobacteria* (53-67%), *Actinobacteria* (2-16%), *β-Proteobacteria* (4-11%), *Chlamydiia* (≤8%), *γ-Proteobacteria* (3-10%), *4C0d-2* (4-7%), *δ-Proteobacteria* (2-7%), and *Bacteroidia* (≤1%). On the other hand, the unsaturated zone was predominated by *Thermotogae* (≤29%), *β-Proteobacteria* (13-18%), *α-Proteobacteria* (11-13%), *δ-Proteobacteria* (1-17%), *γ-Proteobacteria* (7-10%), *Clostridia* (3-13%), *Bacteroidia* (3-8%), *Bacilli* (≤5%), *Flavobacteriia* (2-5%), *Actinobacteria* (1-4%), and *4C0d-2* (1-2%). The increases in anaerobic bacteria in the saturated zone (*Thermotogae*, *Clostridia* and *Bacteroidia*) can be attributed to the significantly reduced oxygen availability in the saturated zone.

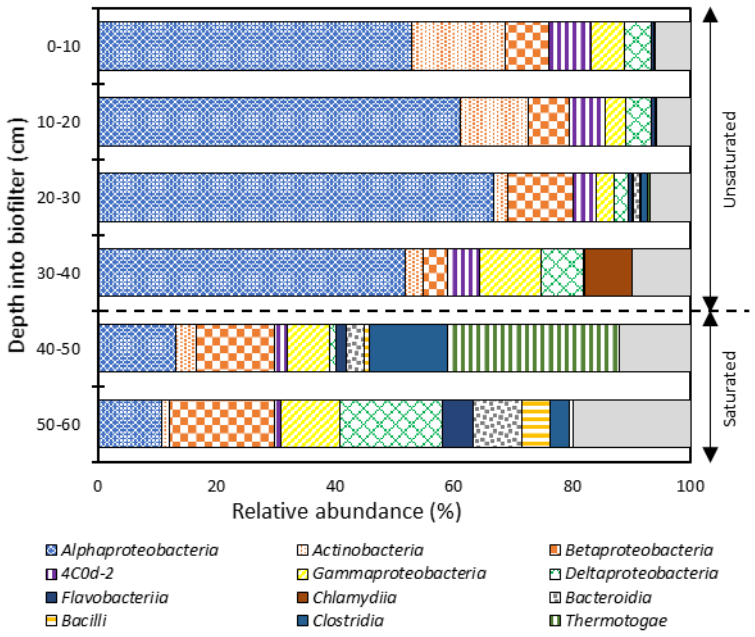

Figure 2.7. Relative abundances of the predominating bacterial phylotypes (> 1.0%) at the class level along the GAC biofilter depth.

The microbial community structure at the genus level is shown in Figure 2.8. The bacterial communities of the unsaturated zone were predominated by the *Oleomonas* genus, ranging from 29% to 49% for the entire zone. *Oleomonas* abundance was constant at 29% throughout the depth of the unsaturated zone except for the third segment at a depth of 20-30 cm where its abundance increased to 49%. *Oleomonas* is a hydrocarbon degrader that belongs to the alpha-*Proteobacteria* class (Kanamori et al. 2002). *Oleomonas* grows well under aerobic conditions, which were present in the unsaturated zone. The increased *Oleomonas* abundance can be attributed to the increased DO at deeper depths in the unsaturated zone due to longer contact between water and air, as DO was measured to be 4.3 mg L^{-1} after the unsaturated zone compared to 2.4 mg L^{-1} in the influent. Nonetheless, *Oleomonas* was also present in the saturated zone with

a relative abundance of <2% despite the zone being un-aerated. This finding is supported by previous studies which reported its growth under low oxygen conditions and possible seeding from the unsaturated zone continuously (Morikawa and Imanaka 1993; Fernández et al. 2008).

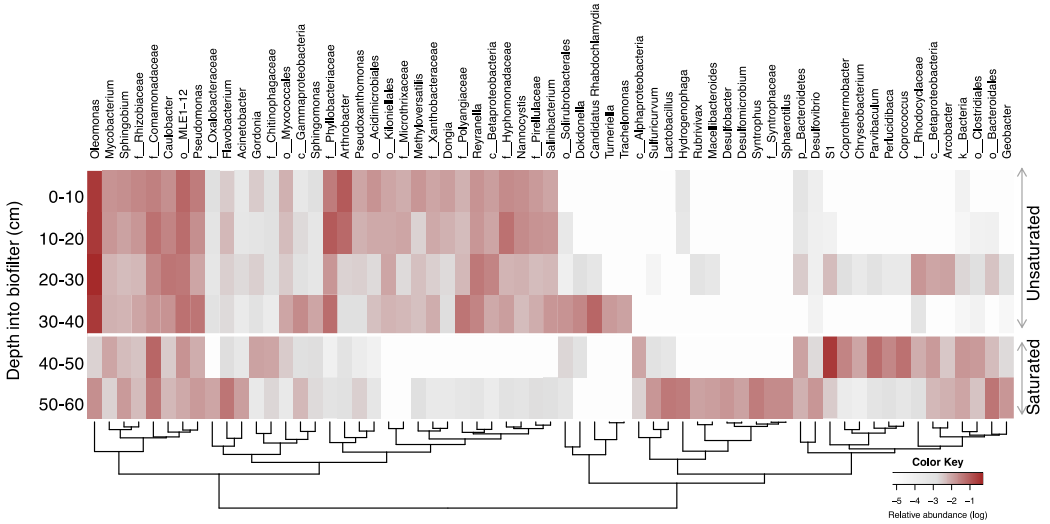

Figure 2.8. Heatmap of the genera with >1% relative abundance. Taxa are shown at genus level or higher (family: f_; order: o_; class: c_; phylum: p_; kingdom: k_) if not identified at genus level. Hierarchical clusters indicate similarities among families based on their fold changes using Euclidean distance method.

There were several genera that were common between the two zones: *Mycobacterium*, *Sphingobium*, *Caulobacter*, *Pseudomonas*, and unidentified genera from the families *Rhizobiaceae*, *Comamonadaceae*, and the order MLE1-12. These are often reported in aerobic wastewater treatment systems (McIlroy et al. 2015) with hydrocarbon-degrading functions (Guo et al. 2019; Kertesz* and Kawasaki 2010; Song et al. 2013).

The saturated zone at 40-50 cm depth was predominated by the genus S1 (29%) from *thermotogaceae* family, which was previously reported to have

predominated a microbial community in a system treating wastewater under anaerobic conditions (Ye and Zhang 2013). At the bottom segment of the biofilter (50-60 cm depth), there was no predominance of a single genus, and a few genera showed similar abundances, *Flavobacterium* (4.5%), unidentified genera in the order *Bacteroidales* (4.5%) and family *Comamonadaceae* (4.4%). Some unique genera with mixed features for oxygen requirements inhabited this layer, including anaerobic bacteria *Syntrophus*, a genus from *Syntrophaceae*, aero-tolerant bacteria *Lactobacillus* (Hammes and Vogel 1995), and facultative-aerobic *Hydrogenophaga* (Chung et al. 2007). Due to the scarcity of substrates, the microbial community may have been developed by opportunistic microorganisms, lacking in dominant species as compared to other layers.

Oleomonas exhibited some attributes that may explain why this genus predominated the microbial community of a biofilter. Firstly, it had a tendency to cluster in aggregates and produce extracellular polymeric substances (EPS), which allows its adhesion to support media- and biofilm formation (Fernández et al. 2008; Kanamori et al. 2002). The abundance of this genus can be advantageous in the early stages of biofilter operation; Fernández et al. (2008) reported that *Oleomonas* predominated the microbial community and initially colonized the media surface due to its characteristic of forming aggregates along with its versatility and broad metabolic flexibility. In addition, *Oleomonas* has the ability to biodegrade recalcitrant hydrocarbons under limited nitrogen conditions, which is the case with greywater as it is composed mainly of surfactants and limited amounts of nutrients. Moreover, being a Gram-negative bacterial genus

(Kanamori et al. 2002), *Oleomonas* possesses an outer membrane that has a natural resistance to detergents due to its structure, which contains lipopolysaccharides (Anderson and Yu 2005; Saimmai et al. 2012). This property allows the *Oleomonas* genus to overcome the high content of surfactants present in the greywater.

2.4. Conclusions

A single-stage unsaturated-saturated granular activated carbon (GAC) biofilter was developed for on-site greywater treatment. With highest hydraulic and organic loadings of 1.2 m^3 m^{-2} d^{-1} and 3.5 g COD m^{-2} d^{-1}, respectively, and a shortest retention time of 2.4 h, the system maintained an average TCOD removal of 98% and complete nutrients removal throughout its 253 days of operation. The system showed a range of reduction towards pathogen surrogates representing human skin-associated and enteric bacteria, viruses, and protozoan cysts and oocysts. A well-functioning biofilm developed within the system, and its mass and activity increased over time with the highest values observed at the top layers. The key microbes within the biofilter were revealed, and the bacterial genus *Oleomonas* predominated the system due to its unique and advantageous attributes.

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Chapter 3 - Mechanisms and Kinetics of Greywater Treatment Using Biologically Active Granular Activated Carbon[2]

3.1. Introduction

Urban growth has placed a burden on fresh water sources (UNESCO and UN-Water 2020), making the reclamation of used waters essential. Greywater is the water generated from domestic sources (e.g., bathing, laundry, dishwashing) that exclude fecal contamination (Li, Wichmann, and Otterpohl 2009). Greywater makes up to 50-80% of domestic water use (Chen, Ngo, and Guo 2013; Al-Jayyousi 2001). Excluding major sources of contamination from greywater (i.e. human excreta and kitchen discharges), as well as its large amounts, makes it a good candidate for reclamation. The major source of contamination in greywater is the surfactants (short for surface active agents) originating from soaps and detergents that would cause problems such as foaming (Mousavi and Khodadoost 2019) and adverse effects on soils when used for irrigation (Wiel-Shafran et al. 2006; Shafran et al. 2005; Mohamed et al. 2018) if greywater was not treated properly prior to reuse.

[2] A version of this chapter has been published in Chemosphere as: Sharaf, Ahmed and Yang Liu. "Mechanisms and kinetics of greywater treatment using biologically active granular activated carbon." *Chemosphere* 263 (January): 128113.

Filters with media covered with biofilm (biofilters) were reported to be an effective solution to treat greywater given their high treatment capacity and low energy requirements (Jenssen & Vrale, 2003; Moges, Todt, Eregno, & Heistad, 2017). Granular activated carbon (GAC) was found to be one of the best media for biofilters due to its ability to adsorb a wide spectrum of pollutants from aqueous solutions (Ahmaruzzaman 2008; Snyder et al. 2007; Toles, Marshall, and Johns 1997; Ahmed et al. 2015) and its capability to support biofilm formation, which help biodegrade pollutants in the bulk solution as well as bioregenerate the GAC media, increasing its lifespan (Leong et al. 2018; Klimenko et al. 2003; Aktaş and Çeçen 2010, 2007).

The superior treatment capacity of GAC biofilters is due to the integrative action of various mechanisms that take place simultaneously within the biofilters' media, including biofilm absorption (biosorption), biological degradation (biodegradation), GAC adsorption, and others (Çeçen and Aktaş 2012). Defining and understanding the functioning mechanisms in GAC biofilters along with their kinetics is essential information for a good design and operation of a system as well as technology improvement. A few recent studies have investigated the adsorption kinetics and equilibrium isotherms of GAC treating greywater (Amiri et al. 2019; Alharbi et al. 2019). However, the media used in these studies was fresh GAC with no biofilm, missing the important role of the biofilm in biodegrading the pollutants existed in the bulk liquid and/or accumulated onto the GAC's surface, bio-regenerating its adsorptive capacity (Aktaş and Çeçen 2007). Another study focused on the biosorption and biodegradation mechanisms of only

biofilm that was previously grown on plastic inert media (Song et al. 2017). Obviously, the focus of all of these studies cannot be directly used to evaluate the mechanisms of GAC biofilters treating greywater. Thus, there is a clear gap in the literature in understanding the mechanisms and kinetics of GAC biofilters treating greywater.

In this context, the overall goal of this research was to understand the mechanisms and kinetics of greywater treatment using biologically active GAC (BGAC) as suggested media for biofilters. This was achieved by: (i) assessing the role of each of the sorption and biodegradation mechanisms to the overall treatment process, (ii) characterizing and modelling the adsorption capacity of the media, and (iii) analysing and modelling the kinetics of adsorption. The knowledge acquired from this study provides broad understanding of the different mechanisms taking place in GAC biofilters, leading to better design and operation.

3.2. Materials and methods

3.2.1. Greywater formulation

The mechanistic and kinetic experiments in this study were performed using synthetic greywater that simulates combined bathing and laundry greywater (NSF/ANSI Standard 350, NSF International 2012) The greywater was prepared by mixing commercial cleaning agents, personal care products, and supplementary chemicals in tap water (Table 3.1). Table 3.2 shows the characteristics of the synthetic greywater.

Table 3.1. Greywater formulation adapted from the NSF/ANSI Standard 350 for combined bathing and laundry waters (NSF International 2012)

Component	Amount per 100 L	Brand
Body wash with moisturizer	15.90	Olay®
Toothpaste	1.59	Crest®
Deodorant	1.06	Gillette®
Shampoo and conditioner	21.20	Dove®
Lactic acid	1.59	ACROS Organics™
Bath cleaner	5.30	Lysol®
Liquid hand soap	12.19	Softsoap®
Liquid laundry detergent (2X)	18.80	Tide®
Liquid laundry fabric softener	9.87	Downy®
Na_2SO_4	1.88	Fisher Chemical™
$NaHCO_3$	0.94	Fisher Chemical™
Na_2PO_4	1.88	Fisher Chemical™

Table 3.2. Characteristics of the synthetic greywater, and contaminant ranges listed in NSF Standard 350 (NSF International 2012)

Parameter	Unit	Value	NSF Standard 350 range
TCOD	mg L^{-1}	389 ± 12	250-400
Total organic carbon (TOC; as C)	mg L^{-1}	67 ± 3	50-100
Total Kjeldahl nitrogen (TKN; as N)	mg L^{-1}	3.4 ± 0.2	3.0-5.0
Total phosphorus (TP; as P)	mg L^{-1}	2.8 ± 0.1	1.3-3.0
pH	-	7.1 ± 0.1	6.5-8.0

3.2.2. Granular activated carbon

Four types of GAC were used in this study to represent different scenarios of treatment mechanisms: fresh GAC (FGAC), BGAC, inhibited BGAC (InBGAC), and ignited BGAC (IgBGAC) as per the following:

- Fresh GAC (FGAC): a commercial acid-washed lignite GAC (Darco®, MilliporeSigma) with a mesh particle size of 4-12 (1.7-4.8 mm). These media have been used freshly with no prior usage in experimental word. Before using, the media was washed using de-ionized (DI) water to wash fines then was let to dry.

- Biologically active GAC (BGAC): GAC with active biomass grown on its surface, obtained from the top unsaturated layer of a laboratory-scale GAC biofilter treating synthetic greywater prepared according to the same formulation used in this study. The origin of these media was FGAC before it is utilized in the biofilter. The media were collected on day 218 of operation when the biofilter was being operated under hydraulic and organic loading rates of 1200 L m^{-2} d^{-1} and 454 g TCOD m^{-2} d^{-1}, respectively and a hydraulic retention time of 2.4 h. The biofilter was operated under intermittent dosing and demonstrated stable operation for reducing the total chemical oxygen demand (TCOD) from greywater by an average of 98%. The BGAC was collected at the end of an operation cycle before dosing greywater to allow stability of sorped compounds. To allow further stability, BCAG was then rinsed and soaked in a phosphate-buffered saline (PBS) and shaken at room temperature (21 ± 1 °C) using a horizontal shaker at 120 rpm for three hours with replacing the solution every hour. The mass of the biofilm was quantified as volatile solids (VS) according to Bruno (2017).
- Inhibited BGAC (InBGAC): BGAC with the biological activity of its biofilm inhibited using sodium azide (Rattier et al., 2012). Sodium azide was added in a concentration of 0.1% to the PBS during the second hour of the stabilization period as well as to the sorption experiments. The biological inhibition was confirmed by measuring and comparing the biological activity before and after the inhibition process using the DSA kit (LuminUltra Technologies Ltd.) according to the manufacturer's protocol.

Ignited BGAC (IgBGAC): BGAC ignited at 350 °C in a muffle furnace for 15 min to destroy its biofilm. This temperature is much lower than the GAC's synthesis temperatures (Ukanwa et al., 2019) and was reported to remove the volatile solids without breaking the chemisorbed surface compounds or affecting the physio-sorption properties of the GAC (Ledesma et al., 2014). Also, it is not likely to affect the properties of the GAC itself because its are usually much higher. The reduction in weight was compared to BGAC samples ignited at 550 °C, which is the temperature suggested by Bruno (2017) to quantify volatile solids (VS), to the effectiveness of destruction. The media were then vortexed in DI water containing sodium azide at a concentration of 0.1% to inhibit any potential biological activity at 150 rpm for 1 h to slough biofilm residues under hydraulic shear forces. The ignition and washing steps were repeated without inhibition. The biological inhibition was confirmed as described earlier.

3.2.3. Batch mechanistic experiment

The mechanistic experiment was conducted in three reactors—one reactor contained greywater and BGAC media, one reactor contained greywater and InBGAC media, and one reactor (the control) contained greywater and no media. The experiment was conducted for 24 h in 200 mL amber glass bottles (the reactors) containing 100 mL of synthetic greywater and a media dose of 50 g L^{-1}. The greywater samples were collected for analysis at time intervals of 0, 0.5, 1, 2, 3, 4, 6, 12, and 24 h, and analysed for the total chemical oxygen demand (TCOD) according to Bruno (2017). Experiments were performed in triplicate at room

temperature (21 ± 1 °C) on a horizontal shaker at 120 rpm. All bottles were capped with foam stoppers during the experiment to minimize water evaporation.

3.2.4. Batch equilibrium isotherms and kinetics experiments

Eight adsorption equilibrium isotherms and kinetics experiments were conducted in parallel for 48 h in 200 mL amber glass bottles containing 100 mL of synthetic greywater and IgBGAC at dosages of 0, 1, 5, 10, 20, 30, 40, and 50 g L^{-1}. IgBGAC media were used rather than FGAC media to better represent the exact conditions occurring in GAC biofilters at an intermediate stage of their lifespan. Bottles were capped with foam stoppers during the experiment to minimize water evaporation. Experiments were performed in triplicate at room temperature (21 ± 1 °C) on a horizontal shaker at 120 rpm. The IgBGAC dose of 50 g L^{-1} was considered for the kinetics study as it demonstrated a satisfactory level of TCOD removal in the equilibrium isotherms experiments. Water samples were collected for analysis at time intervals of 0, 0.5, 1, 2, 3, 4, 6, 12, 24, 36, and 48 h and analysed for TCOD according to Bruno (2017).

3.2.5. Data Analysis

The equilibrium adsorption capacity (q_e; mg g^{-1}), indicating the mass of adsorbate adsorbed per amount of adsorbent mass, was calculated using the following formula:

$$q_e = \frac{(S_0 - S_e)V_0}{M_c} \qquad \text{Eq. (1)}$$

Where S_0 is the initial concentration of organics in greywater (mg L^{-1}); S_e is the equilibrium concentration of substrates in greywater (mg L^{-1}); and V_0 is the

liquid volume (L); M_c is the mass of adsorbent (g). The removal efficiency ($\%R\text{TCOD}$) of TCOD from greywater was calculated using the following equation:

$$\%R\text{TCOD} = \frac{(S_0 - S_e)x100}{S_0} \qquad \text{Eq. (2)}$$

Results of the experiments were assessed using four widely used isotherm models (Table 3.3): Freundlich, Langmuir, Temkin, and Dubinin-Radushkevich (D-R) (Amiri et al. 2019; Alharbi et al. 2019; Song et al. 2017; Ahmadi and Shadizadeh 2012; Barati et al. 2016; Liang et al. 2011; Ghasemi et al. 2019). Linearized forms of these equations were used to plot the relationship between the adsorption capacity of IgBGAC and the equilibrium TCOD concentration. The slopes and intercepts of these plots were obtained to determine the unknown parameters (e.g. isotherm constants) for each isotherm model as indicated in Table 3.3.

The adsorption kinetics were assessed in terms of two kinetics models: pseudo-first order and pseudo-second order rate laws (Ho and McKay 1999) (Table 3.3). Linearized forms of these two models were plotted using the experimental value for q_e ($q_{e,exp}$). The slopes and intercepts of the linear trendlines for these plots were then obtained and the R^2 values and the value calculated for $q_e(q_{e,cal})$ were estimated. The appropriateness of the models was assessed based on the closeness of the calculated value to the experimental q_e and based on the R^2 value. Data with a fractional uptake (F_t) of ≥ 0.95 were excluded to avoid bias of data at or near equilibrium (Simonin 2016). The diffusion of

substrate (i.e., TCOD) into the pores of the IgBGAC media was described using the intraparticle diffusion models listed in Table 3.3 (Simonin 2016).

Table 3.3. Mathematical models used to assess the equilibrium isotherms and kinetics of greywater's TCOD adsorption onto the IgBGAC media

Model	Mathematical formula	Parameter	
		Definition	Unit
Freundlich isotherm	$q_e = K_F {S_e}^{1/n}$	q_e: equilibrium adsorption capacity	mg g^{-1}
		K_F: Freundlich constant related to the adsorbent's capacity	L g^{-1}
		S_e: equilibrium adsorbate concentration	mg L^{-1}
		$1/n$: Freundlich slope	-
Langmuir isotherm	$q_e = \frac{q_{max} b S_e}{(1 + bS_e)}$	q_{max}: maximum amount of adsorbate sorped per unit weight of adsorbent	mg g^{-1}
		b: constant related to the energy of sorption	L mg^{-1}
Temkin isotherm	$q_e = \frac{RT}{b} \ln (K_T S_e)$	R: ideal gas constant	J mol^{-1} K^{-1}
		T: liquid temperature	K
		b: constant related to the heat of sorption	J mol^{-1}
		K_T: Temkin isotherm constant	L g^{-1}
D-R isotherm	$q_e = q_0 - e^{\beta\varepsilon^2}$	q_0: D-R constant	mg g^{-1}
		β: constant related to free energy	-
		ε: Polanyi potential; $\varepsilon = RT \ln (1 + 1/S_e)$	J mol^{-1}
Pseudo-first order kinetics	$q_t = q_e F_t = q_e (1 - e^{-k_1 t})$	q_t: the amount of sorped adsorbate per mass of adsorbent at any time	mg g^{-1}
		F_t: fractional uptake	-
		k_1: pseudo-first order rate constant	h^{-1}
		t: time	h
Pseudo-second order kinetics	$q_t = q_e F_t = q_e \frac{k_2^* t}{1 + k_2^* t}$	k_2: pseudo-second order rate constant; $k_2^* = k_2 q_e$	mg g^{-1} h^{-1}
Intraparticle diffusion	$q_t = K_{diff} \sqrt{t} + C$	K_{diff}: intraparticle diffusion rate constant	mg g^{-1} $h^{-0.5}$
		C: constant	mg g^{-1}

3.3. Results and discussion

3.3.1. Granular activated carbon

The mass of the biofilm in the BGAC samples was quantified to be 178.71 ± 14.12 mg g^{-1} BGAC. The biological activity assessment using the DSA kit resulted in a tATP concentration of 1.03 ± 0.13 µg g^{-1} BGAC. For the InBGAC samples, inhibition of the biological activity was verified, as the tATP was reduced to 3.27×10^{-4} µg g^{-1} BGAC, which is a > 98% reduction compared to the initial concentration of ATP in BGAC. The remaining tATP concentration can be attributed to the extracellular ATP (Hammes et al. 2010). ATP measurement has been widely accepted as a marker of viable cells. Sodium azide inhibits ATP formation (and thus oxidative phosphorylation) by inhibiting the final enzyme (cytochrome oxidase) in the mitochondrial electron transport chain (Harvey, Hardy, and Ashford 1999). Destruction of the biofilm in the IgBGAC samples was verified after two cycles of ignition and washing of BGAC. The weight change of the IgBGAC media was less than 4%, indicating an acceptable level of biofilm destruction. The IgBGAC presented no biological activity after BGAC ignition.

3.3.2. Biodegradation mechanism

The biodegradation mechanism was eliminated in the InBGAC treatment group, while keeping the biofilm intact, by treating the InBGAC media with sodium azide. After the treatment with sodium azide, only biosorption and GAC adsorption were functional in the InBGAC. Therefore, the difference in the level of TCOD reduction between the BGAC and InBGAC was attributed to

biodegradation. Figure 3.1 shows the TCOD concentration in greywater throughout the experiment for the BGAC and InBGAC treatment groups. In general, a higher TCOD removal and removal rate were observed in the BGAC treatment group compared to the InBGAC treatment group throughout the experiment. Figure 3.1 shows that after a one-hour treatment of greywater, a TCOD of 389 mg L^{-1} was reduced to 267 and 298 mg L^{-1}, respectively, in BGAC and InBGAC treatment groups. These values reflect removal efficiencies of 31% and 23%, respectively, from the original TCOD concentration (Figure 3.2). Thus, the microbial activity removal rate in BGAC groups that combined both biodegradation and sorption mechanisms contributed to the TCOD overall removal by 8% compared to InBGAC groups where only sorption mechanisms are functional. After 24 h of greywater treatment, the TCOD was further reduced to 112 and 141 mg L^{-1} in BGAC and InBGAC treatment groups (Figure 3.1), respectively, translating to removal efficiencies of 71 and 64% (Figure 3.2).

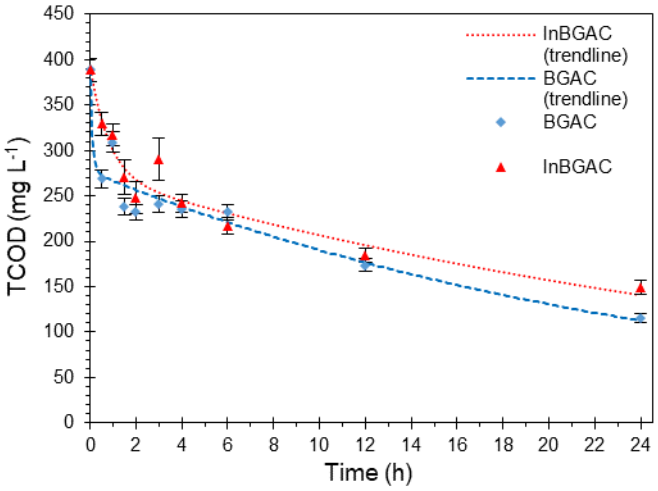

Figure 3.1. TCOD concentration (mg L^{-1}) as a function of time (h) for BGAC and InBGAC treatment groups treating greywater. The error bars represent the standard deviation values for triplicate measurements.

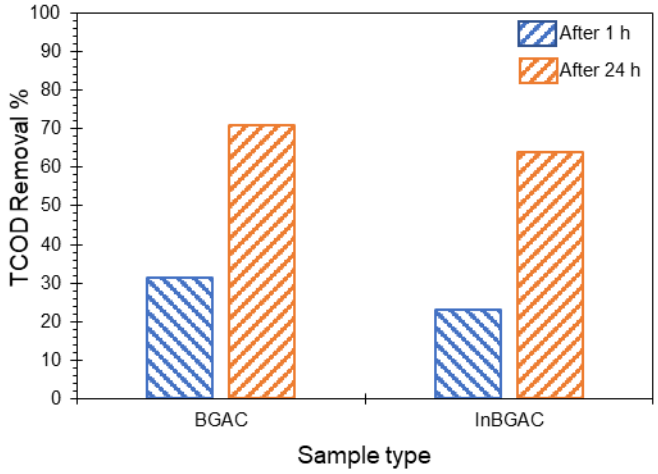

Figure 3.2. TCOD removal efficiency (%) of BGAC and InBGAC treatment groups after 1 h and after 24 h. The columns represent the average values of triplicate measurements

Although the contribution of biodegradation might seem relatively small compared to the sorption mechanisms, it can play a significant role in the design and operation of GAC biofilters. Biodegradation helps to regenerate the adsorptive capacity of the GAC media, allowing for longer operating life without the need to replace the media (Leong et al. 2018; Klimenko et al. 2003; Aktaş and Çeçen 2010, 2007). A considerable amount of the TCOD in greywater comprises surfactants, the majority of which are linear alkylbenzene sulfonates (LAS). Biodegradation of surfactants, including LAS, was reported to reach > 99% (Weiss et al. 2012; Huelgas et al. 2009). This capability helps to bioregenerate GAC media through two mechanisms (Aktaş and Çeçen 2007)—the desorption of sorped compounds due to microbial activity, and diffusion of extracellular

enzymes into the pores of the GAC media, leading to biodegradation in the media pores.

Biodegradation mechanisms and sorption mechanisms allow the bioregeneration of the biofilter media, which means longer operation lifespans can be achieved. To maximize this advantage for greywater GAC biofilters, intermittent dosing of the influent was applied to equalize TCOD removal rates due to biodegradation and sorption mechanisms. This allowed the two bioregeneration mechanisms to occur efficiently and simultaneously, since the media were not continuously loaded with new substrate. Another alternative is to stimulate the growth of biomass on the GAC media to increase biodegradation. However, a study conducted in our laboratory indicated that biofilm cannot significantly increase in systems treating bathing and laundry greywater, possibly because bathing and laundry greywater contains antimicrobial agents.

3.3.3. Adsorption isotherms modelling

The initial and equilibrium TCOD concentrations after treatment using 1 to 50 g L^{-1} of IgBGAC are presented in Figure 3.3. The initial TCOD concentration was 389 mg L^{-1}. Equilibrium TCOD concentrations were 344, 283, 237, 191, 172, 159, and 153 mg L^{-1} for IgBGAC doses of 1, 5, 10, 20, 30, 40, and 50 g L^{-1}, respectively. The equivalent adsorption capacity was calculated using Eq. (1) for each IgBGAC dose and was plotted as presented in Figure 3.4. These concentrations represent the TCOD concentration in the solution at the steady state, where the rates of adsorption and desorption were equal (Edzwald 2011), given that the adsorption of surfactants on GAC is usually reversible (Zhang and

Somasundaran 2006; Wu and Pendleton 2001). The mass of adsorbed organics increased with an increase in IgBGAC in a nonlinear trend that is concave upward. The removal efficiency of TCOD, calculated using Eq. (2), increased from 11 to 27, 39, 51, 56, 59, and 61% for the aforementioned IgBGAC doses, respectively. Clearly, an increase in IgBGAC provided more adsorption sites for TCOD (Xing et al. 2008).

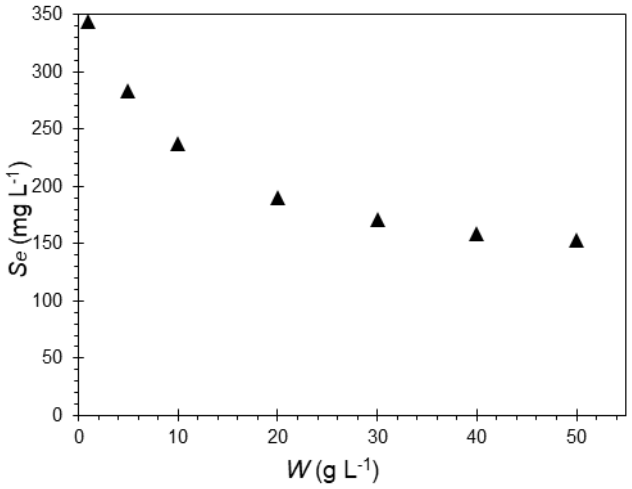

Figure 3.3. Equilibrium TCOD concentration (S_e; mg L^{-1}) as a function of IgBGAC dose (W; g L^{-1}).

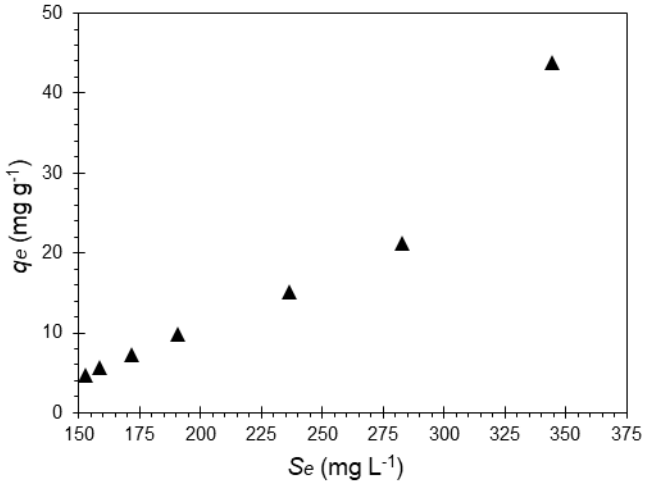

Figure 3.4. Equilibrium adsorption capacity (q_e; mg g^{-1}) of IgBGAC (0.1-5.0 g L^{-1}) for TCOD in greywater as a function of TCOD equilibrium concentrations (S_e; mg L^{-1})

Since the GAC surface is heterogenous in structure and energy, no single mathematical formulation can describe the adsorption process. Therefore, several complex analytical equations have been developed to describe the adsorption process (Çeçen and Aktaş 2012). Freundlich, Langmuir, Temkin, and Dubinin-Radushkevich (D-R) each formulated an isotherm to express adsorption equilibrium (Amiri et al. 2019; Alharbi et al. 2019; Song et al. 2017; Ahmadi and Shadizadeh 2012; Barati et al. 2016; Liang et al. 2011; Ghasemi et al. 2019). These four models were used to describe the adsorption of organics in greywater onto the surface of IgBGAC in Figure 3.5. The four adsorption isotherm models showed coefficient of determination (R^2) values of 0.99, 0.94, 0.84, and 0.95, respectively, indicating that the Freundlich model is the most reasonable isotherm to represent the adsorption characteristics with calculated K_F and $1/n$ values of 1.48×10^{-5} Lg^{-1} and 2.54, respectively (see Table 3.3 for variable definitions).

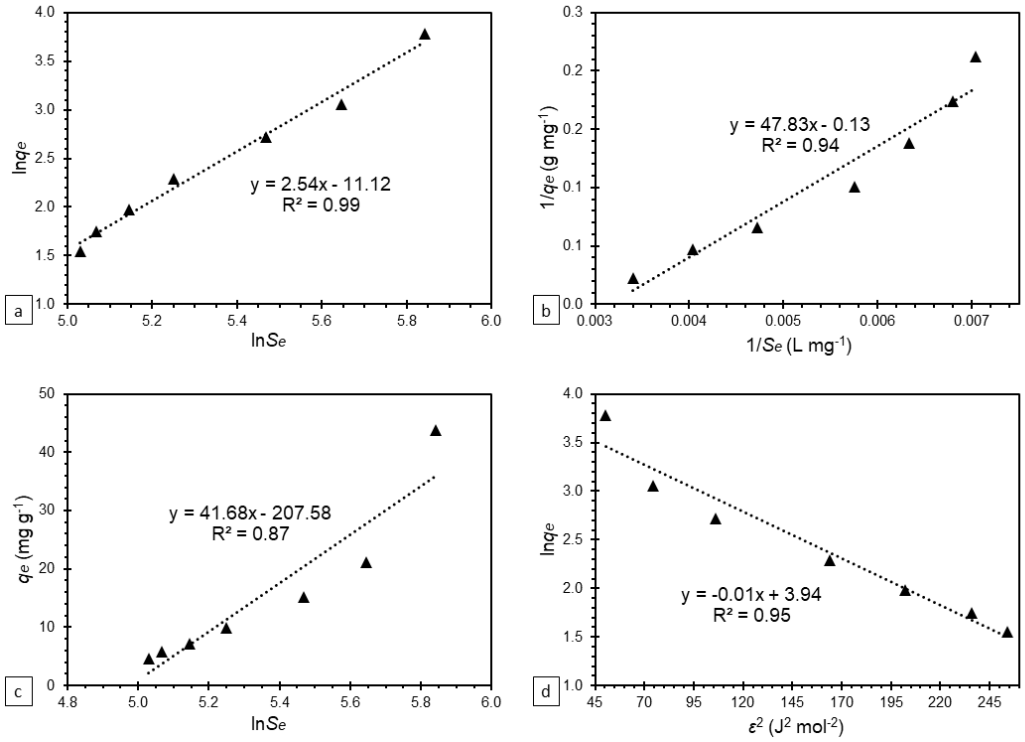

Figure 3.5. Adsorption equilibrium isotherms for TCOD (mg L^{-1}) on IgBGAC media in greywater, described using the linearized forms of the (a) Freundlich, (b) Langmuir, (c) Temkin, and (d) D-R models. The dotted lines represent the linear trendlines of the data sets. The trendlines' formulae and their R^2 values are shown. See Table 3.3 for variable definitions

3.3.4. Adsorption kinetics modelling

Kinetics of TCOD adsorption on IgBGAC media are shown in Figure 3.6. The higher rate of TCOD adsorption observed at the beginning of the experiment decreased gradually until reaching equilibrium after 48 h of treatment. Although data collection continued for 48 h, until equilibrium was reached, data considered in the analysis were limited to 24 h (Figure 3.7) to maintain an F_t value of 0.85 or less (Simonin 2016).

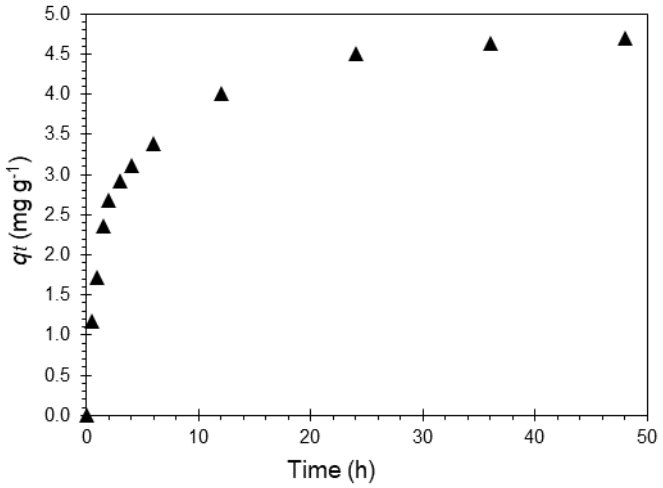

Figure 3.6. Adsorption kinetics of TCOD onto IgBGAC represented as the adsorption capacity (mg g^{-1}) as a function of time (h).

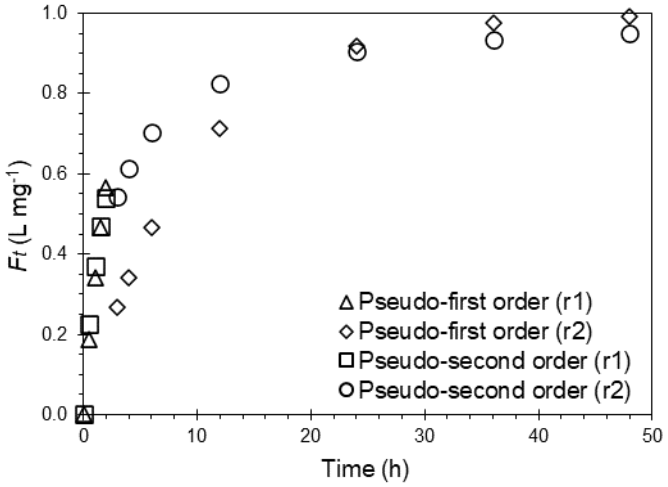

Figure 3.7. Fractional uptake (F_t) of pseudo-first order and pseudo-second order reaction rates as a function of time (h) for phases 1 and 2.

Pseudo-first order, pseudo-second order, and intraparticle diffusion models (Amiri et al. 2019; Alharbi et al. 2019; Barati et al. 2016; Liang et al. 2011; Ghasemi et al. 2019) were used to describe the kinetics of TCOD adsorption onto IgBGAC media. The kinetics data followed two different rates, so further analysis was performed in two phases, where phase 1 ranged from 0 to 2 h and phase 2

ranged from 2 to 24 h (Figure 3.8). The three kinetics models correlated well with the experimental data, with high R^2 values of above 0.96 during both phase 1 and phase 2, as summarized in Table 3.4. In phase 1, the R^2 values were 0.99, 0.97, and 0.99 for pseudo-first order, pseudo-second order, and intraparticle diffusion models, respectively. Calculated q_e ($q_{e,cal}$) values for the pseudo-first order model and the pseudo-second order model were 4.53 and 4.86 mg g^{-1}, respectively. These values are very close to the experimental q_e ($q_{e,exp}$) value (4.71 mg g^{-1}), with small deviations of 3.8 and 3.3%, respectively. Based on the R^2 values and on a comparison between $q_{e,cal}$ and $q_{e,exp}$, the three models represented the adsorption kinetics during phase 1 fairly accurately, with the pseudo-first order and intraparticle diffusion models being slightly more accurate than the pseudo-second order model. The rate constants for the three kinetic models (k_1, k_2, k_{diff}) during phase 1 were 0.42 h^{-1}, 0.12 mg g^{-1} h^{-1}, and 1.91 mg g^{-1} $h^{-0.5}$, respectively.

Table 3.4. Summary of the parameters of the pseudo-first order, pseudo-second order, and intraparticle diffusion models describing the adsorption kinetics of TCOD onto IgBGAC media.

Data source	Parameter										
	$q_{e,exp}$	Phase 1					Phase 2				
		$q_{e,cal}$ *	k_1	k_2	k_{diff}	R^2	$q_{e,cal}$	k_1	k_2	k_{diff}	R^2
Experimental	4.71	-	-	-	-	-	-	-	-	-	-
Pseudo-first order	-	4.53 (3.8)	0.42	-	-	0.99	2.44 (48.2)	0.10	-	-	1.00
Pseudo-second order	-	4.86 (3.3)	-	0.12	-	0.97	4.93 (4.7)	-	0.08	-	1.00
Intraparticle diffusion	-	-	-	-	1.91	0.99	-	-	-	0.50	0.98

*Values in parentheses are the deviation percentage (%) between $q_{e,cal}$ and $q_{e,exp}$.

As shown in Table 3.4, phase 2 R^2 values were 1.00, 1.00, and 0.98 for pseudo-first order, pseudo-second order, and intraparticle diffusion models, respectively. $q_{e,cal}$ values for pseudo-first order and pseudo-second order models were 2.44 and 4.93 mg g^{-1}, respectively. These values indicate a higher deviation of 48.2% between $q_{e,cal}$ of pseudo-first order model. In contrast, the pseudo-second order model maintained a smaller deviation of 4.7%, indicating that this model is more appropriate to express the adsorption kinetics during phase 2. The rate constants for the three models (i.e. k_1, k_2, and k_{diff}) during phase 2 were 0.10 h^{-1}, 0.08 mg g^{-1} h^{-1}, and 0.50 mg g^{-1} $h^{-0.5}$, respectively.

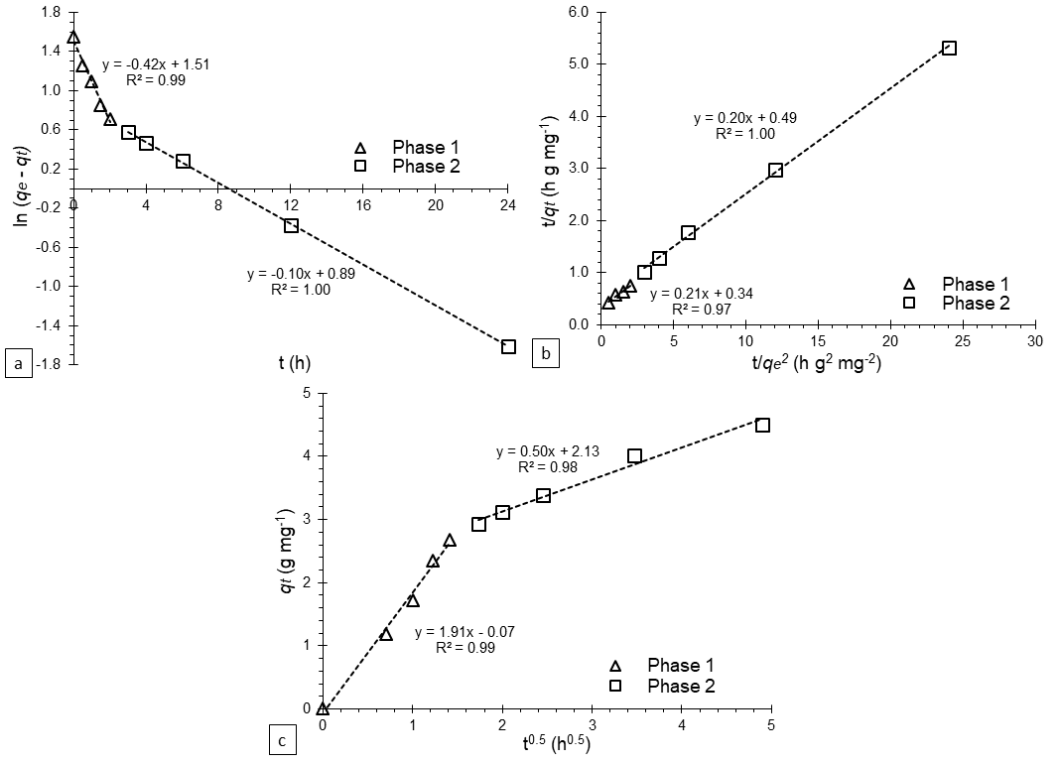

Figure 3.8. Isotherms of the adsorption kinetics of TCOD (mg L^{-1}) on IgBGAC media in greywater constructed using linearized forms of the (a) pseudo-first

order, (b) pseudo-second order, and (c) intraparticle diffusion models. The dotted lines represent the linear trendlines of the data sets. The trendlines' formulae and coefficient of determination (R^2) are shown.

During phase 1 and 2 of greywater treatment, the intercept of the intraparticle diffusion model had values of 0.01 mg g^{-1} and 2.13 mg g^{-1}, respectively (Figure 3.8). Although the intraparticle diffusion model describes internal diffusion of the adsorbate within the pores of the adsorbent, it can give some insights into the external diffusion of the adsorbate as well. The value of intercept in the intraparticle diffusion model is directly proportional to the resistance to the mass transfer of TCOD by diffusing through the stagnant, external liquid film surrounding IgBGAC media until TCOD reaches its surface (McKay, Otterburn, and Aga 1985; Kavitha and Namasivayam 2007; Kannan and Sundaram 2001). The values of the intercept in phase 1 and 2 (0.01 mg g^{-1} and 2.13 mg g^{-1}) indicate minimal external film resistance during phase 1 but greater resistance in phase 2 (Figure 3.8). This resistance can be explained by examining the mechanisms of transport in the current experiment. Regardless of the bulk solution transport of the adsorbate (TCOD), the first transport mechanism is external diffusion through the stagnant liquid film (greywater) surrounding the GAC media particles (Çeçen and Aktaş 2012). Adsorbate transport through this layer is due to molecular diffusion and its kinetics are governed by Fick's law, which states that the transport rate of the liquid (greywater) is a function of the adsorbate (TCOD) gradient. The higher the adsorbate gradient (i.e., the difference in adsorbate concentration between the bulk greywater and the surface of the GAC adsorbent), the greater is the driving force and, hence, the transport rate of

adsorbate (TCOD). Apparently, the washing of the IgBGAC media led to desorption of the reversibly adsorbed TCOD before the adsorption experiment started. During phase 1 of the adsorption experiment, the TCOD gradient was at its highest values due to the washed media, leading to minimal resistance to mass transfer of the TCOD to the adsorbent (GAC). As the experiment proceeded, more TCOD diffused into the stagnant greywater film and IgBGAC media pores resulting in a lower TCOD gradient, which was reflected in the value of the intercept of the intraparticle diffusion model as it increased to 2.13 mg g^{-1} in phase 2 compared to 0.01 mg g^{-1} in phase 1.

3.4. Conclusion

The TCOD biodegradation mechanism contributed less than 10% to the overall removal of TCOD from greywater. This contribution can be a key to maintaining a longer operation lifespan of the GAC media through bioregeneration. The difference in removal rates between sorption and biodegradation mechanisms suggests that adopting an intermittent feeding strategy of greywater to GAC biofilters is recommended to balance this difference. Further research is required to improve the contribution of biodegradation in order to support higher loading rates. The Freundlich isotherm was found to best represent the equilibrium adsorption data. The pseudo-second order and intraparticle diffusion models were found to fit the adsorption kinetics. Intraparticle pore diffusion was found to be the rate limiting step, with some mass transfer resistance due to external film diffusion at lower TCOD gradients during

greywater treatment. Knowledge of the different mechanisms taking place on GAC biofilters leads to better design and operation of these tools.

3.5. References

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Chapter 4 - Comparative Study on Greywater Treatment Using Granular Activated Carbon and Sand as Packing Media for a Single-Stage Unsaturated-Saturated Biofilter

4.1. Introduction

Many treatment technologies have been utilized to treat greywater including physical, chemical, biological, and hybrid systems. Sand filtration is the most commonly used greywater treatment technology which mainly comprises physical treatment with a potential for biological treatment to some extent (Dalahmeh et al. 2012). Nonetheless, previous studies reported the limited capacity of sand filtration in removing pollutants from greywater, resulting in effluents that can be used – in most cases – for limited uses such as toilet flushing under some restrictions (Dalahmeh et al. 2012; March, Gual, and Orozco 2004). Dalahmeh et al. (2012) reported greywater treatment using a sand filter with a depth of 60 cm and under hydraulic and organic loading rates (HLR and OLR) of 32 L m^{-2} d^{-1} and 14 g BOD_5 m^{-2} d^{-1}. The BOD_5, surfactants (as methylene blue active substances; MBAS), total nitrogen (TN), and total phosphorus (TP) were reduced by 75%, 73%, 13%, and 78%, respectively. March, Gual, and Orozco (2004) investigated the treatment of greywater using sand filtration besides sedimentation and hypochlorite disinfection where they achieved reduction of chemical oxygen demand (COD), turbidity, suspended solids (SS) and TN by 54%, 18%, and 84%. In the study of Aizenchtadt, Ingman, and Friedler (2009), they achieved COD, BOD, total suspended solids (TSS), and turbidity of 38%, 10%, 65%, and 46%, respectively, from greywater using a stand-alone sand filter.

In addition to the limited removal capacity for pollutants from greywater, frequent clogging was reported as a common operational problem associated with sand filtration due to the narrow pore size (Spychala and Blazejewski 2004). Therefore, granular activated carbon (GAC) was applied in this research as an alternate biofilter media to overcome the drawbacks associated with sand media. As concluded in the previous chapters, and based on previous research and experimental evidence, GAC possesses high adsorptive capacity and capability to efficiently remove a wide variety of pollutants from water (Ahmaruzzaman 2008; Snyder et al. 2007; Toles, Marshall, and Johns 1997; Ahmed et al. 2015). This adsorptive capacity results in accumulating the substrate onto the surface of the media, stimulating the biological growth on these surfaces (Aktaş and Çeçen 2007). This integration of adsorption and biodegradation leads to a major benefit, which is the regeneration of GAC media by active microorganisms in the biofilm. Longer operation life cycles can be achieved through this synergistic action with a reduced frequency of off-line media regeneration (Aktaş and Çeçen 2007; Leong et al. 2018).

The main objective of this chapter is to compare different aspects of the performance of GAC and sand biofilters as two greywater treatment alternatives. This objective was achieved by evaluating the capacity of both GAC and sand in removing major nutrients from greywater, assessing their capability to support biofilm growth, and analyzing the structure and functionality of the grown microbial community throughout the biofilters' depth.

4.2. Materials and methods

4.2.1. Greywater formulation

Synthetic combined bathing and laundry greywater was prepared in the laboratory following the same formulation described in the previous chapters according to the NSF/ANSI Standard 350 (NSF International 2012).

4.2.2. GAC and sand biofilters setup

The experiments were performed in two identical laboratory-scale cylindrical column reactors (Figure 4.1) having the configuration described in the previous chapters. In this study, in addition to a GAC reactor, a second column reactor packed with sand (mesh particle size 20) was used.

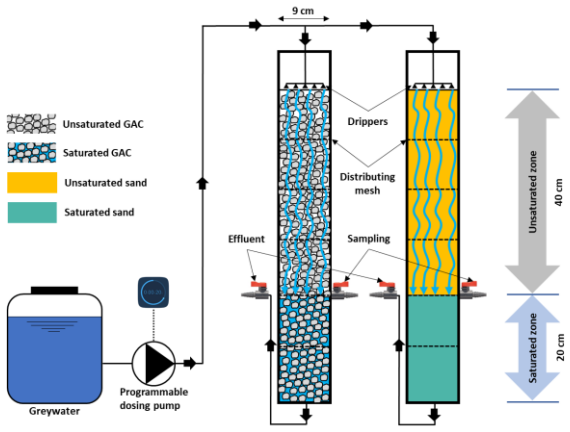

Figure 4.1. Schematic of the experimental setup of the laboratory-scale single-stage unsaturated-saturated GAC and sand biofilters treating greywater.

4.2.3. Feeding strategy and operation stages

Feeding strategy and operating conditions were the same as Chapter 2. HRT in the sand reactor was different from the GAC reactor due to the difference in porosity, as shown in Table 4.1.

Table 4.1. Operating conditions of the GAC and sand biofilters during the different operation stages

Stage	Time (d)			HLR		OLR	HRT (h)	
	Start	End	Duration	(L m^{-2} d^{-1})	(cm d^{-1})	(g COD m^{-2} d^{-1})	GAC	Sand
I	0	30	30	71	7	22	40.3	26.9
II[a]	31	57	26	71	7	24	40.3	26.9
III	58	93	35	100	10	35	28.8	19.2
IV	94	138	44	150	15	54	19.2	12.8
V	139	155	16	250	25	78	11.5	7.7
VI	156	180	24	600	60	189	4.8	3.2
VII	181	195	14	900	90	333	3.2	-
VIII	196	253	57	1200	120	454	2.4	-

[a] Addition of secondary effluent to the synthetic greywater started from stage II as per the NSF/ANSI 350 formulation.

4.2.4. Water sampling and analysis

Water sampling and analysis were performed as described in Chapter 2 for the two reactors.

4.2.5. Biofilm growth assessment

The biofilm growth was qualitatively assessed on day 218 through imaging of the biofilm formed and attached to the GAC and sand media using scanning electron microscopy (SEM). The methods of sampling, preparation, and imaging were described earlier in Chapter 2.

4.2.6. DNA extraction and microbial community analysis

GAC and sand samples were collected on day 218 from the two biofilters at vertical depths of 0-10, 20-30, 50-60 cm to profile and compare the microbial community structures. Sampling and analysis were performed following the same protocol described in Chapter 2.

4.3. Results and discussion

4.3.1. Treatment performance

Figure 4.2 shows the TCOD concentrations in the influent, unsaturated zone effluent, and final effluent as well as its removal efficiencies within the unsaturated and saturated zones throughout the operational stages of the GAC and sand biofilters. Throughout the eight operational stage, the GAC biofilter did not experience any clogging issues due to its larger interparticle pore size, whereas the sand biofilter required several washings before it came to a complete shutdown after a few days into stage VI due to frequent clogging. Clogging can be attributed to the retention of particulate matter from the greywater, organic debris and live microorganisms, blocking the pores between the sand particles (Spychala and Blazejewski 2004; de Vera et al. 2019). Thus, the highest HLR, highest OLR, and lowest HRT achieved in the sand biofilter was 250 L m^{-2} d^{-1}, 78 g COD m^{-2} d^{-1}, and 7.7 h, respectively. The GAC biofilter withstood further loading up to HLR, OLR, and HRT of 1200 L m^{-2} d^{-1}, 454 g COD m^{-2} d^{-1}, and 2.4 h, respectively, which are equivalent to about 5 times higher loading compared to the sand biofilter.

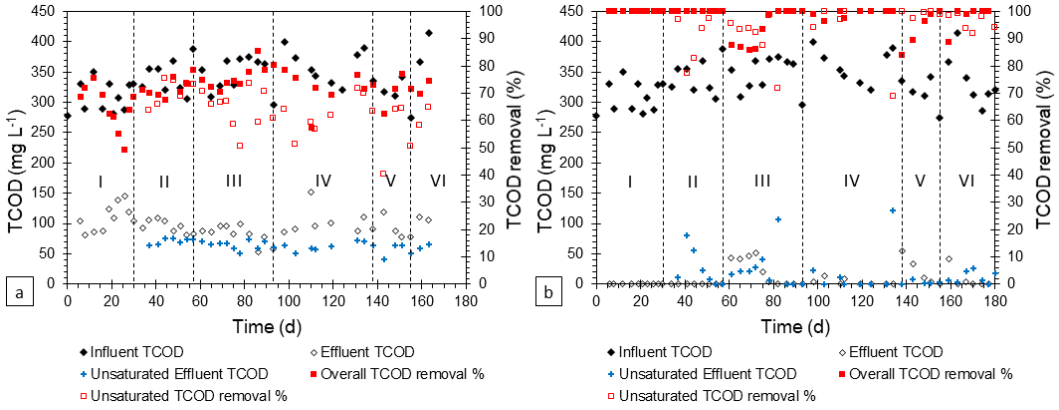

Figure 4.2. Concentration of TCOD (mg L^{-1}) in the influent, unsaturated effluent, and overall effluent as well as the removal percentage in the two effluents as a function of the (a) sand and (b) GAC biofilters' operational time (d). The Latin numerals represent the operation stages.

The treatment capacity varied largely between the GAC and sand biofilters as shown in Figure 4.3 In general, the GAC biofilter achieved higher treatment capacity compared to the sand biofilter. The GAC biofilter maintained an overall TCOD removal between 84% and >99% with an average of 98% during the operation stages. The sand biofilter, on the other hand, achieved an overall TCOD removal between 49% and 85% with an average of 71%. The superiority of GAC over sand in removing TCOD from greywater was a results of the its high adsorptive capacity towards a wide range of pollutants and its capability to support biofilm formation (González-García et al. 2004; Wu and Pendleton 2001; González, Petrovic, and Barceló 2007). The majority of TCOD removal took place in the unsaturated zone in both biofilters.

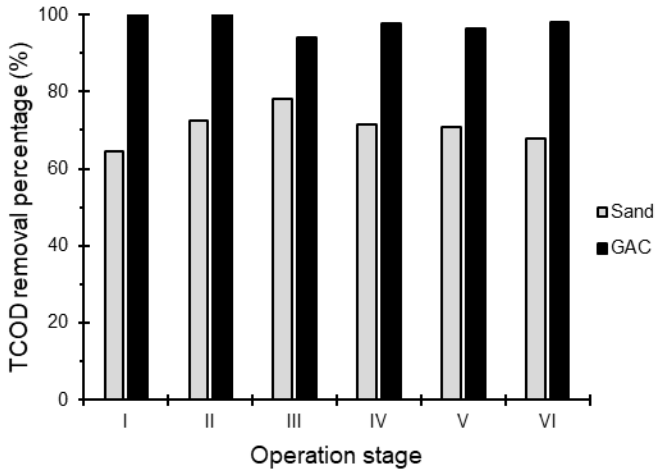

Figure 4.3. Overall TCOD removal percentage of the GAC and sand biofilters during the six operation stages.

4.3.2. Biofilm growth and biological activity

Biofilm formation was assessed using SEM imaging to verify the growth of biofilm on the surface of GAC and sand media in both filters. This verification was important because unfavourable biological growth conditions may present due to the lack of nutrients in greywater as well as the presence of antimicrobial agents. The SEM images showed that biofilm successfully developed on the surface of both the GAC and sand media (Figure 4.4). The images clearly showed microorganisms and extracellular polymeric substances (EPS) attaching to the surface of the GAC and sand, indicating successful biofilm growth despite the conditions indicated earlier.

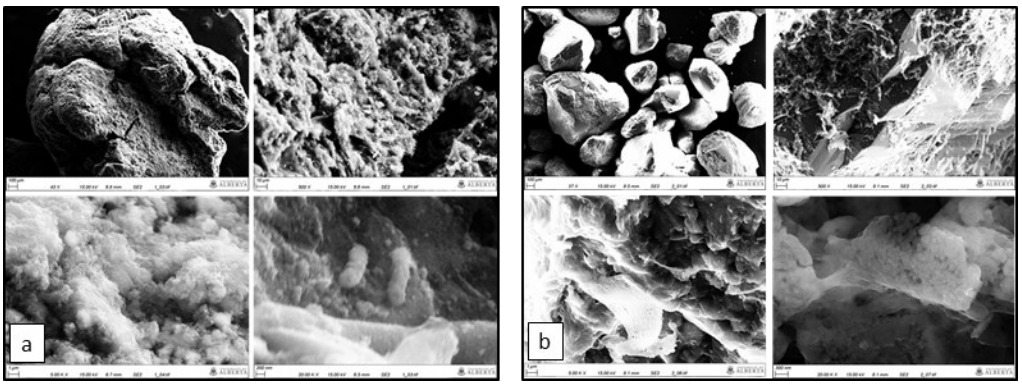

Figure 4.4. Scanning electron microscopy images of the (a) GAC and (b) sand media collected from the GAC and sand biofilters, respectively, on day 218.

4.3.3. Microbial community structure

The microbial community along the GAC and sand biofilters was analysed by sequencing the 16S rRNA gene amplicons sourced from biofilms at various depths. Figure 4.5 shows the relative abundances of the predominating bacterial phylotypes at the class level along the GAC and sand biofilters depth. The unsaturated zone of the GAC biofilter was predominated by *α-Proteobacteria* (53-

67%), *Actinobacteria* (2-16%), *β-Proteobacteria* (7-11%), *4C0d-2* (4-7%), *γ-Proteobacteria* (3-5%), *δ-Proteobacteria* (2-5%), and *Bacteroidia* (≤1%). In the sand biofilter, the bacterial communities were predominated by *α-Proteobacteria* (39-70%), *γ-Proteobacteria* (9-60%), *4C0d-2* (≤4%), *Actinobacteria* (≤4%), *Planctomycetia* (≤4%), and *β-Proteobacteria* (≤3%). The bacterial community of the unsaturated zone in the GAC biofilter comprised all the bacterial classes that were observed in the sand biofilter except for *Planctomycetia*. *Planctomycetia* species are mostly aerobic chemo-heterotrophs that was reported to be the most abundant class in slow sand filters, especially in the top layers (Delgado-Gardea et al. 2019).

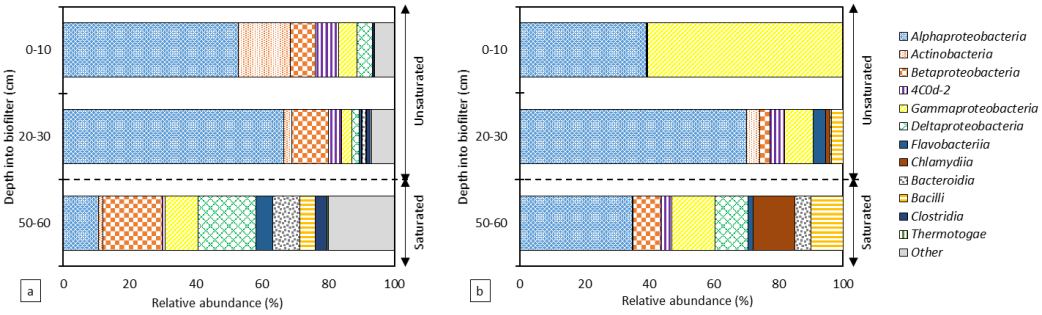

Figure 4.5. Relative abundances of the predominating bacterial phylotypes (> 1.0%) at the class level along the (a) GAC and (b) sand biofilters depth.

The saturated zone of the GAC biofilter was predominated by *β-Proteobacteria* (18%), *δ-Proteobacteria* (18%), *α-Proteobacteria* (11%), *γ-Proteobacteria* (10%), *Bacteroidia* (8%), *Flavobacteriia* (5%), *Bacilli* (5%), *Clostridia* (3%), *Actinobacteria* (1%), and *4C0d-2* (1%), and *Thermotogae* (1%). In the sand biofilter, on the other hand, it was predominated by *α-Proteobacteria* (35%), *γ-Proteobacteria* (13%), *Bacteroidia* (13%), *δ-Proteobacteria* (10%), *β-*

Proteobacteria (9%), *Spartobacteria* (5%), and *4C0d-2* (≤4%). *Bacteroidia* was observed in the saturated zone of the sand biofilter – similar to the GAC biofilter – despite its absence in the unsaturated zone. *Spartobacteria* appeared in the saturated zone of the sand biofilter despite its absence in the GAC biofilter. *Spartobacteria* was reported to exist in soils (Janssen 2006) and in aquatic environments (Freitas et al. 2012) and has the capability to degrade hydrocarbons (Herlemann et al. 2013). The increases in anaerobic bacteria in the saturated zone (*Bacteroidia, Clostridia*, and *Thermotogae*) can be attributed to the reduced oxygen availability in the saturated zone. *Bacteroidia* are capable of degrading complex hydrocarbons under anaerobic conditions (Delgado-Gardea et al. 2019). Sun et al. (2019) found that *Bacteroidia*'s abundance increased significantly in the microbial community of an anaerobic digester treating municipal sludge amid addition of anionic and non-ionic surfactants.

The microbial community structure at the genus level is shown in Figure 4.6. The predominating bacterial genus were dependant on the packing material to a large extent. In the GAC biofilter, the bacterial communities of the unsaturated zone were predominated by the *Oleomonas* genus, ranging from 29% to 49% for the entire zone. *Oleomonas* abundance was constant at 29% throughout the depth of the unsaturated zone except for the third segment at a depth of 20-30 cm where its abundance increased to 49%. *Oleomonas* possesses a tendency to form aggregates through the production of extracellular polymeric substances (EPS), allowing its adhesion to support media and biofilm formation (Fernández et al. 2008; Kanamori et al. 2002). In addition, *Oleomonas* can degrade complex

hydrocarbons under limited nitrogen conditions, which is a key for greywater treatment. Another advantage of *Oleomonas* is its resistance towards detergents because of possessing an outer membrane that has a natural resistance to detergents due to its structure that contains lipopolysaccharides (Anderson and Yu 2005; Saimmai et al. 2012).

Predominance of *Oleomonas* was not previously reported to occur in sand filters, which is the case herein. Instead, the bacterial genus *Pseudoxanthomonas* predominated the top layer of the unsaturated zone in the sand biofilter with a relative abundance of 52%. *Pseudoxanthomonas* was identified in biofilm samples collected from biological reactors treating winery wastewater (de Beer, Botes, and Cloete 2018). The abundance of this genus in these reactors was attributed to the availability surfactants in the wastewater as a result of using detergents containing wetting agents in the winery cleaning process (de Beer, Botes, and Cloete 2018). This observation is consistent with our study which comprises a biofilm reactor (sand biofilter) treating greywater, which contains a considerable amount of surfactants.

In the saturated zone, there was no predominance of a single genus in the GAC or sand biofilters likely due to the depletion of easily biodegradable matter at this depth of the biofilters. A few genera in the GAC biofilter showed similar abundances such as *Flavobacterium* and unidentified genera in the order *Bacteroidales* and family *Comamonadaceae*. *Macellibacteroides* was the most abundant genus in the sand biofilter at about 7%. *Macellibacteroides* is fermentative bacteria that was reported to exist in anaerobic systems and to

possess the ability to hydrolyse and degrade complex compound to simpler forms (Zhang, Xu, and Zhu 2017; Salminen and Rintala 2002).

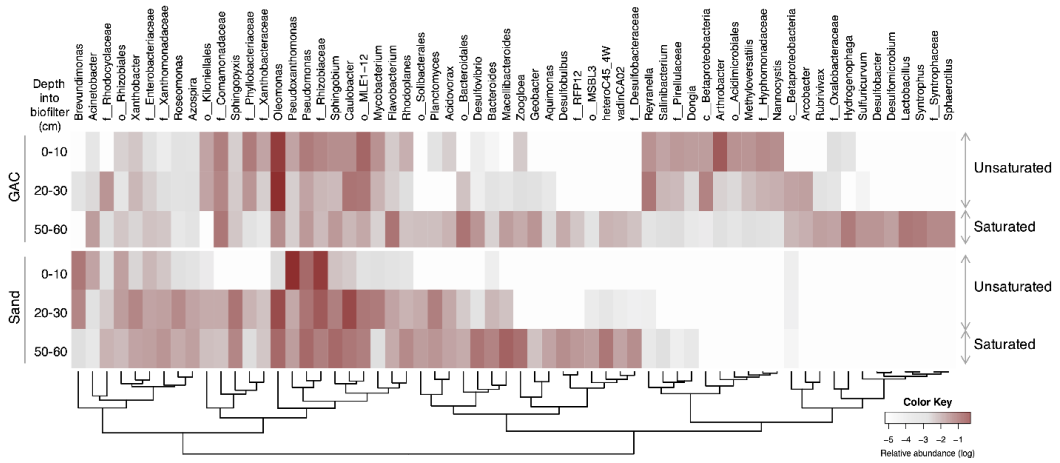

Figure 4.6. Heatmap of the genera with >1% relative abundance. Taxa are shown at genus level or higher (family: f_; order: o_; class: c_; phylum: p_; kingdom: k_) if not identified at genus level. Hierarchical clusters indicate similarities among families based on their fold changes using Euclidean distance method.

4.4. Conclusion

The performance of two biofilters treating greywater packed separately with GAC and sand media was compared to each other. The sand biofilter treating greywater was more susceptible to clogging compared GAC biofilters. The GAC biofilters achieved higher TCOD removal (98% in average) at higher HLR (1200 L m^{-2} d^{-1}), higher OLR (454 g COD m^{-2} d^{-1}), and lower HRT (2.4 h). Most treatment occurred in the unsaturated zone in both filters. SEM imaging showed that biofilm was successfully grown on both the GAC and sand media. Each biofilter had its unique microbial community, which also changed from the unsaturated zone to the saturated zone for each biofilter.

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Chapter 5 - Impact of the Filamentous Fungi Overgrowth on the Aerobic Granular Sludge Process[3]

5.1. Introduction

The aerobic granular sludge wastewater treatment technology has numerous advantages as compared to the conventional flocculent sludge technologies. However, the filamentous fungi overgrowth (FFO) is a major operational problem that is hindering its widespread application. In this study, the behaviour and mechanisms of the FFO were elucidated, and its impact on granulation, treatment performance and microbial community structure was investigated. The overgrown filamentous organisms were identified to be yeast-like fungi belonging to the moulds Geotrichum of the phylum Ascomycota. The FFO was found to disrupt the structural integrity of granules and cause their disintegration and washout. The removal of carbon and nitrogen were slightly affected, while phosphorus removal was largely impacted. In addition, the FFO shifted the microbial community towards a structure lacking bacteria genus that are essential for efficient granulation. Limitations of the currently used routine measurements were discussed.

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Biological wastewater treatment has always been an open and attractive field of research and innovation to achieve more efficient and eco-friendly technologies through optimizing the operation of existing technologies or developing brand-new ones. For more than a century, flocculent sludge-based technologies (e.g. the conventional activated sludge and its various emanated technologies) have been dominating the wastewater treatment market (Guven et al. 2019). A few decades ago, the aerobic granular sludge (AGS) technology started to emerge (Morgenroth et al. 1997; Tay, Liu, and Liu 2001a), bringing to the wastewater treatment market a new alternative which displays superior characteristics compared to the flocculent activated sludge technologies (Bengtsson et al. 2018).

The AGS technology is based on a unique concept of operating the process under specific conditions, allowing for the selective growth of larger, smoother and high-density granular sludge (Y. Liu and Tay 2004). This results in numerous advantages such as the simultaneous removal of organics and nutrients, better settling ability, higher biomass concentration, and significant reduction in footprint and power consumption (Pronk et al. 2015). Therefore, the AGS technology is gaining acceptance worldwide as an effective wastewater treatment technology and it is a promising technology to replace the conventional flocculent activated sludge technologies in the future (Bengtsson et al. 2018). However, some operational challenges, such as the long start-up periods and poor stability of the AGS, are still outstanding to hinder the widespread of the AGS technology worldwide (Franca et al. 2018). The overgrowth of filamentous organisms is the

most common cause for the AGS process instability. This problem is caused by the transformation of dense, smooth granules into fluffy ones, divesting the technology of its distinctive advantages, which may eventually lead to total inhibition of the process (Franca et al. 2018; Y. Liu and Liu 2006; Meunier et al. 2016; Wan et al. 2014; Y.-Q. Liu and Tay 2015).

Maintaining a microbial community with a high abundance of bacteria that can copiously produce extracellular polymeric substances (EPS) is key to grow dense, structurally-stable granules (Y.-Q. Liu, Liu, and Tay 2004). However, the filamentous organisms also play an important role in the granulation process as they act as a backbone that bacteria can grow on, especially at the early stages of granulation (Nancharaiah and Kiran Kumar Reddy 2018; Y. Liu and Liu 2006). Problems associated with filamentous organisms happens when these organisms overgrow and dominate (Franca et al. 2018), impacting the structural stability of granules, treatment performance of the process, and microbial community structure (Aqeel et al. 2016; Franca et al. 2018). Two types of the filamentous organisms have been reported in the literature to cause such problems: bacteria (Meunier et al. 2016; Y. Liu and Liu 2006) and fungi (Wan et al. 2014; Li et al. 2016).

Although both types (i.e. bacteria and fungi) of overgrowth lead to similar operational problems (Li et al. 2016; Y.-Q. Liu and Tay 2015), the overgrowth of filamentous fungi, in particular, is of special concern as it exhibits unique characteristics. For instance, due to the low nutrients' requirements of fungi compared to bacteria (J. Zhang and Elser 2017), removal of nutrients can be

potentially impacted. Also, fungi compete with and may minimize the abundance of bacteria in the microbial community (Mille-Lindblom, Fischer, and J. Tranvik 2006), which significantly impacts the structural integrity of granules as it largely depends on the structural EPS produced by specific species of bacteria (Ding et al. 2015; Y.-Q. Liu, Liu, and Tay 2004; Tay, Liu, and Liu 2001b). Another characteristic of fungi is their ability to reproduce using unique pathways (e.g. fragmentation) that can be stimulated under the AGS normal operation conditions, favouring the overgrowth conditions (Campbell, Johnson, and Warnock 2013). Thus, it was necessary to better understand this problem, given there is a lack of information in the literature about this type of filamentous overgrowth.

To understand the behaviour and consequences of filamentous fungi overgrowth on the different aspects of performance of an AGS reactor, the objectives of this study are to (i) reveal the potential causes and overgrowing mechanisms of filamentous fungi, (ii) assess the impact on biomass and granulation, (iii) appraise the impact on treatment performance, and (iv) elucidate the dynamics of the microbial community structure. The knowledge acquired from this study provides comprehensive understanding of the filamentous fungi overgrowth from different perspectives, which facilitates overcoming this problem, allowing this promising technology to achieve its full potential. In addition, limitations of the current routine analyses were discussed along with giving recommendations for the early detection of this problem.

5.2. Materials and methods

5.2.1. Reactor set-up and operation

The experiments were performed in a laboratory-scale column reactor operating continuously for 274 days at the room temperature. The reactor has an inner diameter of 9 cm and effective height of 63 cm, resulting in a working volume of 4 L and a height-to-diameter ratio of 7. The reactor was operated as a sequencing batch reactor with 3-hours cycles composed of 5 min of feeding, 169 min of aeration, 1 min of settling, and 5 min of decanting (gradually decreased from 20 to 1 min throughout the first two weeks of operation). The influent was introduced at the bottom of the reactor while effluent was withdrawn at the half-height, giving an exchange ratio of 50% and hydraulic retention time of 6 h. Air was introduced at the bottom of the reactor using a fine air diffuser and controlled to introduce a constant superficial air up-flow velocity of 2.4 cm s^{-1}, referenced to the reactor's circular cross-sectional area.

5.2.2. Inoculum and wastewater composition

The reactor was inoculated with its total working volume of activated sludge with a mixed liquor suspended solids (MLSS) concentration of 3,147 mg L^{-1} and sludge volume index at 5 min (SVI_5) of 168.09 mL g^{-1} obtained from a biological nutrient removal (BNR) unit at the urban municipal Gold Bar Wastewater Treatment Plant (WWTP) located in Edmonton, AB, Canada, treating an average daily flow of 265M L d^{-1}. The reactor was fed with high-strength synthetic wastewater prepared according to Tay et al. (2002) and having a chemical oxygen demand (COD) of 2,000 mg L^{-1} (using sodium acetate as a

carbon source; resulting in an organic loading rate [OLR] of 8 g L^{-1} d^{-1}), ammonium of 92 mg N L^{-1}, and phosphate of 11 mg P L^{-1}. The wastewater was prepared using tap water without controlling its pH.

5.2.3. Operation stages and sludge sampling for microbial community analysis

In order to understand the evolution of the microbial community in the aerobic granular sludge reactor (AGS), sludge was sampled and analysed at three different stages throughout the timeline of reactor operation: the start-up stage (stage I), the granulation stage (stage II), and the disintegration stage (stage III). The three stages have been defined, as described in Table 5.1, based on the sludge settling ability (SVI_5) as well as its morphological characteristics. The SVI_5 was used in this study as a measure of sludge settling ability as it is the most commonly used parameter. An SVI_5 value of 50 mL g^{-1} was chosen as a threshold to distinguish between flocculent and granular sludge since the SVI_5 in full-scale applications often lies between 35 and 70 mL g^{-1} (van der Roest et al. 2011; van Dijk, Pronk, and van Loosdrecht 2018; Pronk et al. 2015). The start-up stage was the first stage of operation starting from the reactor inoculation until the SVI_5 was reduced to just above 50 mL g^{-1}. The granulation stage was when the SVI_5 was below 50 mL g^{-1} and the sludge was dominated by granular sludge. The disintegration phase followed the granulation stage and was characterized by the dominance of filamentous granules and increase in the SVI_5 to above 50 mL g^{-1}.

Table 5.1. Reactor operation stages and their characteristics.

Operation stage	Main features	Biomass samples		Duration (d)
		Type	ID	
Stage I (start-up)	– $SVI_5 > 50$ mg L^{-1} – Dominated by flocculent sludge	Flocculent sludge	Seed	45
Stage II (granulation)	– $SVI_5 \leq 50$ mg L^{-1} – Dominated by granular sludge but still comprise some flocculent sludge	Flocculent sludge AGS	II-F II-G	180
Stage III (disintegration)	– $SVI_5 > 50$ mg L^{-1} – Comprised of AGS, impaired AGS, and flocculent sludge	Flocculent sludge AGS Filamentous AGS	III-F III-G III-FG	22

Sludge samples were collected from the inoculum and at the end of stages II and III for the microbial community analysis. The sludge samples were collected using the same method described in section 2.4. Three sludge types were identified throughout the three stages of the reactor's operation as shown in Table 5.1: flocculent sludge, AGS, and filamentous AGS. The flocculent sludge is fluffy dispersed sludge similar to the conventional activated sludge. The AGS is originated from the flocculent sludge but eventually granulated, while the filamentous AGS is AGS but with complete or partial filamentous growth on the outer surface of the granules. The sludge samples were split into these three types prior to downstream analyses to understand the interaction between the different types. The flocculent sludge was separated from the AGS and/or filamentous AGS by allowing the sludge sample to settle and stratify then collecting the top flocculent sludge layer. The AGS was separated from the filamentous AGS by

manually selecting granules according to their morphological characteristics, after washing the flocculent sludge.

5.2.4. DNA extraction, MiSeq sequencing, and analysis

DNA was extracted from the different types of sludge (i.e. flocculent, AGS, and/or filamentous AGS) at each stage using the DNeasy PowerSoil Kit (Qiagen Co., Netherlands) as per manufacturer's instructions. The extracted DNA was then checked for quality and quantity using the NanoDrop™ One Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific Co., USA) as per manufacturer's instructions. The extracted DNA was stored at -70 °C until dispatched for sequencing. The sequencing was performed in the molecular sequencing laboratory RTL Genomics (Texas, USA) on the Illumina MiSeq Next-Generation sequencing platform according to the laboratory's methodology. The seed, II-G, III-G, and III-FG samples were analysed for the bacterial and the eukaryotic community, while the flocculent sludge samples in stages II and III (i.e., II-F and III-F) were analysed only for the bacterial community. The bacterial 16S rRNA genes were amplified using the primers 357wF (CCTACGGGNGGCWGCAG) and 173 785R (GACTACHVGGGTATCTAATCC), while the eukaryotic 18S rRNA genes were amplified using the primers Euk1391F (GTACACACCGCCCGTC) and EukBR (TGATCCTTCTGCAGGTTCACCTAC).

5.2.5. Analytical methods

The treatment performance of the AGS reactor was monitored by analysing both the influent and effluent for organics and nutrients, represented in

the dissolved organic carbon (DOC), ammonium (NH_4^+-N), and orthophosphate (PO_4^{3-}-P). Influent and effluent samples were filtered prior to analyses using borosilicate glass microfiber filters with nominal particle retention of 1.5 μm. The DOC was measured using the TOC-L TOC analyser (Shimadzu Corp., Japan) as per manufacturer's instructions. The ammonium and orthophosphate were measured using Hach kits and spectrophotometer (Hach Co., United States) as per manufacturer's instructions.

Sludge samples were collected at the beginning of the aeration phase from a sampling port located at 21 cm (one third of the total working height) from the reactor bottom to minimize the potential error of inhomogeneous sludge distribution along the reactor depth. MLSS, MLVSS, and SVI are measured according to the Standard Methods (APHA 1998).

5.3. Results and discussion

5.3.1. Filamentous fungi overgrowth and its potential causes

The AGS reactor was continuously operated for 274 days. Dense, chardonnay-coloured (RGB of 255,192,113) granules with smooth surfaces were successfully cultivated from activated sludge after a start-up period of 45 days (Figure 5.1a). The average diameter of AGS was 2.58 mm. On day 155, colonies of white filaments were first observed to occupy specific spots on the surface of a few granules forming what is defined in this study as "filamentous AGS". After day 155, these filaments spread gradually and covered wider spots and more granules (Figure 5.1b). Due to the high hydrodynamic shear forces caused by aeration, a portion of the filaments detach and mix with the flocculent sludge.

Eventually, filamentous AGS disintegrated after extended filaments overgrowth. Disintegrated granules were often washed out of the reactor with the effluent due to their poor settling ability compared to the AGS and even filamentous AGS, given the short settling time.

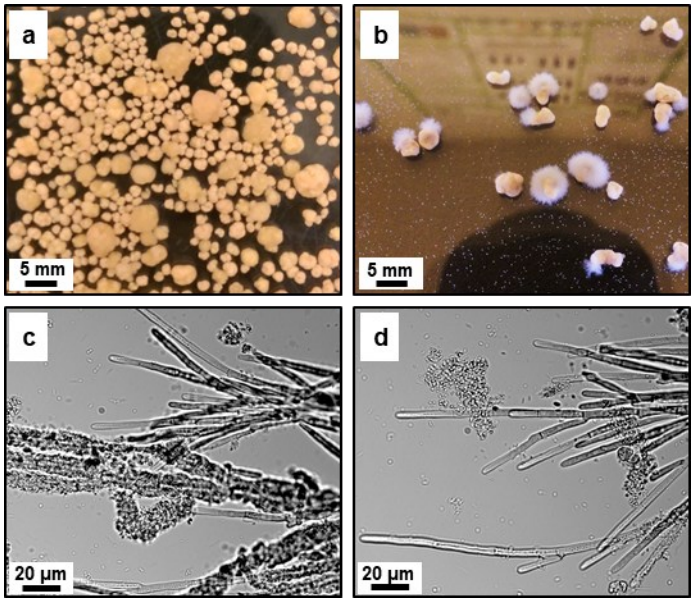

Figure 5.1. Photographs of AGS (a) and filamentous AGS (b), and microscopy images of filamentous fungi around granules (c and d).

The white filamentous organisms were identified to be yeast-like fungi that belong to the arthrosporic moulds *Geotrichum* of the family *Dipodascaceae* and the phylum Ascomycota. The microbial community structure is discussed in more detail in section 5.3.4. Several possible causes have been suggested in previous studies for the overgrowth of such fungi; those which relate to our study include the continuous aeration, high DO, high sludge organic loading rate (SOLR), and dissimilar microbial growth rates. Continuous aeration was suggested to favour the filamentous fungi overgrowth compared to intermittent aeration (Moreira et al. 1996). Despite the cyclic nature of an AGS reactor's

operation, its aeration tends to act as continuous due to its long periods compared to the total cycle time. Another study found that maintaining high dissolved oxygen (DO) through aeration in a high rate led to an increase in the filamentous fungi biomass (Bai et al. 2003).

Cultivating AGS at different SOLRs was investigated in an AGS reactor treating synthetic sugar-containing wastewater (COD of 2,000 mg L^{-1}) where overgrowth of white filamentous fungi of the family *Dipodascaceae* – similar to the fungi identified in our study – was observed at higher SOLRs, leading to deteriorated settling ability (Li et al. 2016). The highest SOLR used in their study was about 1.05 kg COD kg^{-1} MLSS d^{-1} compared to an average SOLR of 2.58 kg COD kg^{-1} MLSS d^{-1} in our study, increasing the likelihood of filamentous domination. According to the competitive exclusion principle (aka the Gause's law; Hardin, 1960), species with a higher growth rate constant would proliferate and dominate in the long term. Comparing the growth rate constants of *Thauera* spp. (B. Liu et al. 2013) and *Geotrichum* spp. (Caldwell and Trinci 1973) as the dominant species in the granulation and disintegration stages, respectively, the growth rate of *Geotrichum* spp. was 2.89 and 1.48 times in average higher than that of *Thauera* spp. under aerobic and anaerobic/anoxic conditions, respectively (B. Liu et al. 2013). It should be noted that *Thauera* spp. can grow under both aerobic and anaerobic/anoxic conditions and have been observed to be distributed all across the AGS where different redox conditions apply (Fra-Vázquez et al. 2016).

The microscopic images revealed that the fruiting bodies of the fungal hyphae were missing, likely due to the high hydrodynamic shear forces caused by aeration (Figure 5.1c and 5.1d). Wilén et al., (2018) have shown that high hydrodynamic shear forces and consequent particle-particle collisions can preferentially erode the biomass located on the granules' surface. However, this was not a barrier for the *Geotrichum* to overgrow, which may be attributed to their ability to reproduce both sexually and asexually (Campbell, Johnson, and Warnock 2013). The main reproduction method of *Geotrichum*, which can be readily favoured under the AGS reactor operation conditions (i.e. strong mixing caused by aeration), is the mycelial fragmentation. This occurs when arthrospores (i.e. chains of individual cells) transport as fungal thallus detach from existing hyphae and land on other spots or granules, forming new colonies (Campbell, Johnson, and Warnock 2013). This interprets the widespread of the filamentous fungi from a few granules to most of them as well as the high rate of reproduction despite the loss of fruiting bodies. As a conclusion, once the filamentous fungi overgrowth is triggered, it is likely to continue spreading unless otherwise an inhibitory action is taken.

5.3.2. Sludge settling ability and morphological and gravimetrical evolution

The SVI_5 has been used in numerous studies ranging from laboratory to full scale to measure the AGS settling ability and the extent of granulation (X. Zhang et al. 2013; Y.-Q. Liu et al. 2016; Ni et al. 2009; Y.-Q. Liu et al. 2010; van der Roest et al. 2011; Pronk et al. 2015; van Dijk, Pronk, and van Loosdrecht 2018). The SVI_5 was used in this study to identify the dominant nature of the

sludge in terms of being flocculent or granular, and, accordingly, define the different stages. Thus, an SVI_5 value of 50 mL g^{-1} was set to distinguish between stages since AGS applications often have an SVI_5 between 35 and 70 mL g^{-1} (van der Roest et al. 2011; Pronk et al. 2015; van Dijk, Pronk, and van Loosdrecht 2018).

As presented in Figure 5.2, the SVI_5 started at 168.42 mL g^{-1} at the beginning of Stage I, reflecting the flocculent nature of the inoculum (i.e. activated sludge). Then the SVI_5 gradually decreased down to 59.37 mL g^{-1} on day 59 due to the applied selection pressures, such as the high shear forces (Y. Liu and Tay 2002) and short settling time (Beun, van Loosdrecht, and Heijnen 2002). The SVI_5 then had a period of stability with minor fluctuation around an average of 17.54 mL g^{-1} before it started to increase again on day 211 in a high rate until it reached 247.02 mL g^{-1} at the end of Stage III and reactor's operation on day 274.

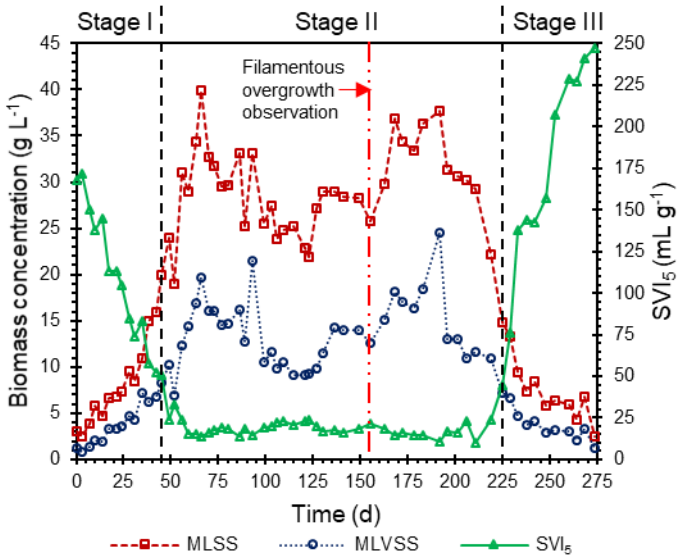

Figure 5.2. AGS settling ability and gravimetrical evolution.

The average settling velocity of the AGS and filamentous AGS were 4.13 and 2.55 m s^{-1}, respectively, indicating deterioration in the settling ability by 38.11%. The high sludge concentration achieved in stage II was then significantly reduced due to the sludge washout, leading to complete failure of the reactor at the end of stage III.

It is worth noting that there was a lag of 78 days between the first observation of the filaments on day 155 and the SVI_5 exceeding beyond 50 mL g^{-1} on day 225 indicating poor granulation and domination of flocculent sludge. This shows that the SVI_5 largely lacks sensitivity towards the physical consequences caused by the filamentous overgrowth, which signals the limitation of depending on SVI_5 as the sole measure of the extent of granulation. Thus, other routine measures should be considered to override or complement the SVI_5 to allow for early detection and intervention before a reactor fails.

5.3.3. Impact of filamentous fungi overgrowth on the treatment performance

The AGS reactor removal performance of DOC, N, and P throughout the three stages of operation is presented in Figure 5.3. The average DOC, N, and P concentrations in the feeding water were 2,129.71, 85.05, and 27.16 mg L^{-1}, respectively, resulting in an average DOC:N:P ratio of 100.0:4.0:1.3. During the first two months of operation, the DOC removal efficiency increased gradually from about 80% until it reached a plateau at almost complete removal (i.e., near 100%) during most of stage II. The DOC removal efficiency during stage III decreased slightly compared to the steady state of stage II, however, it remained above 90% at all times. This indicates that, even under this severe filamentous

overgrowth occurred in this experiment, and the consequent breakdown and washout of sludge, the AGS reactor can still achieve satisfactory DOC removal efficiency, given a minimum sludge concentration in the reactor.

For the nutrients (i.e. N and P) removal efficiencies, they gradually increased during stage I until they reached almost complete removal during stage II, except for some short instability periods. After the filamentous overgrowth occurred, the N removal efficiency was slightly decreased starting from day 183 until it reached a low of 91.46% at the end of stage III. On the other hand, the P removal efficiency was significantly impacted by the filamentous overgrowth starting from day 189 and decreased to 33.36% at the end of stage III. These results are consistent with the previously reported C:N:P ratio of 200:15:1 for the phylum Ascomycota (J. Zhang and Elser 2017), compared to a ratio of 100:7:1 for bacteria (Tchobanoglous et al. 2003).

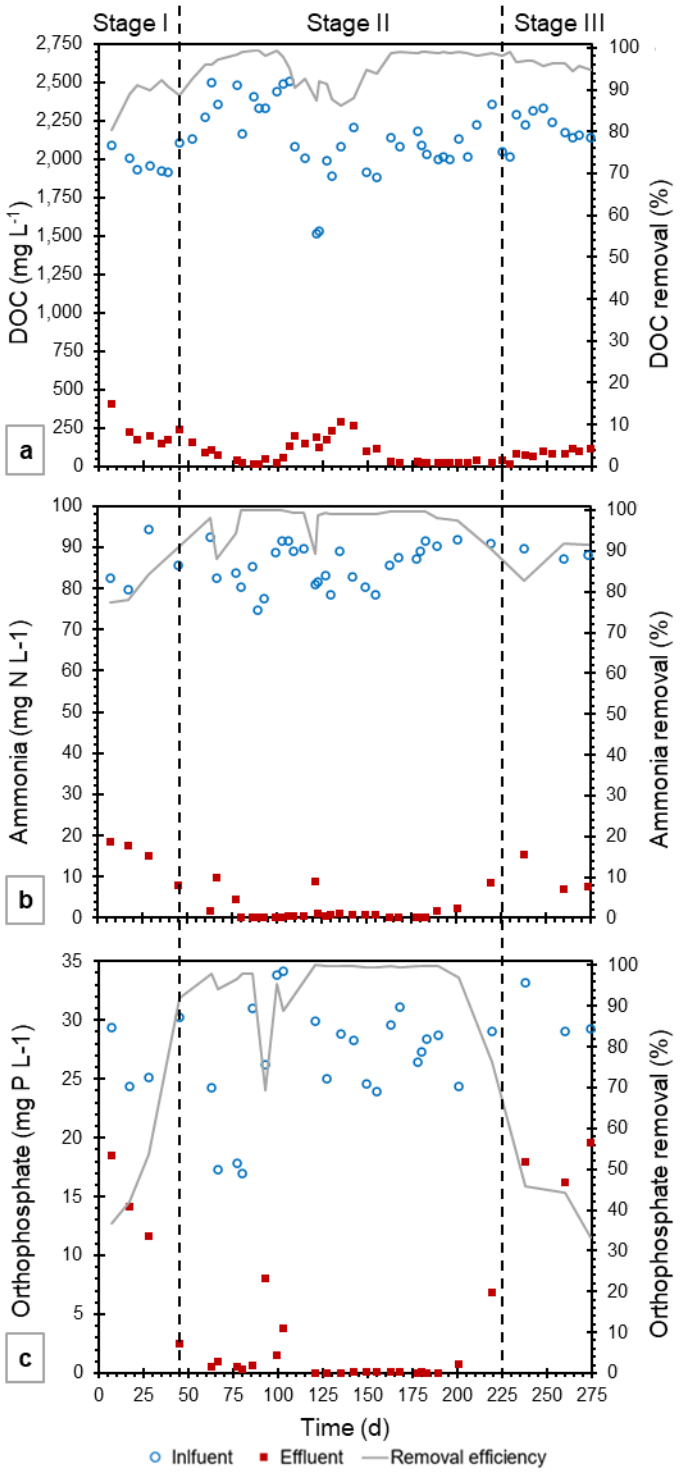

Figure 5.3. AGS reactor removal performance of (a) DOC, (b) ammonia, and (c) orthophosphate.

5.3.4. Impact of filamentous fungi overgrowth on the microbial community structure

5.3.4.1. Eukaryotic community

The predominant eukaryotic taxa changed through different stages (Figure 5.4). In the seed sludge, taxa in the phylum SAR (*Rhogostoma* [41.9%], Thecofilosea [18.2%], *Peronosporomycetes*_uncultured [5.7%], *Rhogostoma*_uncultured [4.7%]) and in Opisthokonta (Bdelloidea [9.5%], *Saccharomyces* [4.6%] and Ploimida [4.0%]) predominated the community. This is consistent with previous studies where Matsunaga et al. (2014) investigated the diversity of eukaryotes in nine samples collected from three different full-scale municipal WWTPs and found that, for the WWTP that supports nitrogen removal as the case in Gold Bar WWTP, the eukaryotic community was predominated with *Alveolata* (19.6-81.4%; belonging to the phylum SAR) and fungi (10.5-56.9%; belonging to the phylum Opisthokonta). These taxa decreased to low-abundance or undetectable levels after granulation in stages II and III.

The AGS in stage II was predominated by Tubulinea group 04 sp. CAL7 (92.7%), that belongs to the phylum Amoebozoa. This is in line with a recent study where the abundance and diversity of higher organisms in AGS was analysed, and they found that the community was predominated by naked Amoeba (Thwaites et al. 2018). The transmission electron microscopy (TEM) imaging has confirmed the high abundance of Amoeba, especially in the outer shell of the granules. Amoeba was suggested to indicate healthy activated sludge at sludge loading of 0.15-0.5 kg BOD kg^{-1} MLSS d^{-1} (Eikelboom 2000) which covered the loading range of stage II. Its dramatic decreases in stage III's AGS

and filamentous AGS suggested its potential use as an indicator of stable AGS, which has the advantage of easy monitoring using microscope (Eikelboom 2000; Thwaites et al. 2018).

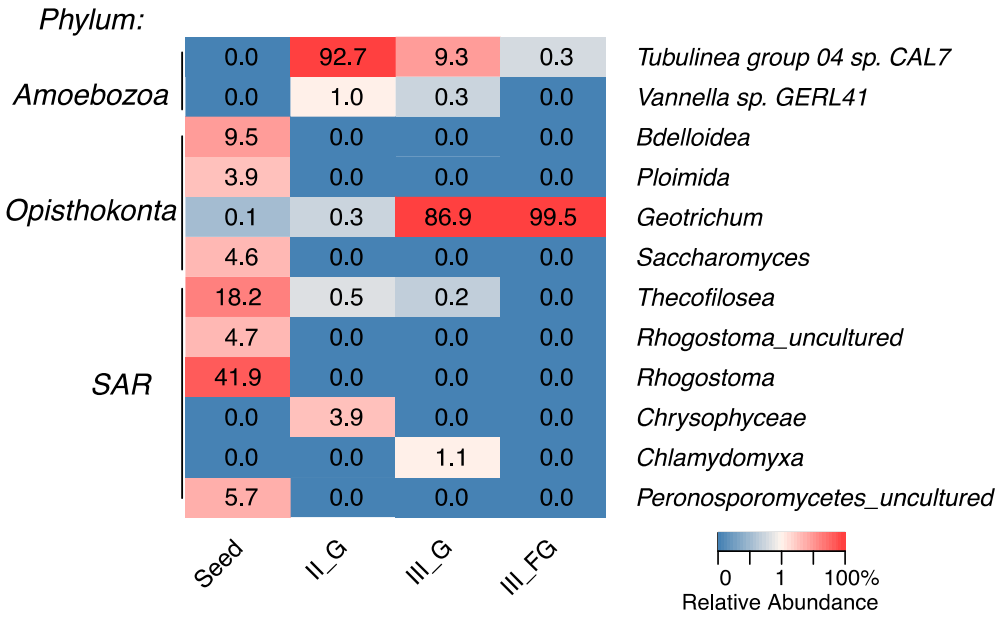

Figure 5.4. Relative abundance of eukaryotes (> 1%) in seed; stage II's AGS (II_G); and stage III's AGS (III_G), and filamentous AGS (III_FG). Taxon names were shown at the identified taxonomic level.

In stage III, the predominant taxon was *Geotrichum* in the phylum Opisthokonta, 86.9% in granular sludge and 99.5% in the filamentous granular sludge. Wan et al., (2014) has previously reported the overgrowth of *Geotrichum* to deteriorate the structural stability of bacterial AGS in the long term operation, leading eventually to the reactor failure. Moreover, Li et al. (2016) has reported the domination of white filamentous fungi of the family *Dipodascaceae* (the same family for the genus *Geotrichum*), which was also associated with deterioration in the sludge settling and compression ability.

The eukaryotic community in the seed showed higher alpha-diversity than in stages II and III as revealed by the faith_pd diversity, the observed OTUs and the Shannon index. It could be explained by that growth of predominant species supressed the growth or the detection of other species in stages II and III, which reduced the alpha-diversity.

5.3.4.2. Bacterial community

The bacterial communities were compared between the seed; stage II's AGS and flocculent sludge; and stage III's AGS, filamentous AGS, and flocculent sludge. Their weighted Unifrac distances are shown in the Principal coordinates analysis (PCoA) plot (Figure 5.5a). From seed to stage II, the microbial community showed a large distance along the PCoA1 (62.2 % of total variance) axis. The AGS and flocculent sludge communities in stage II were similar. In stage III, the AGS and filamentous AGS communities varied largely from stage II's AGS, whereas the flocculent sludge community was similar with the community of stage II's flocculent sludge. The community changes in AGS from stage II to stage III are related with the AGS deterioration process. Therefore, bacterial variation in AGS is suggested to be a better indicator of granular stability and deterioration than in flocs community.

At phylum level (Figure 5.5b), Proteobacteria predominated all samples, increasing from seed to stage II but decreasing in stage III AGS and filamentous AGS. Actinobacteria relative abundance was observed to be minimal in the flocculent (0.09%) and granular sludge (1.40%) prior to filamentous overgrowth, while it has significantly increased coinciding with the filamentous overgrowth

and granules disintegration in all types of biomass in stage II, reaching 16.91, 27.27, and 7.98% in AGS, filamentous AGS, and flocculent, respectively. This was by competing with *Bacteroidetes* in the flocculent sludge and Proteobacteria in the granular sludge. Actinobacteria are known of their capability to produce extracellular enzymes that initiate the degradation of complex polysaccharides (Větrovský, Steffen, and Baldrian 2014), which are considered as an integral component of the EPS matrix and major structural substances for granulation (T. Seviour et al. 2012). This impact of Actinobacteria was confirmed by Luo et al. (2014) as they observed reduction in the polysaccharides fraction of the EPS matrix accompanied with granules disintegration after a significant increase in the Actinobacteria relative abundance in the microbial community of the granules.

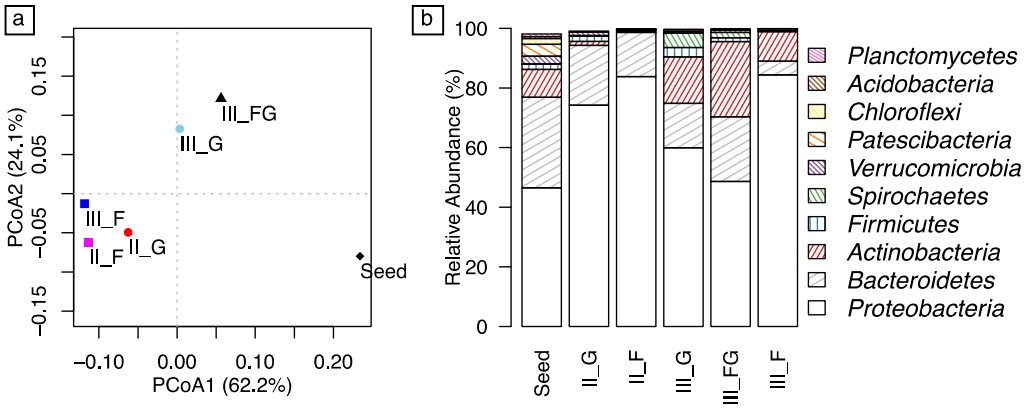

Figure 5.5. (a) Principal coordinates analysis (PCoA) using weight Unifrac distances of bacterial communities in seed; stage II's AGS (II_G) and flocculent sludge (II_F); and stage III's AGS (III_G), filamentous AGS (III_FG), and flocs (III_F); and (b) their relative abundance of (> 1%) at phylum level.

At genus level (Figure 5.6), *Thauera* was the most abundant genus in stage II AGS (57.0%) and flocculent sludge (61.6%), followed by *Flavobacterium* (7.0

and 6.8% respectively). By comparing the bacteria genera in stages II and III's AGS, *Thauera* increased from 0.1% in seed to 57.0 and 61.6% in stage II's AGS and flocculent sludge, respectively. However, it decreased to 16.9% and 10.8% in stage III's AGS and filamentous AGS, respectively, but kept at high level at 57.6% in flocculent sludge. This high abundance of the genus *Thauera* can be attributed to the applied selection pressures resulting from the operating conditions. *Thauera* has been reported to copiously produce extracellular polymeric substances (EPS; Ding et al., 2015), which is a main driver for granulation (Wilén et al. 2018). *Thauera* is a member of the family *Rhodocyclaceae* of the class β-Proteobacteria, which has been known as denitrifiers in wastewater treatment systems such as activated sludge (R. J. Seviour and Nielsen 2010). *Thauera* was also reported to perform denitrification under both aerobic and anaerobic conditions (Shinoda et al. 2004). The relative abundance of *Thauera* in the flocculent sludge was not affected by the filamentous overgrowth, although it was significantly affected in the granular sludge. Comparing the relative abundance of *Thauera* in the flocculent to granular sludge, it was significantly reduced because of competence with other species during the deterioration process. The filamentous overgrowth and granular deterioration showed a stronger association with AGS bacterial abundance changes than flocculent sludge bacterial abundance changes, thus this supports the suggestion for the AGS bacterial community to be a better indicator than flocculent bacterial community.

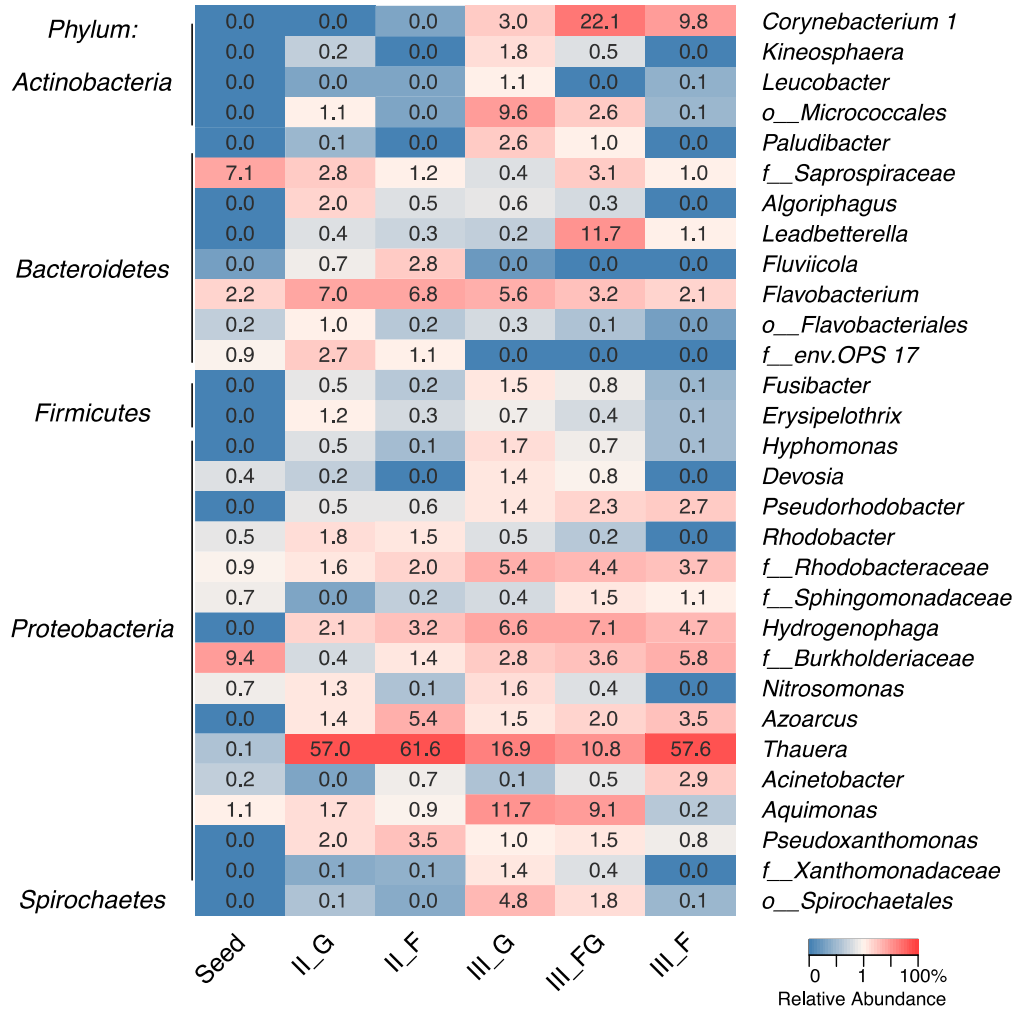

Figure 5.6. Relative abundance of bacterial genera (> 1%) in seed; stage II's AGS (II_G) and flocculent sludge (II_F); and stage III's AGS (III_G), filamentous AGS (III_FG) and flocculent sludge (III_FG). Taxon names are shown at family level (f__) or order level (o__) if not identified at genus level.

The bacterial community alpha-diversity indices (faith_pd diversity, observed OTUs and the Shannon index) were reduced from seed to stages II's AGS and flocculent sludge. The flocculent sludge community in stage III showed further decrease in observed OTUs and the Shannon diversity. The AGS bacterial

community in stage III showed higher alpha-diversity indices than in stage II, likely due to the community shift associated with the filamentous overgrowth.

5.4. Conclusion

Filamentous fungi can overgrow in an AGS reactor treating high strength wastewater after a period of stability. This overgrowth results in AGS poor settling ability and, eventually, biomass disintegration and washout. Conventional routine gravimetric measures lack sensitivity to early detection of this problem. The impact on nutrients removal efficiency is more significant, especially the phosphorus, compared to organics. The eukaryotic community of healthy and filamentous granules were predominated by Tubulinea and fungi, respectively. *Thauera* predominated the bacterial community of healthy granules, being a copious EPS producer, yet it was outcompeted by filamentous overgrowth leading to structurally loose granules.

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Chapter 6 - Conclusions and Direction for Future Work

6.1. Conclusions

6.2. Process evaluation for various technologies

- Two types of treatment technologies were evaluated for greywater treatment, namely biofilters and AGS reactors. For the biofilters, GAC and sand were evaluated as two packing media.
- The AGS technology, despite its superior treatment capacity, failed to exhibit a stable performance due to a commonly reported operational issue, which is filamentous overgrowth. Therefore, AGS was not considered for further research and research on biofilters moved forward.
- A new design of biofilters composed of two zones (unsaturated and saturated) in a single stage was developed for greywater on-site treatment to provide high-quality effluent that is safe for potential domestic uses or safe discharge into the environment.
- The treatment capacity of the developed technology was tested by evaluating its capability in removing major nutrients under different loading rates where the system achieved an average TCOD removal of 98% and complete nutrients removal throughout its 253 days of operation at highest hydraulic and organic loadings of 1.2 m^3 m^{-2} d^{-1} and 3.5 kg COD m^{-2} d^{-1}, respectively.
- The capacity of the system to reduce pathogens was tested against five pathogen surrogates representing four groups of pathogens (human skin-

associated bacteria, human enteric bacteria, viruses, and protozoan cysts and oocysts). The system showed a range of reduction towards the pathogen surrogates ranging from no reduction in viruses to a log reduction of 3.4 in protozoan cysts and oocysts with an intermediate log reduction of 0.26-1.13 in bacteria.

- The individual capacity of each of the unsaturated and saturated zones was identified for reducing the major nutrients and pathogen surrogates.

6.3. GAC process optimization and treatment mechanism evaluation

- Biofilm development and activity were profiled along the biofilter's depth to show that a well-functioning biofilm developed within the system, and its mass and activity increased over time with the highest values observed at the top layers.
- The microbial community structure along the depth of the biofilter was analysed and results were reported at class and genus levels where the key microbes were revealed and the bacterial genus *Oleomonas* was found to predominate the system due to its unique and advantageous attributes.
- The treatment processes taking place within the system were identified and their kinetics were measured to help understand the behaviour of the biofilter and potentially facilitate its design and operation.
- The individual contribution of sorption and biodegradation processes to the overall treatment was quantified.

- In a mechanistic study conducted on BAC media collected from the GAC biofilter, biodegradation was found to contribute 26% and 10% after 1 h and 24 h of treatment, respectively, while the rest was attributed to sorption processes. This finding suggested that intermittent dosing of greywater to the biofilter is preferable due to the difference in removal capacities to allow for bioregeneration of the BAC media by the biodegradation process.
- A new method was developed to study the adsorption equilibrium and kinetics while completely eliminating the impact of the biofilms surrounding the GAC media. The method included exposing the BGAC media to stages of ignition, biological inhibition, and washing.
- Testing the equilibrium adsorption experimental results against four isotherm models revealed that the Freundlich isotherm was found to best represent the equilibrium adsorption data.
- A study on the kinetics of isotherm showed that the pseudo-second order and intraparticle diffusion models were found to fit the adsorption kinetics. Intraparticle pore diffusion was found to be the rate limiting step after a few hours of treatment.

6.4. Direction for future work

This study a GAC biofilter with a single depth of 60 cm and fixed depths of the unsaturated and saturated zones of 40 cm and 20 cm, respectively. Investigating the impact of the biofilter's depth on its performance may result in

achieving effluents with acceptable quality using shorter depth and, thus, less cost.

Although the greywater formulation used in this study was composed of real commercial products, evaluating the performance of the system against real greywater could give more accurate insights about the applicability of the system in the real life and reveal possible limitations. This scenario may trigger the need for upstream physical treatment unit, such as a holding tank, to allow for the separation of settleable and floating matter.

The greywater formulation used in this study was deficient in nutrients, especially nitrogen. Therefore, the capacity of the system in reducing nutrients was not challenged. Future studies that includes feed water with higher loads of nutrients would evaluate the capacity of the system as a whole and the unsaturated and saturated zones individually in contributing to nutrients reduction. Recirculation of effluents could be required in case effluents with acceptable cannot be achieved through a single pass.

Since the GAC biofilter was developed for on-site treatment, it may experience inactive periods where no influent is provided during, for example, vacations. Therefore, evaluating the impact of these inactive periods on the mass and activity of biofilms as well as assessing the reactivations periods could be essential.

The system showed a range of reduction towards pathogen surrogates representing human skin-associated and enteric bacteria, viruses, and protozoan cysts and oocysts. Adding a disinfection/inactivation unit downstream of the

biofilter, such as an ultraviolet lamp, would result in higher reduction of pathogens, providing high-quality effluent that is safe for potential domestic uses or safe discharge into the environment.

The microbial community structure of the GAC biofilter was predominated by the bacterial genus *Oleomonas*. Future research can investigates ways to further enrich for this bacterial genus and its impact on improving the role of the biodegradation treatment mechanism.

The mechanistic study in this research showed that the biodegradation mechanism has less contribution to the overall treatment process as compared to sorption processes. It could be beneficial to stimulate the growth of more biomass to allow for greater loading rates and improved media bioregeneration.

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