Optimization of Moving Bed Biofilm Reactor (MBBR) Operation for Brewery Wastewater Treatment

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A thesis submitted under the supervision of Dr. Chris Kinsley in partial fulfillment of the requirements for the degree of Masters of Applied Science in Environmental Engineering.

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Abstract

The significant rise in the number of micro-breweries in North America has increased the need for efficient on-site industrial wastewater facilities. Brewery wastewater is considered to be a high strength food industry wastewater with high variability in terms of both organic and hydraulic loading. Small breweries require cost-effective, reliable, and simple to operate treatment technologies to properly manage their brewery wastewaters. Moving bed biofilm reactor (MBBR) technology has shown promise at the lab-scale and full-scale with respect to brewery effluent treatment. MBBR systems have the capability for short hydraulic retention times (HRT), high organic loading rates, as well as increased treatment capacity and stability due to biofilm retention, all within a compact reactor size when compared to other aerobic and attached growth treatment options.

Two MBBR systems utilizing two different carrier types (Kaldnes K5 and Kontakt), and a suspended growth (SG) control reactor, were used in this study to investigate the impacts of surface area loading rate (SALR) and HRT on attached growth (AG) and SG kinetics and carrier type for brewery wastewater at 2000 mg-sCOD/L. An increase in SALR from 10-55 g-sCOD/m^2/d while at an HRT of 12 hr resulted in no significant impact in total volumetric removal rates between the MBBR systems and the SG control reactor; however, MLSS concentrations were lower for the MBBR systems at SALRs below 55 g-sCOD/m^2/d, which indicated AG contribution. Over 92% soluble chemical oxygen demand (sCOD) removal was achieved at each SALR in each of the three reactors. These results indicated that the reactors were substrate limited and SG controlled. Due to the SG dependency, the difference between the two types of carriers was indeterminate. A decrease in HRT from 12-3 hr while maintaining an SALR of 40 g-sCOD/m^2/d resulted in a shift from SG to AG dependency in the MBBR systems. The total volumetric removal rates for the MBBR systems were significantly higher at HRTs of 3 and 4 hr as compared to the SG control reactor. The AG volumetric removal rates from both MBBR systems were highest at an HRT of 3 and 4 hr. At an HRT of 12 hr all three reactors maintained over 92% sCOD removal; however, at an HRT of 4 hr the SG control reactor dropped to 88% and at 3 hr to 61%, whereas the MBBR systems maintained 95% removal at an HRT of 4 hr and only decreased to 73% at 3 hr. These results indicated that the MBBR systems were more effective at lower HRT than the SG control reactor, with no significant difference observed between the two carrier types tested.

Biofilm morphology and viability from each of the two carriers utilized in the study of moving bed biofilm reactor (MBBR) treatment of brewery wastewater were investigated using stereoscopy and confocal laser scanning microscopy (CLSM) in combination with live/dead cell staining. Both carriers demonstrated thicker and more viable biofilms at high SALR and denser and less viable biofilms at low SALR. At lower HRT, the carriers reacted differently resulting in thicker, but less dense biofilms on the Kontakt carriers and thinner, but more dense biofilms on the K5 carriers. However, no trend in cell viability was observed with change in HRT. Although the systems were suspended growth (SG) dominated, based on the MBBR kinetics and carrier biofilm morphology and cell viability, either carrier would be a viable choice for an MBBR treating brewery wastewater at HRTs between 4 to 12 hr and SALRs between 10-55 g-sCOD/m^2/d.

Acknowledgments

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List of Abbreviations

AD	Anaerobic Digestion
AG	Attached Growth
AnMBR	Anaerobic Membrane Bioreactors
BOD	Biological Oxygen Demand
CH_4	Methane
CIP	Clean in Place
CLSM	Confocal Laser Scanning Microscopy
CMAS	Complete Mix Activated Sludge
C:N	Carbon to Nitrogen Ratio
COD	Chemical Oxygen Demand
CO_2	Carbon Dioxide
CSAD	Continuously-Stirred Anaerobic Digester
EGSB	Expanded Granular Sludge Bed
EPS	Extracellular Polymeric Substances
HDPE	High Density Polyethylene
HRT	Hydraulic Retention Time
IFAS	Integrated Fixed-Film Activated Sludge
MBRR	Moving Bed Biofilm Reactor

MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
ODF	Over-Strength Discharge Fee
RAS	Return Activated Sludge
R1	Reactor 1
R2	Reactor 2
R3	Reactor 3
SA	Surface Area
SALR	Surface Area Loading Rate
SARR	Surface Area Removal Rate
sCOD	Soluble Chemical Oxygen Demand
SG	Suspended Growth
TKN	Total Kjeldahl Nitrogen
TP	Total Phosphorus
TSS	Total Suspended Solids
UASB	Up-flow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acid

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

The average person consumes 80 L of beer per year, which contributes to beer being the third most consumed beverage worldwide (Ritchie & Roser, 2018). The brewing industry is a major part of the consumer world; the way beer has been brewed has seen minimal changes in the last five thousand years (Radu et al., 2014). However, the North American brewery structure has seen many changes over the past couple of decades. Micro-breweries (produce 17,000 hL/yr) and craft breweries have become more popular than large consumer breweries (produce 5,000,000+ hL/yr) (The Government of Canada, 2013; Brewers Association, 2017). As of 2017, there were 817 craft and microbreweries in Canada, which was an increase of 215% from 2013 (Beer Canada, 2018). The United States has also seen a dramatic increase in craft breweries, increasing from 2000 to 6300 craft breweries between 2010 and 2017, with 99% being microbreweries (Dowling, 2017; Brewers Association, 2017). These changes have resulted in municipalities focusing on the environmental impacts associated with beer production, where a major point of concern is the treatment and discharge of brewery wastewater.

1.1.1 Beer Production

A major issue with beer production is that it requires a large quantity of water and thus, produces substantial amounts of wastewater. A brewery's water and wastewater usage can be described by the ratio of water:beer:wastewater, where the respective quantities are approximately 9:1:8 m^3 (Connaughton et al., 2006; Chen et al., 2016). This ratio demonstrates that for every 1 m^3 of beer produced there is eight times the amount of wastewater produced.

Beer is produced by means of batch production with multiple stages. There are three main steps required for producing beer that every brewery will employ (Fillaudeau, et al., 2006;

Saila & Hasan, 2017). The first step is the malt production and handling. Malt is made from grain germination. The grain is steeped in water, which produces rootlets and enzymes. Once the rootlets and enzymes are fully developed the starch will be converted into maltose. The rootlets will then be removed, resulting in the end malt product. The second step is wort production. The previously made malt is ground with water in a mash tun to produce grist or mash. The mash tun is heated to help activate the enzymes in the mash. The residual grist (solids) are separated from the liquid, which is the wort. At this stage any number of ingredients for flavour may be added and boiled. Hops, or other natural or artificial anti-microbial products, will also be boiled into the wort. The trub (precipitated solids), the spent hops and any other solids left are removed and the wort is cooled. The final step is the beer production. The cooled wort is mixed with yeast and fermented for a selected amount of time. The spent yeast and any other solids present are then filtered out, producing the end product: beer.

There are three solid-liquid separation phases resulting in waste solids and wastewater: wort separation, wort clarification and rough beer clarification (Fillaudeau et al., 2006). After each stage of the process a clean-up and disinfection of the containers is also required. The beer industry is governed by the food and beverage industry regulations and thus, must meet strict sterilization requirements, which are denoted as clean in place (CIP) procedures. After beer production, the equipment utilized must be fully sterilized, where extremely caustic and acidic solutions and agents (some of which contain phosphorus or nitrogen (i.e. phosphoric acid and nitric acid) or surfactants) are alternated with rinse water ranging in temperature between 60-80°C (García & Díaz, 2011).

The primary constituents of brewery wastewater consist of total suspended solids (TSS), which are made up from spent grains, kieselguhr, waste yeast and trub, as well as dissolved constituents comprising: sugars, soluble starch, ethanol, volatile fatty acids (VFAs) and nutrients (phosphorus and nitrogen) (Chen et al., 2016; Saila & Hasan, 2017).

1.1.2 Brewery Wastewater Characteristics

Brewery wastewater is considered to be a high strength food industry wastewater with high variability in terms of both organic and hydraulic loading (Table 1.1). Many micro-breweries are located in towns where wastewater management options typically include pre-treatment and discharge to a municipal sewer (when available) or on-site wastewater treatment and discharge to a soil infiltration system. If not managed properly, beer production effluent can be extremely detrimental to the natural environment or to the operation of municipal wastewater systems, often lagoons, which are not designed to handle such high strength waste, nor the volumes with which it is discharged (Simate et al., 2011; Olajire, 2012). Municipalities have started to charge micro-breweries with over-strength discharge fees (ODFs), which can result in fines upwards of hundreds of thousands of dollars per year on top of standard sewer use fees (The Government of Canada, 2013). The large surcharges associated with direct discharge and the pressure applied by the municipalities to lower the strength of the wastewater, has thus prompted many breweries to implement on-site wastewater treatment systems.

Table 1.1: Typical brewery effluent characteristics.

Parameter	**Unit**	**Brewery Effluent Composition**	**This Study: Beau's Brewery** *Range (Average)*	**References**
COD	mg/L	1000-125,000	5000-40,000 (8000)	Connaughton et al., 2006; Fillaudeau, et al., 2006; WEF, 2010; Simate et al., 2011; Metcalf & Eddy, 2014; Radu et al., 2014; Choi, 2015; Chen et al., 2016; Manyuchi & Chikwama, 2016; Saila & Hasan, 2017
BOD	mg/L	600-110,000	n/a	
TSS	mg/L	200-3000	300-15,000 (4000)	
Tot. Nitrogen	mg/L	10-80	10-100 (25)	
Tot. Phosphorus	mg/L	10-100	50-1330 (70)	
Temperature	°C	15-80	20-50	
pH	-	2-14	3-10	

Note: Typical sewer discharge limits for the Ottawa region (By-law No. 2003-514): 300 mg-BOD/L, 300 mg-TSS/L, 10 mg-TP/L, 100 mg-TKN/L.

1.1.3 Brewery Wastewater Treatment Methods

The treatment strategy for brewery wastewater will depend on the individual plant operation; however, all brewery wastewaters will include high concentrations of soluble organics which will require some form of biological treatment (Saila & Hasan, 2017). Additionally, pH adjustment will always be necessary due to the high concentration of acids and bases used in the CIP procedures and primary settling may also be required depending upon TSS concentrations. The nature of beer production (i.e. batch process) and the subsequent need for sterilization, as well as the potential for weekly operational downtime, also makes volume equalization a necessity to ensure consistent flow through the wastewater system.

There are two fundamental biological means of treatment: anaerobic and aerobic. Anaerobic digestion (AD) involves the conversion of organic matter to methane gas (CH_4) and carbon dioxide (CO_2) by methanogenic bacteria under anaerobic conditions, while aerobic digestion involves the conversion of organic matter to CO_2 by heterotrophic bacteria in the presence of excess oxygen, often provided through mechanical aeration. AD of wastewater has been increasingly applied over the past 20 years to treat high strength wastewaters (Power & Jones, 2016) and is typically the primary method for the treatment of brewery wastewater (Simate et al., 2011; Olajire, 2012). However, due to the lower chemical oxygen demand (COD) removal percentages (≈80%) typically seen in anaerobic treatment, it is most often followed by an aerobic process (Metcalf & Eddy, 2014). The following sections will highlight the operational characteristics as well as the advantages and disadvantages of anaerobic and aerobic treatment.

1.1.3.1 Anaerobic Treatment

AD is a wastewater treatment process that is an environmentally sustainable method (Bocher et al., 2008). Anaerobic processes are typically employed when wastewater COD concentrations are greater than 2000 mg/L (Metcalf & Eddy, 2014) and are best suited for mesophilic (25-45 °C) wastewaters (Saila & Hasan, 2017). Operational advantages, such as no aeration requirements and utilization of CH_4 to offset energy consumption, make AD a favourable treatment method for large-scale breweries. However, the lack of nutrient removal, complexity dealing with CH_4 and long hydraulic retention times (HRT) can have an impact on their effectiveness for small-scale breweries. The following are examples of anaerobic treatment strategies that are capable of attaining high COD removal efficiencies (75-85%) when treating high strength industrial wastewaters (Saila & Hasan, 2017).

1.1.3.1a Continuously-Stirred Anaerobic Digester (CSAD)

Continuously-stirred anaerobic digester (CSAD), is a reactor containing anaerobic biomass and wastewater that is continuously mixed to achieve the appropriate treatment. CSADs are typically followed by a settling tank. The CSAD shears the organic matter which helps to break up the larger particles and allow them to be more accessible to the microbes. The continuous stirring also promotes contact between the microbes and particles, while simultaneously maintaining a constant temperature, pH and bulk composition within the digester (Bocher et al., 2008).

Unfortunately, anaerobic microbes do not precipitate as easily as aerobic microbes and with CH_4 gas bubbles in the tank, the anaerobic sludge will float, creating settling problems. Therefore, assisted settling is needed for the biomass to effectively treat the wastewater. A CSAD will normally include a de-gassing system and some sort of filtration or inclined plates in the settling tank to promote biomass settling (Bocher et al., 2008).

1.1.3.1b Up-flow Anaerobic Sludge Blanket (UASB)

In an up-flow anaerobic sludge blanket (UASB), the wastewater is fed into the bottom of the system and passes upwards through the sludge blanket. UASB typically utilise seed or inoculum of previously developed anaerobic sludge to accelerate the acclimation or start-up period (Chen et al., 2016, Parawira et al., 2005). The denser the sludge blanket, the more organic and nutrient removal possible. The anaerobic sludge is made up of granules, which are very dense and have complex methanogenic microbial activity (Wheatley et al., 1998; Emiliano et al., 2006), and have very good settling characteristics (Saila & Hasan, 2017). Biomass concentrations within the sludge bed are usually between 60-70 kg/m^3 (Ibrahim et al., 2012).

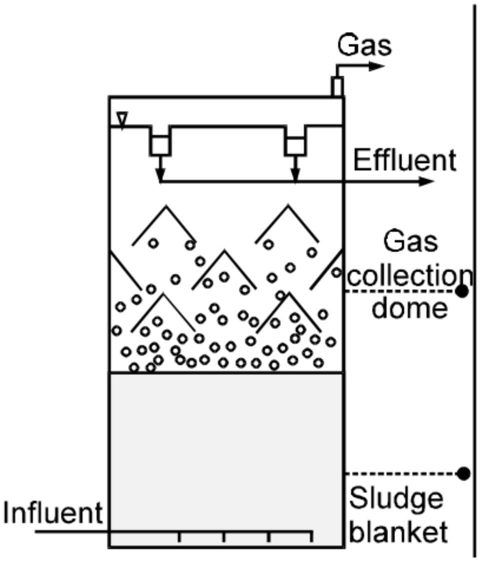

Figure 1.1: Up-Flow Anaerobic Sludge Blanket (UASB) process schematic (Ibrahim et al., 2012).

As can be seen from Figure 1.1, water and gas-containing sludge are drawn upward creating a natural circulation within the reactor. Biogas, made primarily of CH_4 and CO_2, is then produced from the break-down of soluble chemical oxygen demand (sCOD) (Ibrahim et al., 2012; Metcalf & Eddy, 2014). Once the gas has been removed from the sludge, the particles will slowly move back down the reactor, completing the natural circulation process. The continuous movement of sludge out and into the sludge blanket creates convection, which enables effective contact time between the sludge and the wastewater. High loading rates from 10-14 kg-COD/m^3/d and lower HRT (i.e. less than 48 hr) can be seen in UASB systems (Ibrahim et al., 2012). However, a major disadvantage to the UASB system, is that there is a constant build-up of suspended solids from the influent stream. The solids build-up reduces the reactor capacity and thus, its efficiency. A secondary disadvantage is that these reactors can be inhibited by pH values outside of the 6.8 to 7.8 range and usually require a post treatment to meet discharge regulations (Metcalf & Eddy, 2014; Saila & Hasan, 2017).

1.1.3.1c Expanded Granular Sludge Bed (EGSB)

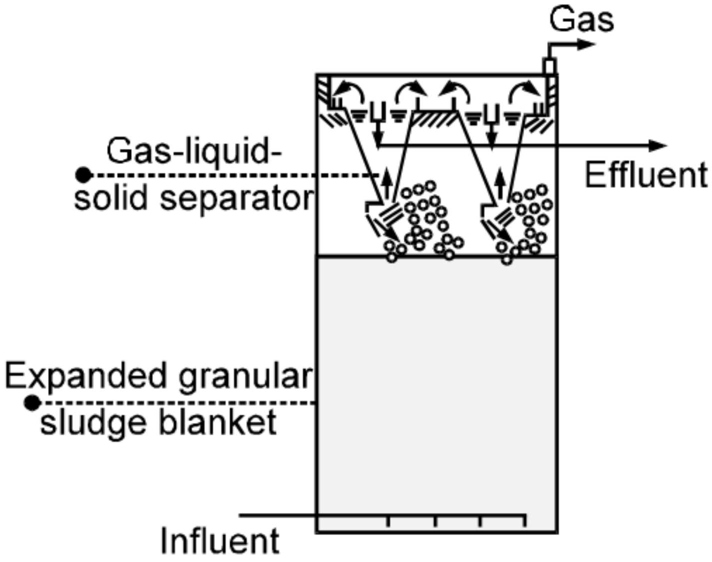

Figure 1.2: Expanded granular sludge blanket (EGSB) process schematic (Ibrahim et al., 2012).

The expanded granular sludge bed (EGSB) (Figure 1.2) works much the same as an UASB reactor, except the up-flow velocities used in an ESGB are much higher due to the large height to diameter ratio and high sludge recirculation rate (Jeison & Chamy, 1999). The higher up-flow velocities cause much more mixing and thus, much more contact between the sludge and the wastewater allowing for higher loading rates. These systems can run at organic loading rates of 20-30 kg-COD/m^3/d, which is substantially larger than many other anaerobic treatment systems (Radu et al., 2014).

1.1.3.2 Aerobic Treatment

Aerobic treatment is typically employed for low strength (<2000 mg/L COD) wastewater due to operational costs associated with aeration; however, it has the capacity to be used for industrial wastewaters with upwards of 6000 mg/L COD (Rusten et al., 1996; Johnson et al., 2000; Metcalf & Eddy, 2014). A major advantage for aerobic treatment is that it allows for 95% COD removal, in comparison to the 80-85% seen for anaerobic operations (Metcalf & Eddy, 2014). Anaerobic treatment, although it is employed by many large-scale breweries, may not be optimal for the increasing number of micro-breweries due to longer HRTs (days to weeks as compared to a couple hours for aerobic) and increased operational complexity when dealing with CH_4. For micro-breweries with medium strength wastewater (2000-8000 mg/L COD) (Aygun et al., 2007; Enitan et al., 2015; Saila & Hasan, 2017), it is possible that aerobic treatment can provide an appropriate solution. The following sub-sections will highlight the operational characteristics as well as the advantages and disadvantages of suspended growth (SG) (i.e. activated sludge) and attached growth) (AG) (i.e. MBBR technology) for treatment of brewery effluent.

1.1.3.2a Conventional Activated Sludge Technology

One of the most widely used SG systems is activated sludge. The most basic activated sludge process contains an aeration tank, a settling tank, an activated sludge recycle line and an activated sludge waste line. The aeration tank aerates (and keeps suspended) free floating microorganisms responsible for wastewater treatment. The settling tank necessitates the liquid-solid separation, where the biomass settles and the treated effluent flows from the top of the tank. The recycle stream is used to pump a portion of the settled biomass from the settling tank back into the aeration tank. The typical MLSS concentrations range between 1000-5000 mg/L in activated sludge processes (EPA, 1997; Metcalf & Eddy, 2014).

The conventional construction of activated sludge systems are to promote plug flow hydraulics, but this became problematic with the introduction of industrial wastewater treatment due to the toxicity of some effluents and was ultimately difficult to obtain in real world applications (Metcalf & Eddy, 2014). Due to more strict treatment objectives, discharge regulations and the need for treating industrial wastewaters, as well as for simplicity, the complete mix activated sludge (CMAS) system was developed (Figure 1.3). CMAS systems utilize rapid mixing in the aeration tank that allows for substrates, microorganisms and dissolved oxygen (DO) to be uniform throughout the tank. These types of activated sludge systems are typically used for industrial wastewater because they are more effective at handling fluctuations, as well as diluting toxicity within the influent; however, they typically require post-treatment to meet water discharge regulations. Drawbacks of SG aerobic treatment include high aeration costs, significant sludge production, and inability to respond to high fluctuations in both hydraulic and organic loading, which is inherent to beer production.

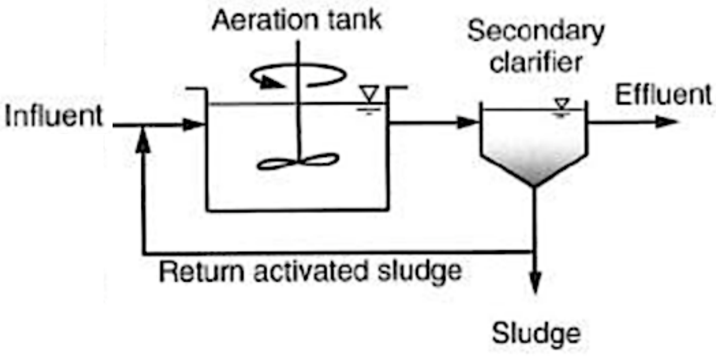

Figure 1.3: Activated sludge process schematic demonstrating complete mix activated sludge (CMAS) design (Metcalf & Eddy, 2014).

1.1.3.2b Moving Bed Biofilm Reactor (MBBR)

Moving bed biofilm reactor (MBBR) technology was invented in Norway in the late 1980s and has since been implemented over 36 times in North America (Qiqi et al., 2012). An MBBR is an aerated, AG biological wastewater treatment system that utilizes free floating media, otherwise known as carriers (Figure 1.4). MBBR systems are also often followed by a settling tank.

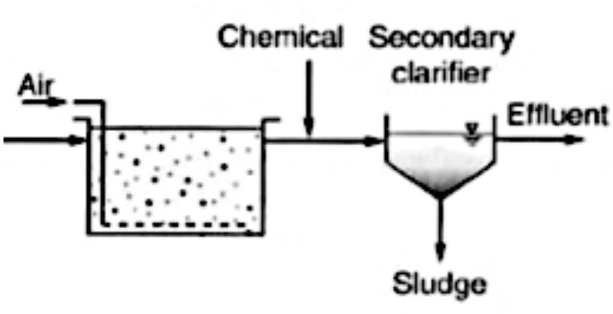

Figure 1.4: Moving bed biofilm reactor (MBBR) process schematic (Metcalf & Eddy, 2014).

The carriers are made of numerous materials including high density polyethylene (HDPE) and are the most fundamental component of a MBBR: they provide the surface area for biofilm attachment (Figure 1.5). Carriers are able to move freely within the bulk liquid even at maximum filling fractions of 70+% (Ødegaard, 1999; Ødegaard et al., 2000). The percent fill, or filling fraction, pertains to the amount of volume occupied by the carriers within the reactor and is usually between 25-70% of the total reactor volume (Ødegaard, 1999; Ødegaard et al., 2000). Carriers allow for more biomass to be maintained in the reactor due to the surface area available for microbial growth and the intrinsic separation of HRT and cell retention time associated with AG systems. One of the main design parameters, apart from the carrier percent fill, is the surface area loading rate (SALR), which is the quantity of organic carbon applied to the surface of the carriers and thus, the biofilm. The available surface area within a reactor can also be manipulated for specific treatment objectives by simply adding or removing carriers or using carriers with different specific surface areas (Ødegaard, 1999; Ødegaard et al., 2000). The biofilm is also retained in the reactor because the carriers do not leave with the treated effluent, which typically results in low MLSS concentrations ranging between 10-800 mg/L (Rusten et al., 1992;

Ødegaard, 1999; Johnson et al., 2000; Ødegaard et al., 2000; Metcalf & Eddy, 2014; Bassin et al., 2016). There are many advantageous properties associated with MBBRs: short HRTs, high organic loading rates, large surface areas for mass transfer, compact reactor size, increased treatment capacity due to biofilm retention, enhanced process stability and reduced sludge production (Ødegaard, 1999; Ødegaard et al., 2000; Aygun et al., 2007; Ibrahim et al. , 2012; Qiqi et al., 2012).

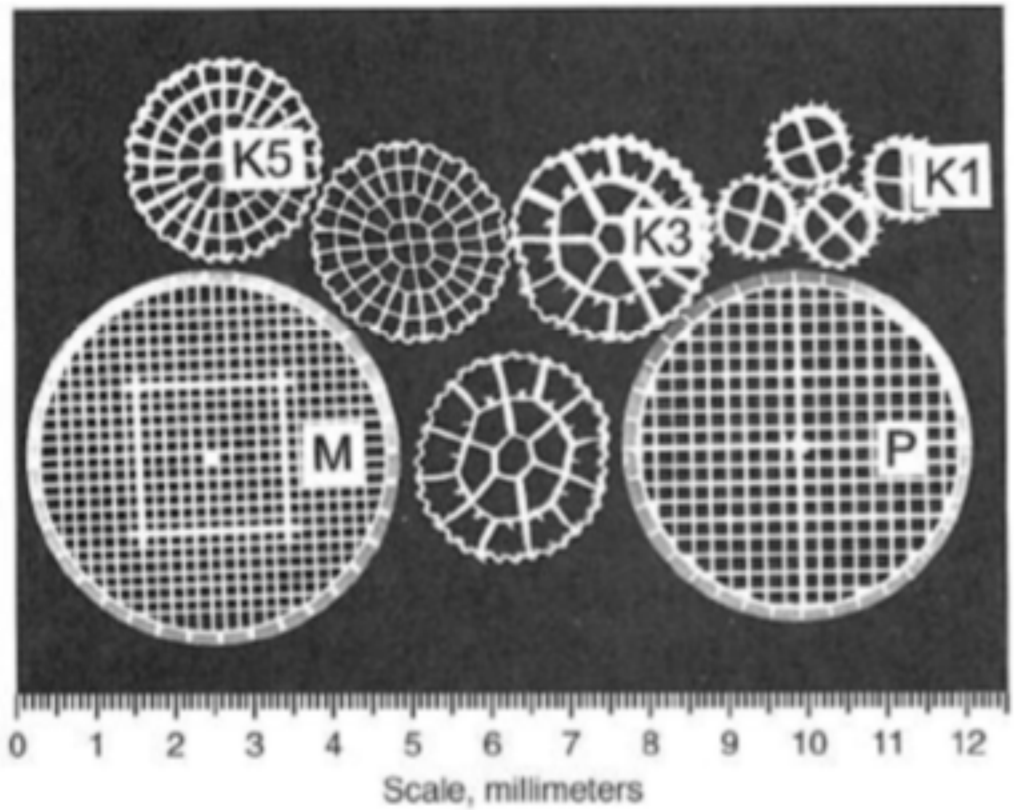

Figure 1.5: Different types of media used for attached growth treatment strategies such as moving bed biofilm reactor (MBBR) systems (Metcalf & Eddy, 2014).

MBBR systems are commonly used for municipal wastewater treatment; however, in recent years they have become more prominent in industrial wastewater treatment (Table 1.2). MBBR technology can be used in a variety of ways: they can be used for pre-treatment, as a standalone treatment, post-treatment or as a retrofit to upgrade current systems for further constituent removal. MBBR technologies have been used in some capacity for the treatment of brewery wastewater (Manyuchi & Chikwama, 2016); dairy wastewater (Rusten et al., 1992; Andreottola et al., 2003); cheese wastewater (Rusten et al., 1996); municipal wastewater (Ødegaard, 1999; Ødegaard et al., 2000; Canziani et al., 2006; Young et al., 2017); coal gasification wastewater (Li et al., 2011); oilfield wastewater (Dong et al., 2011); pesticide wastewater (Chen et al., 2007); hospital wastewater (Shokoohi et al., 2017); petrochemical/chemical wastewater (Cao & Zhao, 2012; Hassani et al., 2014; Cao et al., 2016); and landfill leachate (Canziani et al., 2006; Chen et al., 2008).

Table 1.2: Industrial wastewater treatment studies that have utilized moving bed biofilm reactor (MBBR) technology.

Wastewater Type	**Inf COD (mg/L)**	**COD Rem (%)**	**MLSS (mg/L)**	**AG (mg/L)**	**HRT (hr)**	**SALR (g-COD/m^2/d)**	**Media SA [%fill] (m^2/m^3)**	**Reference**
Brewery*	673	58 93 94	2156 1887 1293	0.2 5.8 6.9	6 12 24	n.a.	3000 [n.g.]	(Manyuchi & Chikwama, 2016)
Coal Gasification	1000-2000	71 76 73-79	700 1000 1000-1900	900 1100 700-1400	32 40 48	n.a.	n.g. [50]	(Li et al., 2011)
Dairy (2 MBBRs in series)	3310	60-85	MBBR1: 620-1790 MBBR2: 650-880	(1) 2210-3100 (2) 3340-2230	3.5-11.2	34.8-43.2	276 [n.g.]	(Rusten et al., 1992)
Cheese (2 MBBRs in series)	3070-4420	80-95	n.g.	n.g.	n.g.	n.a.	335 [n.g.]	(Rusten et al., 1996)
Hospital	750-850	88	n.g.	n.g.	84-253	2.4-8.1	500 [60]	(Shokoohi et al., 2017)
Oilfield (2 MBBRs in series)	343-365	63 77 77	n.g.	n.g.	10 18 36	n.a.	n.g.	(Dong et al., 2011)
Pesticide	3000-3500	86 85 85 84 82 72	n.g.	6900 7200 6900 7000 6200 4800	24	7.5 9.38 12.5 18.75 25 37.5	800 [50] 800 [40] 800 [30] 800 [20] 800 [15] 800 [10]	(Chen et al., 2007)
Petro-chemical	280-315	88	n.g.	n.g.	8	6.3	300 [50]	(C. Y. Cao & Zhao, 2012)
Petroleum Refinery	283-540	62	272-380	n.g.	2.4	10-51	150 [30]	(Johnson et al., 2000)
Slaughter-house (2 MBBRs in series)	5141	86	MBBR1: 1797 MBBR2: 1867	n.g.	n.g.	20-45	250 [50]	(Johnson et al., 2000)

*Batch process
n.g. = not given, n.a. = not applicable, SG = suspended growth, AG = attached growth

Previous work has demonstrated that HRT can be decreased and SALR increased in MBBR systems to enhance the removal efficiencies. The food and beverage wastewaters, such as dairy, cheese and slaughterhouse, would be the most comparable to brewery wastewater and these sources utilized SALRs between 20-45 g-COD/m^2/d. Although the HRT was not given for the cheese and slaughterhouse wastewater studies, the dairy wastewater study varied the HRT between 3.5-11.2 hr. It suggests that higher SALRs and lower HRTs may be the optimal conditions for food and beverage wastewater treatment. Interestingly, the dairy and slaughterhouse studies also demonstrated high MLSS concentrations within the reactors under these conditions, suggesting that at high SALR and low HRT more suspended growth may be present than is typically seen for MBBR technology.

1.1.4 Brief Overview of Suspended and Attached Growth Kinetics

Biological growth is described by Monod kinetics, where growth is limited by substrate availability and thus, the rate of biodegradation within a biological system is directly related to the substrate concentration (Monod, 1949; Metcalf & Eddy, 2014). This is inherently true for suspended growth systems; however, attached growth kinetics are more complex due to the fact that they are not free-floating bacteria, but a biofilm. All biofilms are mass transfer rate limited through the stagnant liquid boundary layer or through the biofilm itself.

The biofilm is protected from the bulk liquid by a stagnant liquid boundary layer (Figure 1.6). Substrates within the bulk layer must diffuse first across the stagnant liquid layer and then through the biofilm to be made available to the bacteria within the biofilm (Ødegaard, 1999; Ibrahim et al., 2012; Qiqi et al., 2012; Metcalf & Eddy, 2014). This diffusion of particles through the biofilm may result in concentration gradients within the biofilm. The substrate concentrations within the biofilm will decrease with depth as they diffuse through and are consumed, thus resulting in a diffusion limitation. There are many factors that will dictate the rate of diffusion across the biofilm: biofilm porosity, concentration of substrate in the bulk liquid, mass transfer limitations through the stagnant liquid layer and through the biofilm and the intrinsic cell reaction rates of the embedded biomass within the biofilm (Ibrahim et al., 2012; Qiqi et al., 2012).

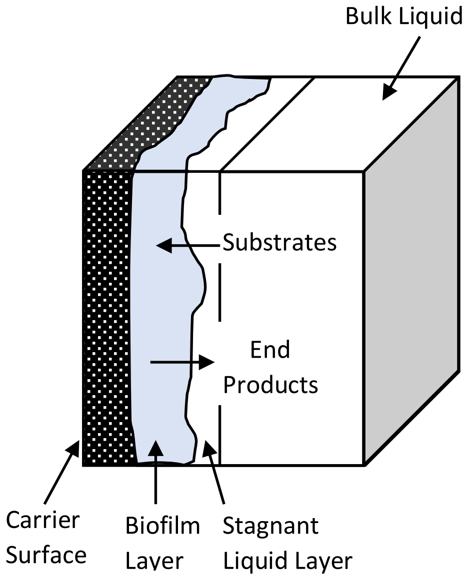

Figure 1.6: Biofilm cross section schematic showing the stagnant liquid film (i.e. the diffusion layer).

1.1.5 Biofilm Growth

Biofilm can be described as many cells adhering to one another on a surface with the help of extracellular polymeric substances (EPS). In other terms, biofilm is an intricate assortment of organism's co-habitating together in a protected community that are dictated by substrate diffusion gradients; these gradients cause stratification and clustering of different bacterial species (Ibrahim et al., 2012; Qiqi et al., 2012). Planktonic cells will form a biofilm under a variety of environmental conditions: lack of nutrients, antibiotic presence or simply due to attachment site recognition on a surface. Planktonic cells of the same microorganism, which by contrast will be free-floating in the bulk liquid, are physiologically different than the same species in a biofilm (Ibrahim et al., 2012; Qiqi et al., 2012).

There are three main stages of biofilm growth (Figure 1.7): attachment (reversible and irreversible), growth and dispersion (Cunningham et al., 2008; Ibrahim et al., 2012; Qiqi et al., 2012). Initial attachment is when planktonic or free-floating bacteria encounter a surface and begin to colonize by releasing EPS. There are two types of attachment: reversible and irreversible. Reversible attachment is the very initial stages when the free-floating cells have not yet fully formed a biofilm complex or polymeric matrix, but have started releasing EPS. Irreversible attachment occurs once the polymeric matrix is complete, thus resulting in the cells becoming embedded in the matrix. The second step is the growth and maturation of the biofilm, where the community of cells form a complex and dynamic structure allowing for optimal substrate diffusion. The third and final step is the dispersal and detachment of the biofilm in order to propagate on other available surfaces. Sections of the biofilm or individual cells will be released during this stage.

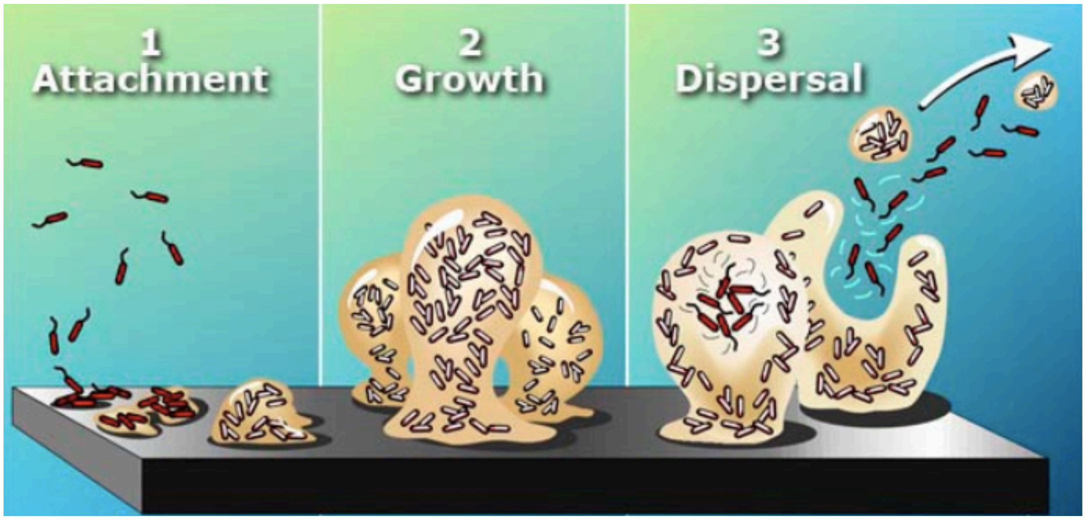

Figure 1.7: The three main stages of biofilm growth: attachment, growth and dispersal (Cunningham et al., 2008).

The growth, stability and detachment rate of biofilms will differ depending on the different types of microorganisms present in the biofilm, as well as the environmental conditions (Mahendran et al, 2012). Typically, in MBBR operations where carriers are used as the attached growth surface, biofilms will grow predominantly on the internal surfaces, rather than the external surfaces of the carriers. This is due to the fact that the external surfaces are exposed to liquid shearing and abrasions caused by carrier-carrier contact (Ødegaard et al., 2000; Mahendran et al., 2012).

Aerobic biofilms, or heterotrophic biofilms, have been documented as being very heterogeneous, meaning there are many different types of cell clusters and micro-colonies, as well as substantial void spaces present within the biofilm (i.e. porosity) (De Beer et al., 1994). Heterotrophic biofilms, which are what normally grow in carbon-based, aerobic, attached growth treatment operations, have been documented with having vast ranges of thicknesses and densities. Mahendran et al. (2012) found thicknesses ranging from 139 to 253 μm with densities ranging from 77 to 559 kg/m^3 and Liang (2005) found thicknesses ranging from 186 to 300 μm. However, other studies, have found much smaller thicknesses in the range of 72 to 90 μm (Huang et al., 2017).

According to literature, ideal densities are within 34 to 76 kg/m^3 for heterotrophic biofilm (Tanyolaç & Beyenal, 1997). However, biofilm in different stages of development will have variable density because immature and developing biofilms are much more susceptible to shear forces prompting the organisms to cluster more closely together (Chang & Rittrnann, 1991). Hoehn and Ray (1973) showed densities of 105 kg/m^3 during the initial stages of biofilm development; however, they eventually decreased with biofilm maturation to 20-40 kg/m^3. Also, it has been found that the closer the biofilm is to the surface of the carrier the more dense it becomes; studies have observed the inner layers to be 5-10 times more dense than the outer biofilm layers (Zhang & Bishop, 1994).

1.2 RESEARCH AREA

The growing number of micro-breweries in North America, and subsequent need for small scale brewery wastewater treatment and disposal, has created a research opening in the field of environmental engineering. MBBR technologies are starting to be implemented at small-scale breweries without clear design criteria. MBBR systems have proven to be a practical and cost-effective technology to treat municipal and industrial wastewaters; however, there has not been sufficient research and testing for brewery wastewater applications. This juxtaposition in implementation with lack of understanding has created a fundamental need for research in the area of MBBR technology for the treatment of brewery wastewater.

1.3 STUDY OBJECTIVES

This research will provide a better understanding of chemical oxygen demand (COD) treatment of brewery wastewater using MBBR technology. However, beyond the study of carbon removal rates, the production of mixed liquor suspended solids (MLSS) and biofilm response to high strength brewery effluent will also be investigated. The aim of the study is to provide new and critical knowledge on the performance of MBBRs in an otherwise untested area of industrial wastewater treatment. The specific research objectives are as follows:

- Study the impact of SALR (10 - 55 g-sCOD/m^2/d) on MBBR carbon oxidation kinetics using authentic brewery effluent for two types of MBBR carriers compared to a suspended growth control reactor;
- Study the impact of HRT (3-12 hr) on MBBR carbon oxidation kinetics using authentic brewery effluent for two types of MBBR carriers compared to a suspended growth control reactor;
- Characterize the effects on the MLSS as a function of SALR and HRT for two types of MBBR carriers compared to a suspended growth control reactor;

- Characterize and compare the morphology and viability of MBBR carbon oxidizing biofilms, in terms of thickness, mass, density and percentage of live embedded cells at each condition for two types of MBBR carriers;
- Investigate and identify the threshold for effective treatment and the limitations of MBBR technology for brewery wastewater treatment.

1.4 THESIS ORGANIZATION

Chapter 1 outlines the objectives of the study while also giving a brief introduction to the problems faced by small scale breweries. Chapter 2 presents the first article to be published from this research called, *Influence of SALR and HRT on carrier types and attached and suspended growth kinetics using MBBR technology for the treatment of brewery wastewater*. Chapter 3 presents the second article to be published from this research called, *Response of carbon removal MBBR biofilm across carrier type, SALR and HRT*.

The final chapters, Chapter 4 and 5, present a summation and synthesis of the research and the significant conclusions made with regards to MBBR carbon oxidation of high strength brewery effluent under varying conditions, as well as the biofilm morphology and viability of different MBBR carriers at those same conditions. It also gives recommendations for future research in the area as well as for the full-scale operation.

1.5 CONTRIBUTION OF AUTHORS

The work gleaned from this research has resulted in two scientific manuscripts prepared for publication. Listed below are the titles of the articles and an overview of the authors' contributions.

Article 1:

Boyle, K.; Kinsley, C.; Delatolla, R.; Abbassi, B. *Influence of SALR and HRT on carrier types and attached and suspended growth kinetics using MBBR technology for the treatment of brewery wastewater.* In preparation for journal submission.

K. Boyle: Conducted literature review, developed and conducted experimental procedure, analyzed results and wrote manuscript.

C. Kinsley: Provided supervision in the development of experimental procedure, analysis of results and reviewed manuscript.

R. Delatolla: Provided supervision in the development of experimental procedure, analysis of results and reviewed manuscript.

B. Abbassi: Provided supervision and project funding.

Article 2:

Boyle, K.; Kinsley, C.; Delatolla, R; Abbassi, B. *Response of carbon removal MBBR biofilm across carrier type, SALR and HRT*. In preparation for journal submission.

K. Boyle: Conducted literature review, developed and conducted experimental procedure, analyzed results and wrote manuscript.

C. Kinsley: Provided supervision in the development of experimental procedure, analysis of results and reviewed manuscript.

R. Delatolla: Provided supervision in the development of experimental procedure, analysis of results and reviewed manuscript.

B. Abbassi: Provided supervision and project funding.

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CHAPTER 2: INFLUENCE OF SALR AND HRT ON CARRIER TYPES AND ATTACHED AND SUSPENDED GROWTH KINETICS USING MBBR TECHNOLOGY FOR THE TREATMENT OF BREWERY WASTEWATER

2.1 ABSTRACT

Two moving bed biofilm reactor (MBBR) systems utilizing two different carrier types (Kaldnes K5 and Kontakt), and a suspended growth (SG) control reactor, were used in this study to investigate the impacts of surface area loading rate (SALR) and hydraulic retention time (HRT) on attached growth (AG) and SG kinetics and carrier type for brewery wastewater at 2000 mg-sCOD/L. An increase in SALR while at an HRT of 12 hr resulted in no significant impact in total volumetric removal rates between the MBBR systems and the SG control reactor; however, mixed liquor suspended solids (MLSS) concentrations were lower for the MBBR systems at low SALR, which indicated AG contribution. Over 92% soluble chemical oxygen demand (sCOD) removal was achieved at each SALR in the MBBR and SG systems. These results indicated that the reactors were substrate limited and SG controlled. Due to the SG dependency, the difference between the two types of carriers was indeterminate. A decrease in HRT while maintaining an SALR of 40 g-sCOD/m^2/d resulted in a shift from SG to AG dependency in the MBBR systems. At an HRT of 12 hr all three reactors maintained over 92% sCOD removal; however at an HRT of 4 hr the SG control reactor dropped to 88% and at 3 hr to 61%, whereas the MBBR systems maintained 95% removal at an HRT of 4 hr and decreased to 73% at 3 hr. These results indicated that the MBBR systems were more effective at lower HRT than the SG control reactor, with no significant difference observed between the two carrier types tested.

2.2 SETTING THE CONTEXT

The article presented in Chapter 2 is entitled *Influence of SALR and HRT on carrier types and attached and suspended growth kinetics using MBBR technology for the treatment of brewery wastewater* by K. Boyle, C. Kinsley, R. Delatolla and B. Abbassi. This article quantifies the AG and SG kinetics for two laboratory scale MBBR systems, utilizing two different carrier types, and compares the kinetics to a SG control reactor. It addresses the impacts of SALR and HRT on AG and SG kinetics, in conjunction with sCOD removal and MLSS concentrations, as well as carrier type for brewery wastewater at 2000 mg-sCOD/L. Full-scale scale-up validation was also demonstrated with practical brewery wastewater treatment suggestions provided.

2.3 INTRODUCTION

The North American brewery structure has seen many changes over the past decade. As of 2017, there were 817 craft breweries in Canada, which was an increase of 215% from 2013 (Beer Canada, 2018). The United States has also seen a dramatic increase in craft breweries, going from 2000 to 6300 craft breweries between 2010 and 2017, with 99% being microbreweries (Brewers Association, 2017; Dowling, 2017). These changes have resulted in municipalities focusing on the environmental impacts associated with beer production, where a major point of concern is the treatment and discharge of brewery wastewater.

Brewery wastewater is considered to be a high strength food industry wastewater with high variability in terms of both organic and hydraulic loading (Table 2.1). The issue with beer production is that it requires a large quantity of water and thus, produces substantial amounts of wastewater. A brewery's water and wastewater usage can be described by the ratio of water:beer:wastewater, where the respective quantities are approximately 9:1:8 m^3 (Connaughton, et al., 2006; Chen, et al., 2016). Many small breweries are located towns where wastewater management options typically include pre-treatment and discharge to a municipal sewer (when available) or on-site wastewater treatment and discharge to a soil infiltration system. If not managed properly, beer production effluent can be extremely detrimental to the natural environment or to the operation of municipal wastewater systems, often lagoons, which are not designed to handle such high strength waste, nor the volumes with which it is discharged

(Simate et al., 2011; Olajire, 2012). Municipalities have started to charge small breweries with Over-Strength Discharge Fees (ODFs), which can result in fines upwards of hundreds of thousands of dollars per year on top of standard sewer use fees.

Table 2.1: Typical brewery effluent constituents and concentrations.

Parameter	**Unit**	**Brewery Effluent Composition**	**This Study:** **Beau's Brewery** *Range (Average)*	**References**
COD	mg/L	1000-125,000	5000-40,000 (8000)	Connaughton et al., 2006; Fillaudeau, et al., 2006; WEF, 2010; Simate et al., 2011; Radu et al., 2014; Choi, 2015; Metcalf & Eddy 2014; Chen et al., 2016; Manyuchi & Chikwama, 2016; Saila & Hasan, 2017
BOD	mg/L	600-110,000	n/a	
TSS	mg/L	200-3000	300-15,000 (4000)	
Tot. Nitrogen	mg/L	10-80	10-100 (25)	
Tot. Phosphorus	mg/L	10-100	50-1330 (70)	
Temperature	°C	15-80	20-50	
pH	-	2-14	3-10	

Anaerobic digestion (AD) is typically used for the treatment of brewery wastewater (Simate et al., 2011; Olajire, 2012). However, for the increasing number of micro-breweries AD may not be the optimum solution due to long HRTs and increased operational complexity dealing with methane gas, as compared to aerobic treatment. Small breweries require cost-effective, reliable, and simple to operate treatment technologies to properly manage their brewery wastewaters. MBBR technology can potentially provide an effective solution for small flow applications either as a stand-alone technology for medium strength effluent streams, or as a post-treatment technology for higher strength applications following an AD system. Advantages of the technology include: compact reactor size resulting in smaller land footprints, short HRTs and high organic loading rates, increased treatment capacity and stability due to biofilm retention, and reduced sludge production (Ødegaard, 1999; Ødegaard et al., 2000; Ibrahim et al., 2012; Qiqi et al., 2012). These benefits can potentially make MBBR technology a viable option for decentralized brewery wastewater treatment.

Although there is very limited information on MBBR technology for treatment of brewery wastewater, other food and beverage wastewater studies have demonstrated the advantages of MBBR technology. Dairy, cheese and slaughterhouse wastewater treatment studies showed that high SALRs and low HRTs may be optimal treatment conditions for MBBR systems treating food and beverage wastewaters (Rusten et al., 1992; Rusten et al., 1996; Johnson et al., 2000). SALRs between 20-45 g-COD/m^2/d and HRTs between 3.5-11.2 hr resulted in favourable removal efficiencies (i.e. upwards of 90%). Interestingly, the dairy and slaughterhouse studies also demonstrated high MLSS concentrations within the reactors under elevated SALR conditions, suggesting that at high SALR more suspended growth may be present than is typically seen for MBBR technology treating low carbon wastewater. These findings are supported by fundamental studies of the MBBR technology that have shown that carrier type, SALR and HRT effect the kinetics, the production of solids and hence the MLSS of the system (Karizmeh et al., 2014; Forrest et al., 2016). Hence, in order to develop an understanding of the potential of the MBBR technology for brewery wastewaters, further information on the effect of carrier type, SALR and HRT on the performance of the system is necessary.

The objective of this research is to evaluate the effectiveness of the MBBR technology for the treatment of medium strength (2000 mg-sCOD/L) brewery effluent common to small-scale breweries (Aygun et al., 2007; Enitan et al., 2015; Saila & Hasan, 2017) and enhance the understanding of the effects of different carrier types, SALR and HRT on brewery wastewater treatment. The treatment of brewery wastewater using MBBR technology was investigated at the lab-scale as well as at a full-scale industrial application.

2.4 MATERIALS AND METHODS

2.4.1 MBBR Laboratory Reactors

Three lab-scale reactors were operated in parallel to evaluate the treatment for brewery wastewater using two MBBR systems with different commercial carriers and to compare with a SG control reactor (Figure 2.1). Each reactor had a working volume of 2 L and was maintained at a neutral pH, room temperature and aeration rate of 5 Lpm. Reactor 1 (R1) contained K5 (AnoxKaldnes, Lund, Sweden) (protected surface area 800m^2/m^3) biofilm carriers and Reactor 2 (R2) contained Kontakt (Jaeger Environmental, Houston, USA) (protected surface area 500 m^2/m^3) biofilm carriers (Figure S2.1). Reactor 3 (R3) was maintained as a control SG system. The SG control reactor did not operate with a return activated sludge (RAS) line to simulate the full-scale facility, where HRT is equal to SRT.

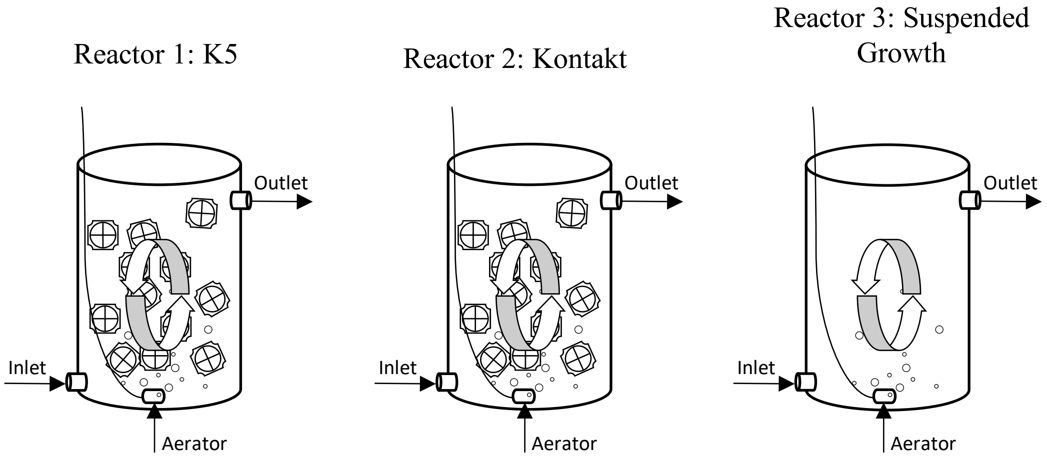


Figure 2.1: Laboratory schematic of MBBR and suspended growth reactors.

A sample of high strength wastewater was collected from the Beau’s All Natural Brewing Company in Vankleek Hill, Ontario, stored at 4°C, and used as the wastewater source for the lab experiments (Table 2.2).

Table 2.2: Beau's All Natural Brewing Company wastewater sample taken to conduct the laboratory analyses.

Constituent	Before Dilution	After Dilution
Soluble COD (mg-sCOD/L)	37500±1020	2034±63
Total Nitrogen (mgN/L)	117±6	6.3±2.2
Ammonia (mgNH$_3$-N/L)	105±4	6.1±2.7
Nitrate (mgNO$_3$-N/L)	n.d.	n.d.
Nitrite (mgNO$_2$-N/L)	n.d.	n.d.
Total Phosphorus (mgP/L)	1328±28	69.9±1.2
Orthophosphate (mgPO$_3^4$-P/L)	951±15	50.7±4.1
TSS (mgTSS/L)	1620±102	88±49
VSS (mgVSS/L)	1459±89	76±16

n.d. = non detect (Nitrate 0.01mg/L, Nitrite 0.01mg/L)

The objective of the laboratory experiment was to compare the efficiencies of three separate systems for the removal of organic matter from raw brewery wastewater. Two distinct trials were conducted wherein four SALRs and three HRTs were tested on each of the three reactors (Table 2.3). The SALRs were chosen based on the information obtained from food and beverage MBBR studies (SALRs 20-45 g-sCOD/m^2/d) (Rusten et al., 1992; Rusten et al., 1996; Johnson et al., 2000), as well as what is typically seen in carbon-based MBBRs. Typical SALR values found for carbon-based MBBRs range between 1-45 g-sCOD/m^2/d (Rusten et al., 1992; Ødegaard, 1999; Ødegaard et al., 2000; Cao et al., 2016). Above 20 g-sCOD/m^2/d is considered to be a high loading rate, 5-15 g-sCOD/m^2/d are moderate loading rates and anything less than 5 g-sCOD/m^2/d are low loading rates (Ødegaard, 1999; Ødegaard et al., 2000).

During each of the four SALRs an HRT of 12 hr was maintained and during the three HRTs an SALR of 40 g-sCOD/m^2/d was maintained. The corresponding carrier percent fill for

K5 and Kontakt carriers is demonstrated for each loading rate in Table 2.3. The first experiment was operated at 12 hr HRT to replicate the full-scale system at the participating brewery.

Table 2.3: Operational conditions applied to each reactor during two separate trials.

Trial 1 – SALR Effects				**Trial 2 – HRT Effects**			
SALR (g-sCOD/m^2/d)	HRT (hr)	K5 %fill	Kontakt %fill	SALR (g-sCOD/m^2/d)	HRT (hr)	K5 %fill	Kontakt %fill
10	12	48	76	40	3	50	80
25	12	20	32	40	4	38	60
40	12	13	20	40	12	13	20
55	12	9	15				

The reactors were fed with the same effluent for the duration of the testing. 400 L of highly concentrated wastewater (approximately 38,000 mg-sCOD/L) was retrieved from Beau's All Natural Brewing Company and diluted to produce the feed (2000 mg-sCOD/L) for the lab-scale reactors to simulate MBBR 2 at the full-scale. The reactors were also dosed with concentrated ammonium sulfate to achieve a 10:1 ratio of carbon to nitrogen (with phosphorus already in excess). The feed tank consisted of a 100 L container with an attached mixer. The reactors were run for two months during the initial start-up phase and then run for three weeks between each condition to allow for acclimatization. The SALR trial was run starting with SALRs of 25, 40 and 55 g-sCOD/m^2/d and then 10 g-sCOD/m^2/d at the end. The change in SALR from 55 to 10 g-sCOD/m^2/d required an acclimatization period of five weeks. The HRT effects were run in decreasing order from 12 hr to 3 hr. Once steady state was reached for each condition, the reactors were sampled daily to obtain ten consecutive data points. Steady state was defined as the period during which the carbon removal rate and effluent concentration varied less than 10% in terms of sCOD. Reactor operation was maintained for a total of 302 days.

2.4.2 Biodegradability Study

The biodegradability study of Beau's All Natural Brewing Company's effluent was run using two 2 L unseeded, aerated (5 Lpm) batch reactors fed with 2000 mg-sCOD/L. One reactor was dosed with a 10:1 ratio of C:N and the other was maintained with no nitrogen addition. The sCOD was tested twice a day in both reactors and the reactors ran for a total of 3.5 days (85 hrs).

2.4.3 Full-Scale Treatment Facility

The full-scale wastewater treatment system located at Beau's All Natural Brewing Company in Vankleek Hill, Ontario, Canada was constructed in October 2016. It consists of a volume equalization tank, a pH dosing tank and two MBBRs in series each followed by a settling tank (Figure 2.2). Solids (yeast and spent grains) are diverted from the process, so only the liquid waste streams go through the treatment train. The average flow rate for the wastewater system is 40 m^3/d Monday through Saturday. For the purposes of this study only MBBR 2 was modelled in the lab-scale experiments.

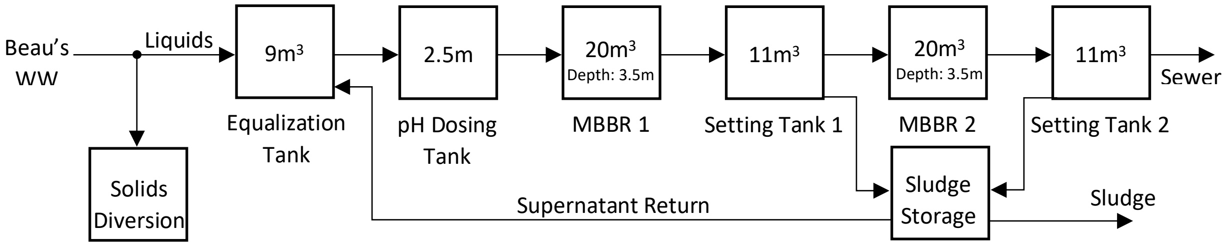


Figure 2.2: Beau's All Natural Brewing Company wastewater treatment train schematic showing volumes and depth.

The full-scale system was monitored and evaluated for the duration of this project; however, there were many issues that needed to be rectified before a comparison could be made to the lab-scale reactors. Due to strict regulations imposed on the food and beverage industry the brewery effluent entering the treatment system has temperatures ranging upwards of 50°C, extremely variable pH of 3-10 (due to the caustic and acidic solutions used for sterilization), as well as hydraulic and organic load variability due to the batch nature of brewery production. Additionally, it was observed that there was insufficient dissolved oxygen (DO) in the two MBBRs due to a combination of a lack of blower/diffuser capacity and high wastewater temperature. These issues were addressed by the brewery by installing a heat exchanger, improving the pH balancing system, replacing the diffusers and upgrading the aeration units.

Once the full-scale system had reached stable operating conditions, two sampling campaigns were completed: composite samples were collected every three hours for three days using ISCO-6712 auto-samplers (April 2018) and grab samples collected daily for a month (July 2018).

2.4.4 Analytical Methods

Samples were filtered through a 0.45μm filter before testing for soluble constituents to limit the effects of suspended solids on the measured values. Influent and effluent concentrations of total nitrogen, ammonia, nitrate, nitrite, total phosphorus and orthophosphate were measured according to HACH “Test N Tube™ Vials” standard methods (HACH, 2013): 10072, 10031, 10020, 10019, 10127, 8114, respectively. Total suspended solids (TSS) and volatile suspended solids (VSS) were measured according to Standard Methods (APHA; AWWA; WEF, 2012); 2540 D and 2540 E, respectively. Soluble chemical oxygen demand (sCOD) was measured according to HACH “Test N Tube™ Vials” standard methods 8000 (HACH, 2013), which is equivalent to Standard Methods 5220 D (APHA; AWWA; WEF, 2012).

The DO, temperature and pH were measured in all three reactors twice a day (morning and night) during both trials using a symphony Multi Parameter Meter with attached DO and pH probes (VWR, Ontario, Canada).

Suspended growth (SG) volumetric removal rate for R1 (K5) and R2 (Kontakt) were calculated based on Equations 1a-b:

$$\text{R1 SG Vol. Rem. Rate (g-sCOD/m}^3\text{/d)} = \text{R3 (g-sCOD Consumed/g-MLSS Produced/d)*R1 MLSS Conc. (g-MLSS/m}^3\text{)} \quad (1a)$$

$$\text{R2 SG Vol. Rem. Rate (g-sCOD/m}^3\text{/d)} = \text{R3 (g-sCOD Consumed/g-MLSS Produced/d)*R2 MLSS Conc. (g-MLSS/m}^3\text{)} \quad (1b)$$

Attached growth (AG) volumetric removal rates for R1 (K5) and R2 (Kontakt) were calculated based on Equation 2a-b:

$$\text{R1 AG Vol. Rem. Rate (g-sCOD/m}^3\text{/d)} = \text{R1 (Total Vol. Rem. Rate – SG Vol. Rem. Rate) (g-sCOD/m}^3\text{/d)} \quad (2a)$$

$$\text{R2 AG Vol. Rem. Rate (g-sCOD/m}^3\text{/d)} = \text{R2 (Total Vol. Rem. Rate – SG Vol. Rem. Rate) (g-sCOD/m}^3\text{/d)} \quad (2b)$$

2.4.5 Statistical Methods

Statistical significance for constituent data was tested using single-factor ANOVA with p values less than 0.05 considered significant.

2.5 RESULTS AND DISCUSSION

2.5.1 Biodegradability Study

A biodegradability study of Beau's All Natural Brewing Company's effluent was conducted to determine if the wastewater was appropriate for biological treatment (Figure 2.3).

The study was first conducted using a 2 L unseeded, aerated reactor with no nitrogen addition. The reactor was tested twice a day and ran for a total of 3.5 days (84 hrs). The initial C:N ratio was found to be 1000:1. The test was then re-run under the same conditions with nitrogen addition, resulting in an initial 10:1 ratio of C:N.

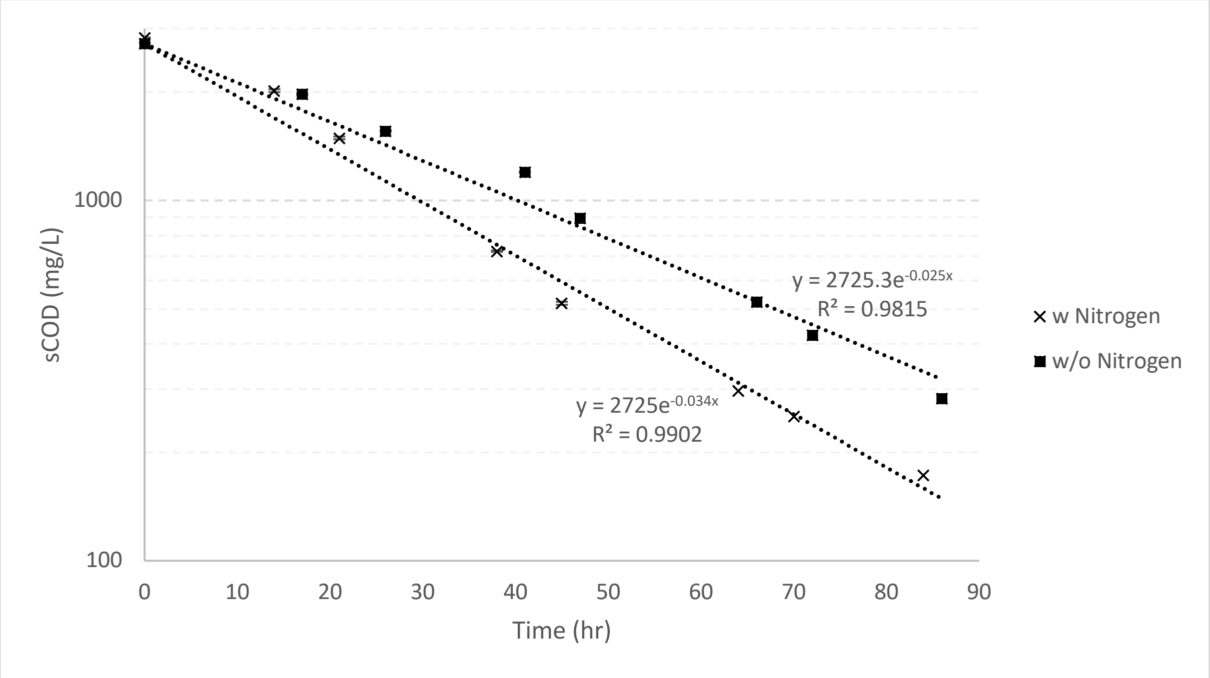


Figure 2.3: Biodegradability study on Beau's All Natural Brewing Company's effluent using a 2L unseeded, aerated reactor with and without nitrogen addition.

The batch tests both showed first order reaction kinetics, which follow Equation 1, where C is the final concentration (mg/L), C_o is the original concentration (mg/L), k is the rate constant (hr^{-1}) and t is time (hr).

$$C = C_o e^{-kt} \tag{1}$$

The rate constant with no nitrogen addition was 0.025 hr^{-1}, whereas the rate constant was 0.034 hr^{-1} with nitrogen addition. Typical k values for multi-component industrial wastewater biodegradability tests in lab-scale aerated batch reactors range from 0.029 to 0.147 hr^{-1} over a 30 hr period (Argaman et al., 2010) and specifically, for pulp and paper wastewater, the range is from 0.006 h^{-1} to 0.019 h^{-1} over a 40 hr period (Wang et al., 1997). However, in both of these cases acclimatized biomass was added to seed the reactors, whereas in this study no biomass was added. Without biomass addition and taking into account the longer biodegradation period of 4d, the k values seem to be within the expected literature values for industrial wastewater.

In other carbon dominated wastewater streams, such as pulp and paper, a lack of nitrogen is a common occurrence and is similar to what was seen with Beau's effluent. In the pulp and paper industry, nitrogen addition or selection of nitrogen fixing bacteria is often imperative for effective biological treatment (Grapes et al., 1999). However, with Beau's effluent there was still effective treatment without nitrogen addition, which suggests that there may be nitrogen fixing bacteria present in the wastewater or that the nitrogen present was being recycled.

Since the k values were similar to literature and approximately 90% sCOD was removed with no inoculum this indicates that the brewery wastewater has a high level of treatability. However, the biodegradability was increased when nitrogen was added. Due to this test the 10:1 ratio of carbon and nitrogen was used throughout the trials to allow for optimal removal.

2.5.2 SALR Effects on Brewery MBBR Performance

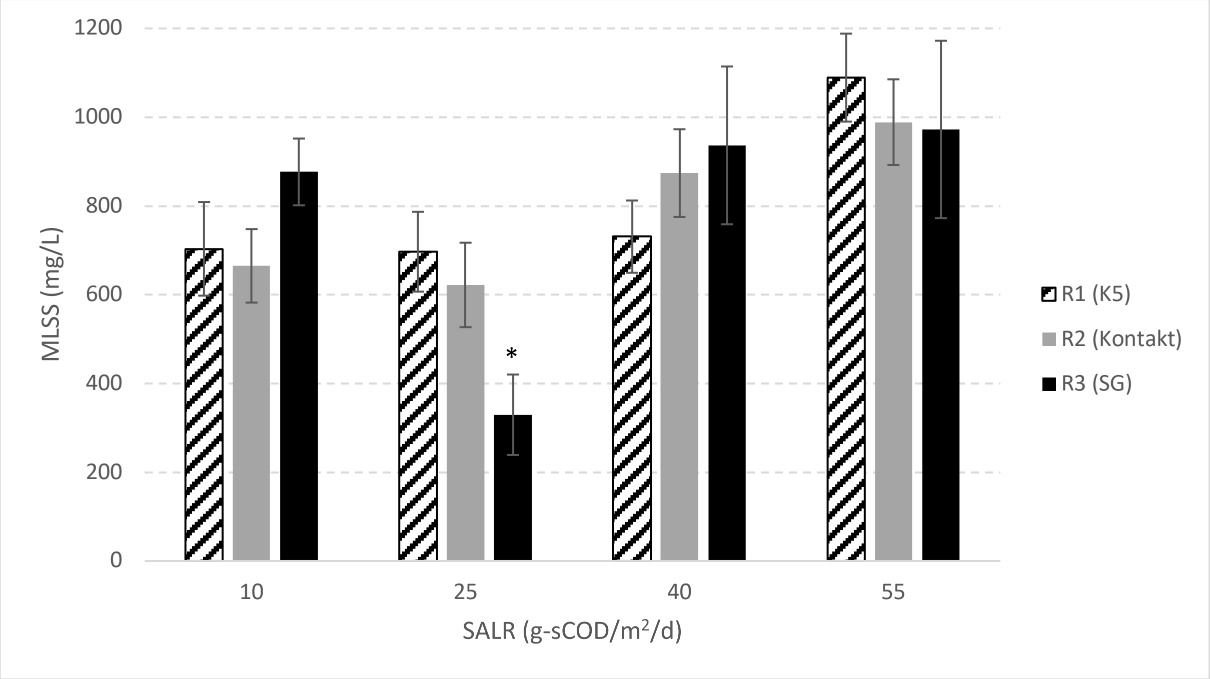
During the trial, DO was in excess and ranged from 5.2 to 7.2 mg/L, temperature ranged from 20 to 24°C and pH ranged from 6.9-7.7. These operating parameters did not vary significantly across the experimental conditions and were maintained at all points in the trial.

2.5.2.1 SALR Effects on MLSS

The MLSS concentrations for all three reactors at four different SALRs are presented in Figure 2.4. The mixed liquor volatile suspended solids (MLVSS) were originally measured; however, a ratio of 0.95 MLVSS to MLSS was found, therefore MLSS was chosen to be the evaluated constituent.

The MLSS concentrations varied with changing loading rate. MLSS in R1 remained consistent for SALRs of 10-40 g-sCOD/m^2/d with 697-731 mg/L, while a significant increase to 1089 mg/L was observed at the highest SALR of 55 g-sCOD/m^2/d ($p<0.05$). MLSS concentrations in R2 at SALRs of 10 and 25 g-sCOD/m^2/d were 698 mg/L and 692 mg/L; however, showed significant increases at higher SALRs of 40 and 55 g-sCOD/m^2/d to 874 mg/L and 989 mg/L ($p<0.05$), respectively. R3 remained consistent with values ranging from 880-990 mg/L, which are at the lower end of the typical range for suspended growth systems (EPA, 1997). Literature shows that the typical MLSS ranges seen for carbon-based MBBRs are between 10-800 mg/L (Rusten et al., 1992; Ødegaard, 1999; Ødegaard et al., 2000; Cao et al., 2016) . However, MLSS ranges will depend on the various constituents present in the wastewater, as well as the concentrations, and the HRT being maintained within the reactor. Many food and beverage wastewaters being treated with MBBR technology resulted in MLSS concentrations upwards of 1000-2000 mg/L at high SALR and low HRT, supporting the MLSS concentrations seen in this study (Rusten et al., 1992; Johnson et al., 2000).

The MLSS values in R1 and R2 were not statistically different at any SALR, but were statistically lower than R3 at SALRs of 10 and 40 g-sCOD/m^2/d ($p<0.05$). The difference between the MLSS concentrations in R2 and R3 at an SALR of 40 g-sCOD/m^2/d were statistically significant with a p value of 0.049. Only at the highest SALR of 55 g-sCOD/m^2/d did the three reactors all have statistically similar MLSS values. The MLSS results suggest that at an SALR of 55 g-sCOD/m^2/d there was no difference in MLSS between the MBBR and SG reactors; however, at lower SALR, the MBBR reactors produced lower solids, which would reduce sludge production and related sludge management costs at the full-scale.



*The acclimatization period for R3 was still underway during the SALR of 25 g-sCOD/m^2/d, so the data is not representative of steady state MLSS concentrations.

Figure 2.4: MLSS (mg/L) for R1 (K5), R2 (Kontakt) and R3 (SG) at SALRs of 10, 25, 40 and 55 g-sCOD/m^2/d at a 12 hr HRT.

At all SALR conditions significant suspended solids developed in the MBBR reactors, suggesting that the systems were in fact operating more similarly to Integrated Fixed-Film Activated Sludge (IFAS) systems, due to the fact that the MBBRs were using both AG and SG to remove the organic components from the wastewater. A trend of increasing suspended solids was observed with SALR, which was also seen in other trials when MBBRs were exposed to high loading rates (Bassin et al., 2016; Shokoohi et al., 2017).

2.5.2.2 SALR Effects on Kinetics

The sCOD removal rates attributable to SG and AG for R1, R2 and the control reactor (R3) with increasing SALR are presented in Table 2.4. The control reactor demonstrated similar SG volumetric removal rates to the MBBR reactors R1 and R2, hence the SG accounted for the majority of treatment with only 19%, 18% and 26% sCOD removal being attributed to the AG for R1 and 31%, 28% and 7% for R2 at SALRs of 10, 25 and 40 g-sCOD/m^2/d, respectively. AG removal rates were not statistically different between 10-40 g-sCOD/m^2/d for K5, while at 55 g-sCOD/m^2/d there was no attributable removal due to the AG media; likely due to the low 9% carrier fill in the reactor. Typical carrier fills are 25-70% of the total reactor volume (Ødegaard, 1999; Ødegaard et al., 2000). Kontakt AG removal rates were similar from 10-25 g-sCOD/m^2/d, but decreased to negligible at 40 g-sCOD/m^2/d and zero at 55 g-sCOD/m^2/d, again likely due to low carrier fills of 20% and 15%. Interestingly, despite the variation in the AG contribution the total sCOD removal remained consistent for all three reactors, indicating that SG was removing the majority of the sCOD. These results are indicative of the systems being under loaded and the systems being SG dependent, as no trend in treatment with SALR was observed. In a pure AG system, the SARR is expected to transition from first order to mixed order to zero order in relation to the SALR; however, in this study the SARR showed no trend with SALR. Therefore, under these conditions, the SARR and SALR are not valid ways of describing or designing the system.

Table 2.4: Volumetric and surficial removal rates for R1 (K5), R2 (Kontakt) and the control reactor (R3) at SALRs from 10-55 g-sCOD/m^2/d at an HRT of 12 hr and DO in excess.

SALR (g-sCOD/m^2/d)	**R3 (Control)**	**R1 (K5)**			
	SG (g-sCOD/m^3/d)	**Total** (g-sCOD/m^3/d)	**SG**[a] (g-sCOD/m^3/d)	**AG**[b] (g-sCOD/m^3/d)	**SARR**[c] (g-sCOD/m^2/d)
10	3888±79	3898±75	3142±516	756±330	2.0±0.9
25	3812±36	3629±135	2961±404*	667±420	4.2±2.6*
40	3746±58	3679±73	2923±106	756±130	7.3±2.4
55	3768±59	3800±60	4239±509	0	0

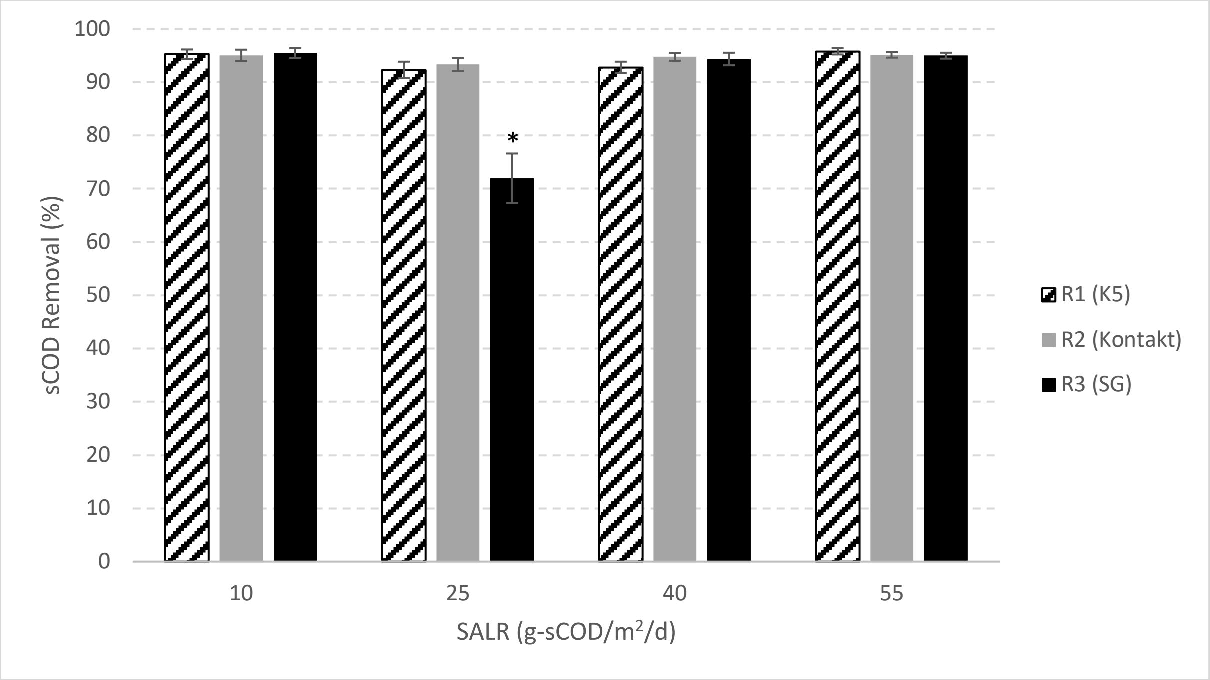
SALR (g-sCOD/m^2/d)	**R3 (Control)**	**R2 (Kontakt)**			
	SG (g-sCOD/m^3/d)	**Total** (g-sCOD/m^3/d)	**SG**[a] (g-sCOD/m^3/d)	**AG**[b] (g-sCOD/m^3/d)	**SARR**[c] (g-sCOD/m^2/d)
10	3888±79	3891±69	2971±370	921±385	2.3±0.9
25	3812±36	3660±105	2644±436*	1016±411	5.9±2.6*
40	3746±58	3765±72	3496±434	269±116	2.5±0.8
55	3768±59	3774±64	3849±396	0	0

[a] The removal rate attributed to SG in R1 and R2 was calculated by multiplying R3 (g-sCOD consumed/g-MLSS produced/d) rate by the MLSS concentrations found in R1 and R2, respectively.
[b] The removal rate attributed to AG in R1 and R2 was calculated by subtracting the SG removal rates from the total removal rates observed in R1 and R2, respectively.
[c] The SARR was calculated by dividing the AG removal rate by the carrier percent fill and the specific surface area of the carrier.
*Calculated based on the data obtained in SALRs of 10 and 40 (value used to estimate the SG and SARR at an SALR of 25 for R1 and R2).

2.5.2.3 SALR Effects on Percent Removal

The sCOD removal percentages for all three reactors at four different loading rates are presented in Figure 2.5. These findings demonstrate that changes in SALR from 10-55 g-sCOD/m^2/d did not have an impact on the removal efficiencies for the two MBBR reactors and demonstrated similar removal efficiencies to the control SG reactor. Given the high removal efficiencies observed, these results indicate that all three reactors were under loaded, and due to the comparable results from the SG control reactor, it suggests that treatment was primarily in the SG phase in both the MBBR reactors.

The SALR of 25 g-sCOD/m^2/d loading was the first experiment conducted. It was observed that treatment in the SG reactor (R3) was lower than both MBBR reactors during this trial, which can be attributed to the statistical difference in MLSS ($p<0.05$). The carriers present within R1 and R2 likely acted as an inoculum, with the resultant biofilm sloughing attributing to the increased MLSS observed within R1 and R2. Although R3 was thought to have reached steady state at this point in the experiment (<10% variation in effluent sCOD), consequent trials exhibited actual steady-state conditions with higher MLSS concentrations. The data at an SALR of 25 g-sCOD/m^2/d supports literature findings that MBBRs have a faster acclimatization period than SG or activated sludge systems (WEF, 2010; Metcalf & Eddy, 2014).



*The acclimatization period for R3 was still underway during the SALR of 25 g-sCOD/m^2/d, so the data is not representative of steady state sCOD removal

Figure 2.5: Soluble COD removal percentages for R1 (K5), R2 (Kontakt) and R3 (SG) at SALRs of 10-55 g-sCOD/m^2/d at a 12 hr HRT.

Ultimately, both carriers showed the same trend with AG removal becoming insignificant at high SALR. At an HRT of 12 hr the majority of the sCOD removal was attributed to SG in R1 and R2, thus neither carrier could be accurately determined as being more optimal than the other. However, the optimal carrier percent fill for K5 carriers ranged between 13-50% and 32-80% for Kontakt carriers. Biofilm attachment and development is typically stimulated when bacteria are stressed due to lack of substrate or limited time in the reactor, so the long operating HRT may have limited the contribution of the AG removal.

2.5.3 HRT Effects on Brewery MBBR Performance

During the HRT trial, DO ranged from 4.1 to 7.2 mg/L, temperature ranged from 20 to 24°C and the pH ranged from 6.5-7.3. The temperature and pH showed no significant changes at the various HRTs; however, the DO decreased with lower HRT, but remained in excess.

2.5.3.1 HRT Effects on MLSS

The MLSS concentrations for all three reactors at three different HRTs are presented in Figure 2.6. The MLSS concentrations for both R1 and R2 were within the typical MBBR MLSS values found in dairy and slaughterhouse wastewaters (Rusten et al., 1992; Johnson et al., 2000). R1 had MLSS concentrations of 731, 849 and 947 mg/L and R2 had MLSS concentrations of 874, 738 and 929 mg/L, at HRTs of 12, 4 and 3 hr, respectively. R3 remained constant across all three HRT conditions with MLSS concentrations ranging from 929 to 986 mg/L.

R3 MLSS concentrations were significantly higher than R1 and R2 at a 12 hr HRT and R2 at 4 hr HRT ($p<0.05$), indicating a potential benefit of MBBR systems in terms of reduced sludge production. Both R1 and R2 exhibited some variability with HRT, with R1 MLSS at a 12 hr HRT being statistically lower than at a 3 hr HRT ($p<0.05$) and R2 MLSS at a 4 hr HRT being statistically lower than both 3 and 12 hr HRTs ($p<0.05$). However, R1 and R2 had statistically similar MLSS concentrations at each HRT. This data does not present any clear trend of MLSS with HRT or carrier type.

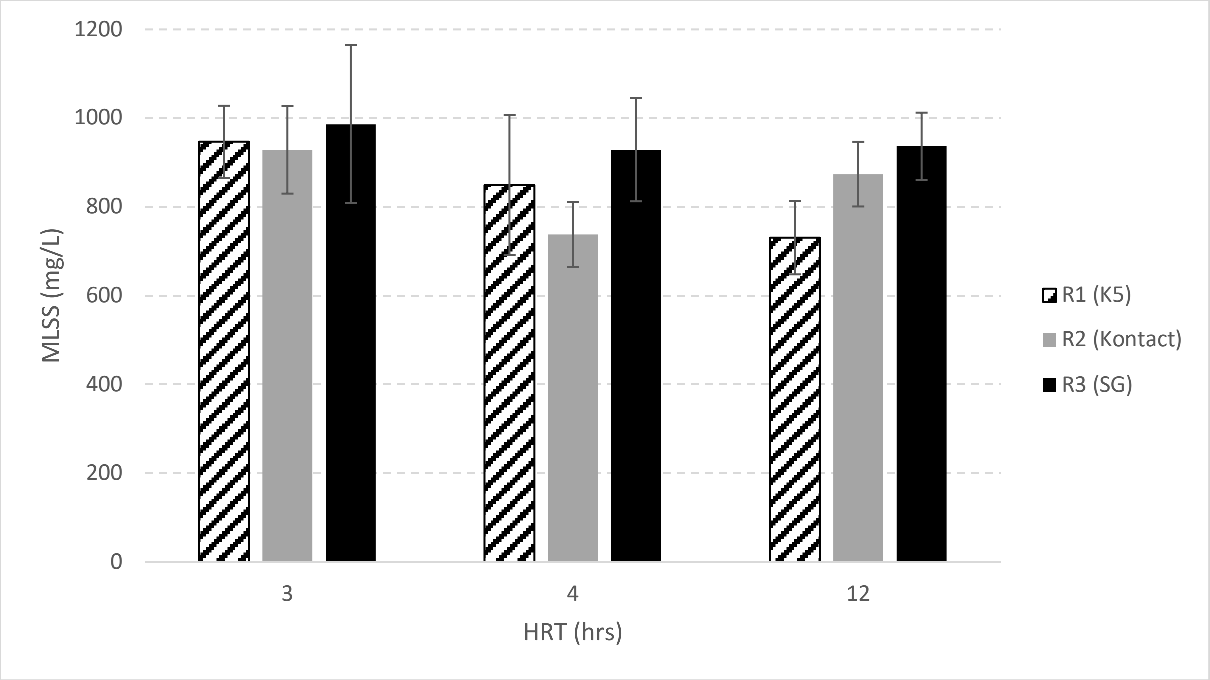


Figure 2.6: MLSS (mg/L) for R1 (K5), R2 (Kontact) and R3 (SG) at a 3, 4 and 12 hr HRT at an SALR of 40 g-sCOD/m^2/d.

2.5.3.2 HRT Effects on Kinetics

The AG and SG removal rates were calculated for R1, R2 and the control reactor (R3) for each change in HRT in Table 2.5. The reduction in HRT from 12 to 4 hr caused a significant increase in the total volumetric removal rates for all three reactors ($p<0.05$); however, the increase in the total volumetric removal rates for R1 and R2 were significantly higher than the control reactor ($p<0.05$). At HRTs of 3 and 4 hr the MBBR systems had significantly higher total volumetric removal rates than the SG control reactor ($p<0.05$), indicating that the AG was having a significant impact on the sCOD removal. The highest AG removal rates for R1 and R2 occurred at a 3 and 4 hr HRT with both reactors being statistically the same. The AG removal rates significantly increased with the change in HRT from 12 to 4 hr ($p<0.05$). At low HRT there was a clear shift from SG to AG dependency, which demonstrates that at a low HRTs the systems became more AG dependent.

Table 2.5: Volumetric and surficial removal rates for R1 (K5), R2 (Kontakt) and the control reactor at HRTs from 3-12 hr with an SALR of 40 g-sCOD/m^2/d and DO in excess.

HRT (h)	**R3 (Control)**	**R1 (K5)**			
	Total (g-sCOD/m^3/d)	**Total** (g-sCOD/m^3/d)	**SG**[a] (g-sCOD/m^3/d)	**AG**[b] (g-sCOD/m^3/d)	**SARR**[c] (g-sCOD/m^2/d)
3	9964±592	11722±332	9560±503	2162±642	5.4±1.6
4	10749±277	11348±241	9441±923	1907±926	6.4±3.1
12	3746±58	3679±73	2923±106	756±130	7.3±2.4
HRT (h)	**R3 (Control)**	**R2 (Kontakt)**			
	Total (g-sCOD/m^3/d)	**Total** (g-sCOD/m^3/d)	**SG**[a] (g-sCOD/m^3/d)	**AG**[b] (g-sCOD/m^3/d)	**SARR**[c] (g-sCOD/m^2/d)
3	9964±592	11787±352	9380±781	2407±643	5.6±1.5
4	10749±277	11434±134	8399±795	3035±770	9.5±2.4
12	3746±58	3765±72	3496±434	269±116	2.5±0.8

[a] The removal rate attributed to SG in R1 and R2 was calculated by multiplying R3 (g-sCOD consumed/g-MLSS produced/d) rate by the MLSS concentrations found in R1 and R2, respectively.
[b] The removal rate attributed to AG in R1 and R2 was calculated by subtracting the SG removal rates from the total removal rates observed in R1 and R2, respectively.
[c] The SARR was calculated by dividing the AG removal rate by the carrier percent fill and the specific surface area of the carrier.

2.5.3.3 HRT Effects on Percent Removal

The sCOD removal percentages for all three reactors at three different HRTs are presented in Figure 2.7. R1 and R2 showed no significant change from a 12 to 4 hr HRT, while R3 showed a significant decrease in removal from 94.4 to 88.2% ($p<0.05$). However, once the HRT was further reduced to 3 hrs, all three reactors exhibited a significant decrease in removal compared to the 4 hr HRT ($p<0.05$). R1 and R2 dropped from 93.9 and 94.5% to 72.9 and 73.6%, respectively, and R3 dropped from 88.2 to 61.1%. No significant differences between R1 and R2 were observed at each HRT; however, they showed significantly higher removal percentages when compared to R3 at both 3 and 4 hr HRTs ($p<0.05$). At a 3 hr and 4 hr HRT the control reactor was not capable of maintaining the same sCOD removal efficiency as the MBBRs. This demonstrates that the MBBRs are more effective than the SG reactor at lower HRTs, which is consistent with the kinetic findings and to literature (WEF, 2010; Metcalf & Eddy, 2014).

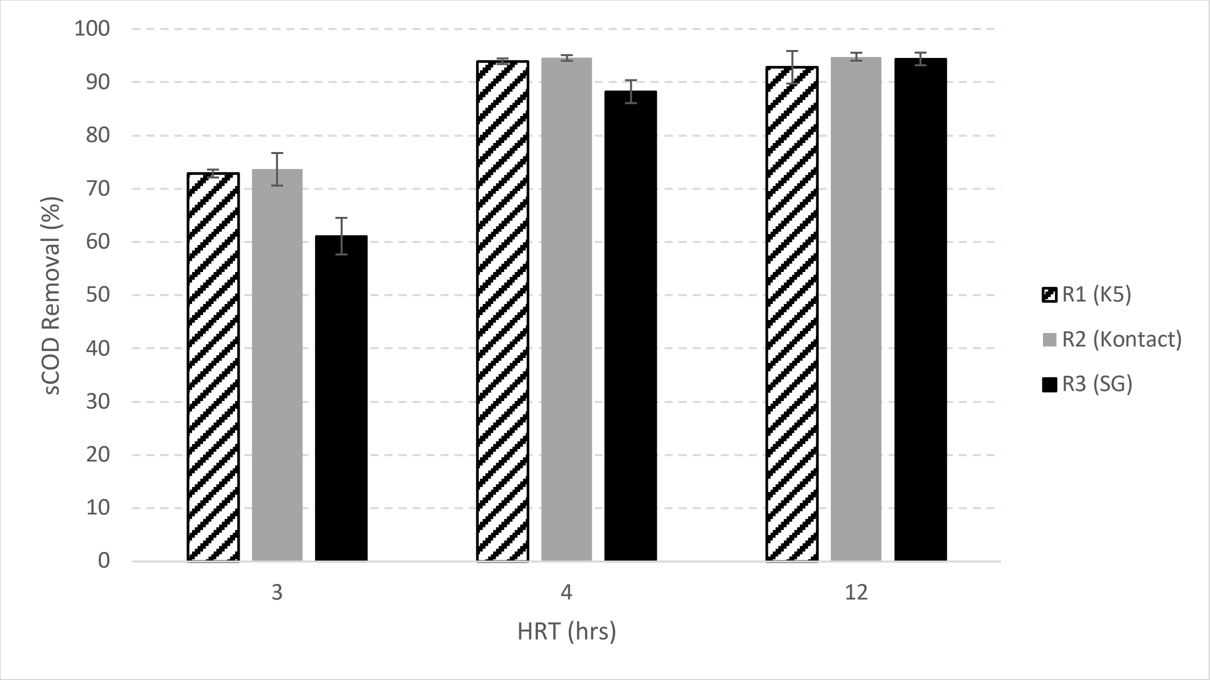


Figure 2.7: Soluble COD removal percentages for R1 (K5), R2 (Kontakt) and R3 (SG) at a 3, 4 and 12 hr HRT at an SALR of 40 g-sCOD/m^2/d.

Due to the fact that there was significantly higher sCOD removal in the MBBR systems as opposed to the SG reactor during the 3 and 4 hr HRT ($p<0.05$), this is indicative that the AG was impacting the removal and thus, was causing a microbial shift from SG to AG dependency. Both MBBR systems operated best at a 4 hr HRT under an SALR of 40 g-sCOD/m^2/d with excess DO, neutral pH and room temperatures. Overall, both MBBR systems performed equally under the experimental conditions, thus either carrier would be an appropriate choice.

2.5.4 Scale-up Validation of Lab-Scale Data

The full-scale system was monitored and evaluated for the duration of this project; however, there were many issues that needed to be rectified before a comparison could be made to the lab-scale reactors. Due to strict regulations imposed on the food and beverage industry the brewery effluent entering the treatment system has temperatures ranging upwards of 50°C, extremely variable pH of 3-10 (due to the caustic and acidic solutions used for sterilization), as well as hydraulic and organic load variability due to the batch nature of brewery production. Additionally, it was observed that there was insufficient dissolved oxygen (DO) in the two MBBRs due to a combination of a lack of blower/diffuser capacity and high wastewater temperature. These issues were addressed by the brewery by installing a heat exchanger, improving the pH balancing system, replacing the diffusers and upgrading the aeration units.

Once the full-scale system had reached stable operating conditions, two sampling campaigns were completed: composite samples were collected every three hours for three days using ISCO-6712 auto-samplers (April 2018) and grab samples collected daily for a month (July 2018). Table 2.6 compares the kinetics for MBBR 2 during the two separate sampling runs (April and July 2018) to the lab-scale reactors under similar operating conditions. The volumetric removal rate is the largest difference between the two full-scale sample runs: 3326 and 1576 g-sCOD/m^3/d. The influent entering MBBR 2 was significantly lower in July than in April leading to substrate limited removal and thus, lower removal rates.

The lab-scale operation showed comparable results to the first collection data set (April). The total volumetric removal rate and MLSS concentration for MBBR 2 were statistically the same as the total volumetric removal rates and MLSS concentrations for all three lab reactors. This demonstrates that the lab-scale reactors effectively modelled the full-scale MBBR 2.

However, the full-scale data differs in that its operational variability is significantly higher. This is due to fluctuations in the concentrations and volumes of brewery effluent entering the on-site treatment train. Due to the batch production and the varying types of beer being produced on any given day there will be fluctuation in the organic content as well as the flowrate of the wastewater entering the system, while the lab-scale reactors were operated under constant hydraulic and organic loading conditions.

Table 2.6: A comparison of the environmental conditions and constituents of MBBR 2 during April and July 2018 and the lab-scale reactors during similar conditions.

	Full-Scale		**Lab-Scale**		
	MBBR 2 (Kontakt)		R1 (K5)	R2 (Kontakt)	Control
Sampling Period	April 2018	July 2018	-	-	-
Sample Type	Composite	Grab	Grab	Grab	Grab
Theoretical SALR (g-sCOD/m^2/d)	8	8	10	10	N/A
Carrier Fill (%)	70	70	50	80	N/A
Reactor Volume (m^3)	20	20	0.002	0.002	0.002
HRT (hr)	12*	12*	12	12	12
Total Volumetric Removal Rate (g-sCOD/m^3/d)	3326±942	1576±486	3898±75	3891±69	3888±79
Influent (mg-sCOD/L)	1927±472	1183±342	1939±38	1939±38	1939±38
Effluent (mg-sCOD/L)	264±39	395±197	79±15	83±13	83±19
MLSS (mg-TSS/L)	833±329**	960±431	703±105	665±83	870±163
DO (mg/L)	4.2-6.1	3.4-5.7	5.9-7.6	5.9-7.6	5.9-7.6
Temperature (°C)	22.9-27.8	23.1-29.7	21.8-24.5	21.8-24.5	21.8-24.5
pH	7.0-8.5	7.0-8.5	6.7-7.8	6.7-7.8	6.7-7.8

*Nominal

**Used averaged MLSS data from MBBR2 as there was an error in MLSS sampling during the April sampling period

The April sample run had an average influent concentration of 1927 mg-sCOD/L and an average effluent concentration of 264 mg-sCOD/L (Figure 2.8), while the July sample run had an average influent concentration of 1183 mg-sCOD/L and an average effluent concentration of 395 mg-sCOD/L (Figure 2.9). During both of these sampling periods, the system's effluent organic concentration was close to the local municipality's sewer use discharge limit of 300 mg-BOD$_5$/L (Bylaw No. 2003-514), which was the system's treatment objective for carbon.

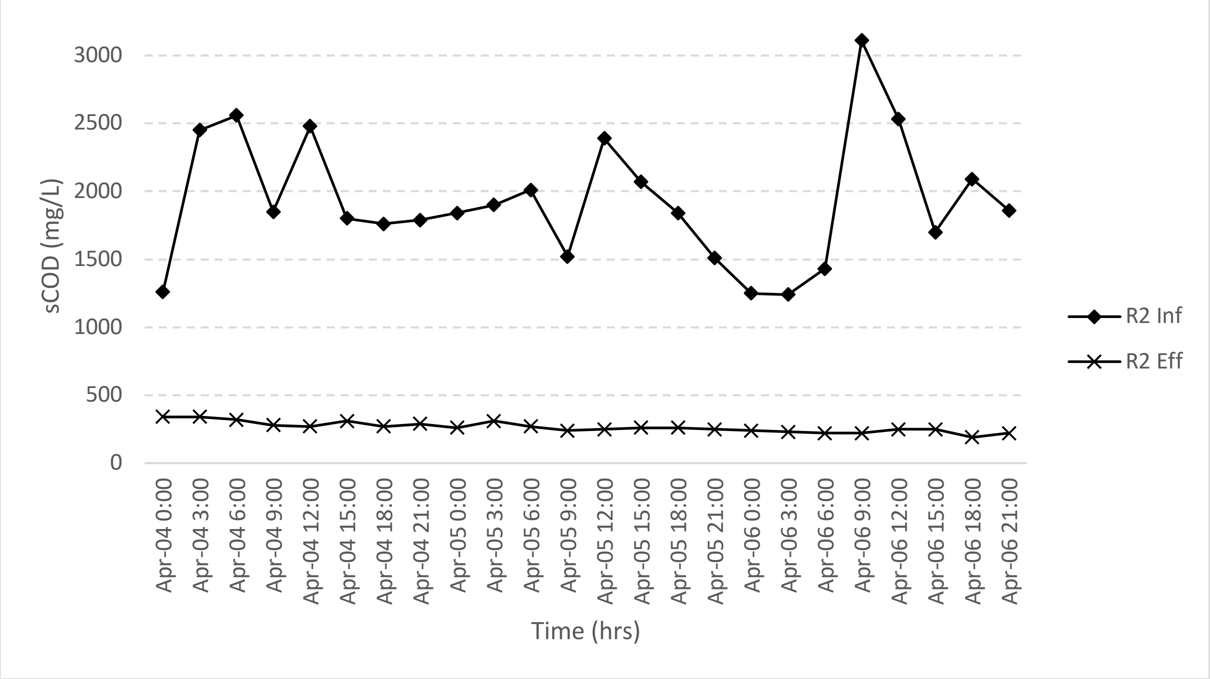


Figure 2.8: Influent and effluent sCOD concentrations (mg/L) of the full-scale MBBR 2 (Kontakt) reactor for April 4^{thd}-6th, 2018.

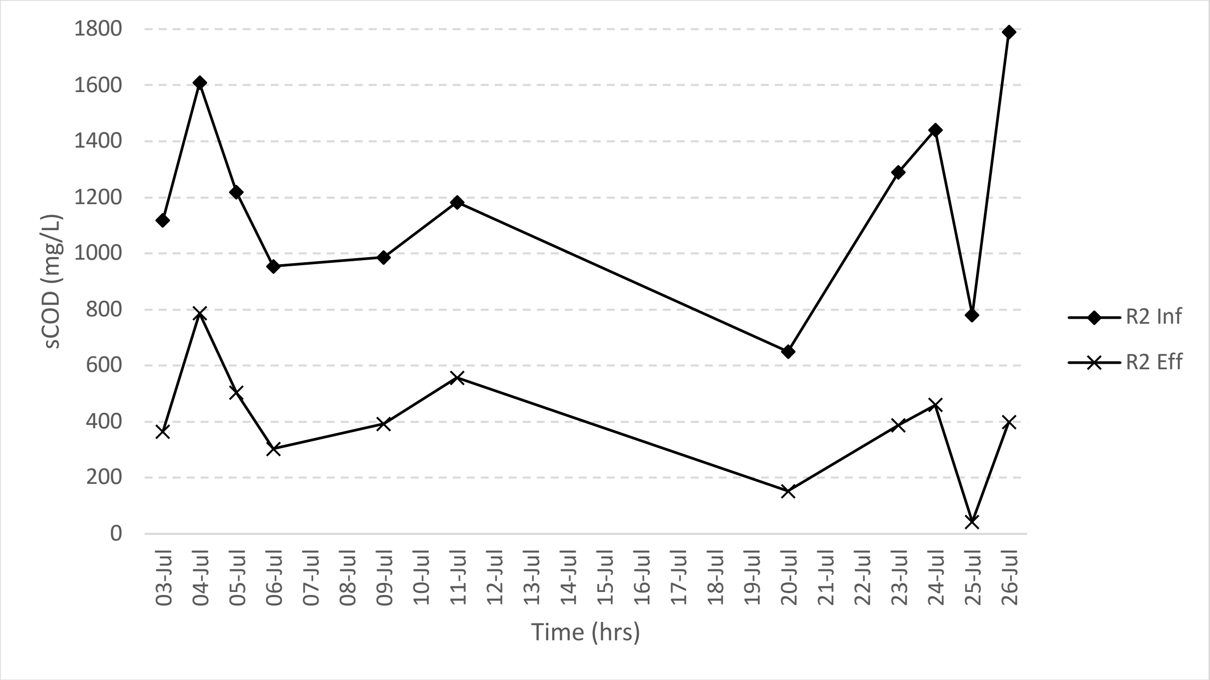


Figure 2.9: Influent and effluent sCOD concentrations (mg/L) of the full-scale MBBR 2 (Kontakt) reactor for the month of July 2018.

The April data set indicates that the system was substrate limited (under loaded) as the influent concentrations varied widely while the effluent concentrations remained stable with low sCOD concentrations, likely constituting refractory compounds. Similarly, at an HRT of 12 hr with ideal DO, temperature and pH conditions, the lab-scale reactors were also substrate limited. The July data set; however, indicates that the system was operating at zero-order kinetics as a constant removal rate of 65±9% was observed, which is independent of the influent concentration and is likely MLSS dependent.

A notable difference between the full-scale and lab-scale reactors was the almost complete absence of AG observed on the carriers (Figure 2.10). Therefore, the full-scale system appeared to rely almost exclusively on SG rather than AG mechanisms. This is consistent with the lab-scale results, which demonstrated no differences between the SG and MBBR reactors at a 12 hr HRT and suggests the potential to decrease HRT as the full-scale production expands.

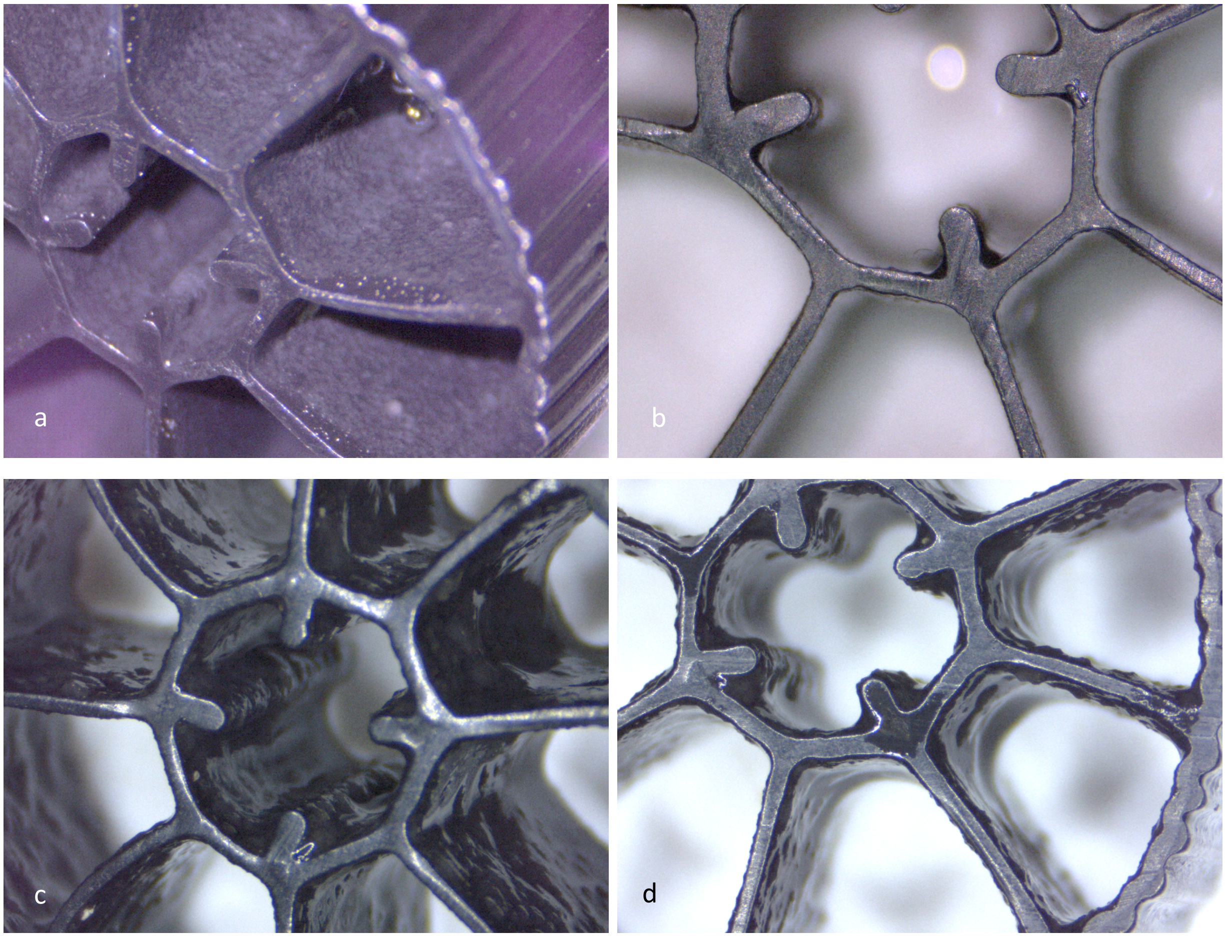


Figure 2.10: Stereoscopic images of the biofilm growth on Kontakt carriers under similar conditions (HRT 12 hr and DO in excess) at the full-scale (a,b) and at the lab-scale(c,d).

Limited biofilm attachment at the full-scale could be the result of many factors. The CIP cleaning products, anti-foaming agents and the naturally occurring anti-microbial agents, such as hops and bog myrtle (Rückle & Senn, 2006; Flythe, 2009; Bocquet et al., 2018), present in beer could all be contributing to the lack of attachment. The CIP cleaning products and anti-foaming agents may contain surfactants, which would severely inhibit biofilm attachment. The anti-foaming agent also has upwards of 150,000 mg-COD/L, which could be causing the biofilm sloughing due to high carbon loading (Chmielewski & Frank, 2003). Notably, the anti-foaming agents are directly dosed into the full-scale MBBRs, so the lab-scale reactors were never exposed to their properties or any combination of the anti-foaming agents with the CIP cleaning products and other anti-microbial constituents.

The temperature ranges in the full-scale reactor were also 1-5°C higher than in the lab-scale reactors. While both temperature ranges fall within the mesophilic bacterial range, wastewater temperatures approaching 30°C could be impacted by mesophilic and thermophilic bacterial competition. It could also be possible that the slightly higher temperatures seen in the full-scale system could cause a degree of mesophilic bacterial denaturing (Chmielewski & Frank, 2003).

2.6 CONCLUSION

Trial 1 studied the impact of increasing SALR on AG and SG removal rates in two MBBR systems utilizing two different carrier types (K5 and Kontakt) with a SG reactor operating as a control. At all SALRs the MBBR systems had statistically similar total volumetric removal rates when compared to the control. At low SALR, the MBBR systems had smaller MLSS concentrations than the control reactor; however, they were still able to obtain the same total removal rates due to the AG contribution. At high SALR the attributed AG removal rates decreased to zero and the MLSS concentrations in the MBBR systems were equivalent to the control. The overall sCOD removal remained consistent between 93-96% across all three reactors, indicating the systems were underloaded and SG dependent.

Trial 2 studied the impact of decreasing HRT on AG and SG removal rates in two MBBR systems utilizing two different carrier types (K5 and Kontakt) with a SG reactor operating as a control. At HRTs of 3 and 4 hr the total volumetric removal rates were significantly higher in the

MBBR systems when compared to the control reactor, indicating that the systems that the AG contributed to the treatment at lower HRT. The highest AG removal rates occurred at a 3 and 4 hr HRT and both MBBR systems exhibited optimum performance at a 4 hr HRT under an SALR of 40 g-sCOD/m^2/d.

The full-scale first collection data set (April 2018) validated the results shown in the lab-scale reactors. The total volumetric removal rates and MLSS concentrations were statistically the same for the full-scale MBBR 2 and all three lab-scale reactors. Interestingly, despite the similar operating conditions between the full-scale and the lab-scale, there was minimal AG on the carriers at the full-scale throughout the project. More testing and monitoring of the full-scale system is required understand the reasons for the lack of AG present.

The results of this study reveal that MBBRs are a potential technology for the treatment of brewery wastewater; however, both AG and SG will be factors. Future research into varying the influent concentration and investigating the biomass response may improve the understanding of optimal AG conditions for MBBR systems and potentially help to minimize the need for accompanied SG removal.

2.7 SUPPLEMENTAL MATERIALS

Two types of carriers were utilized in the study to evaluate the impact of carrier surface area and dimensions on treatment effectiveness and biofilm morphology: Kaldness K5 and Kontakt (Figure S2.1). K5 carriers have a specific surface area of 800 m^2/m^3 and Kontakt carriers have a specific surface area of 500 m^2/m^3. K5 carriers have a diameter of 25mm and a depth of 3.5mm. Kontakt carriers have a diameter of 20mm and a depth of 10mm.

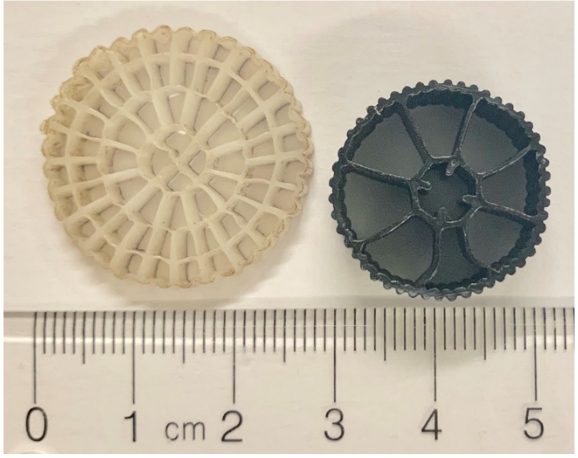


Figure S2.1: Example of a clean Kaldnes K5 carrier (left) and a Kontakt carrier (right).

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CHAPTER 3: RESPONSE OF CARBON REMOVAL MBBR BIOFILM ACROSS CARRIER TYPE, SALR AND HRT

3.1 ABSTRACT

Biofilm morphology and viability from each of the two carriers utilized in the study of moving bed biofilm reactor (MBBR) treatment of brewery wastewater were investigated using stereoscopy and confocal laser scanning microscopy (CLSM) in combination with live/dead cell staining. Both carriers demonstrated thicker and more viable biofilms at high SALR and denser and less viable biofilms at low SALR. At lower HRT, the carriers reacted differently resulting in thicker, but less dense biofilms on the Kontakt carriers and thinner, but more dense biofilms on the K5 carriers. However, no trend in cell viability was observed with change in HRT. Although the systems were suspended growth (SG) dominated, based on the MBBR kinetics and carrier biofilm morphology and cell viability, either carrier would be a viable choice for an MBBR treating brewery wastewater at HRTs between 4 to 12 hr and SALRs between 10-55 g-sCOD/m^2/d.

3.2 SETTING THE CONTEXT

The article presented in Chapter 3 is titled *Response of carbon removal MBBR biofilm across carrier type, SALR and HRT* by K. Boyle, C. Kinsley, R. Delatolla and B. Abbassi. This paper presents a combination of two microscopic analyses for the determination of biofilm viability for a laboratory scale brewery wastewater MBBR system. Quantification of mass, thickness and density as well as live and dead cell analyses were conducted for every change in surface area loading rate (SALR), hydraulic retention time (HRT) and carrier type.

3.3 INTRODUCTION

Brewery wastewater is highly variable in terms of organic and hydraulic loading. The organic content can range between 1000-125,000 mg-COD/L and 200-3000 mg-TSS/L, and the temperature and pH can vary from 15-80°C and pH 2-14. However, even though influent variability is a factor to consider, the majority of brewery wastewater is highly biodegradable. This high biodegradability makes biological treatment ideal and one of the most used wastewater treatment processes in the brewing industry (Saila & Hasan, 2017).

Although anaerobic digestion (AD) is commonly used for brewery wastewater treatment, it has large land footprints, high capital costs and operational complexity due to methane gas production, making it difficult to implement for small scale breweries (Simate et al., 2011; Olajire, 2012). Aerobic treatment, such as moving bed biofilm reactor (MBBR) technology, with its compact reactor size and operational stability at various loadings due to biofilm retention, is a potential solution for decentralized brewery wastewater treatment.

An MBBR is an aerated, attached growth (AG) biological wastewater treatment system that utilizes free floating biological carriers. The carriers provide the surface area for biofilm attachment. Ødegaard et al. (2000) stated that the size and shape of a carrier has minimal impact on MBBR performance and only the specific surface area of the carrier will dictate the effectiveness of the system. However, other studies have found that the shape, size and surface of the carriers are also important parameters to take into account when designing an MBBR system because it will impact the growth and viability of the biofilm (Karizmeh et al., 2014; Bassin et al., 2016; Forrest et al., 2016).

Aerobic, heterotrophic biofilms, have been documented as being heterogeneous – meaning there are many different types of cell clusters and micro-colonies, as well as substantial void spaces present within the biofilm (i.e. porosity) (De Beer et al., 1994). Heterotrophic biofilms, which are what frequently grow in carbon-based, aerobic, AG treatment operations, have been documented with having vast ranges of thicknesses and densities. Tanyolaç & Beyenal

(1997) stated that ideal densities for heterotrophic biofilm are within 34 to 76 kg/m^3. Municipal wastewater studies utilizing carriers for biofilm growth have found thicknesses ranging from 139 to 300 μm with densities ranging from 77 to 559 kg/m^3 (Liang, 2005; Mahendran et al., 2012). However, other studies treating chemical wastewaters have found much smaller thicknesses in the range of 72 to 90 μm (Huang et al., 2017).

Overall, there is limited information on biofilm growth and viability in MBBR systems for industrial wastewater treatment, let alone brewery wastewater treatment. The study by Boyle et al. (20xx) analyzed the impacts of SALR and HRT on brewery wastewater fed MBBR systems with the study finding that the MBBR systems operated similarly to integrated fixed-film activated sludge (IFAS) systems in that there was a dependence on both suspended growth (SG) and AG. SALRs of 10-55 g-sCOD/m^2/d at an HRT of 12 hr resulted in minimal AG with both MBBR systems relying on SG sCOD removal. Biofilm growth can be stimulated by stressing the planktonic bacteria with a lack of substrate or limiting the time exposed to the substrate, which was achieved by reducing the HRT. At low HRTs of 3 and 4 hr at an SALR of 40 g-sCOD/m^2/d, the AG present in the MBBR systems had an impact on sCOD removal; however, there was still significant sCOD removal in the SG phase. Ideally, in MBBR systems there would be minimal SG and the majority of the removal would be as a result of the AG; however, according to the results of the study it is unclear how to lower the SG dependence, while also promoting biofilm growth.

The results at the macroscale are influenced by the dynamics of the bacterial cells at the microscale, thus to advance the understanding of the operation and optimization of MBBR technology for brewery wastewater treatment, an enhanced understanding of the response of the biofilm and the biomass should be investigated. In particular, this article will examine the biofilm thickness, mass and density, as well as the viability of the embedded biomass in the biofilm, for the brewery wastewater fed MBBR systems operating with different carriers and at various SALRs and HRTs.

3.4 MATERIALS AND METHODS

3.4.1 MBBR Laboratory Reactor Set-Up

Three lab-scale reactors were operated in parallel to evaluate the treatment for brewery wastewater using two MBBR systems with different commercial carriers and to compare with a SG control reactor (Figure 3.1). Each reactor had a working volume of 2 L and was maintained at a neutral pH, room temperature and aeration rate of 5 Lpm. Reactor 1 (R1) contained K5 (AnoxKaldnes, Lund, Sweden) (protected surface area 800m^2/m^3) biofilm carriers and Reactor 2 (R2) contained Kontakt (Jaeger Environmental, Houston, USA) (protected surface area 500 m^2/m^3) biofilm carriers (Figure S3.1). Reactor 3 (R3) was maintained as a control SG system. The SG control reactor did not operate with a return activated sludge (RAS) line to simulate the full-scale facility, where HRT is equal to SRT.

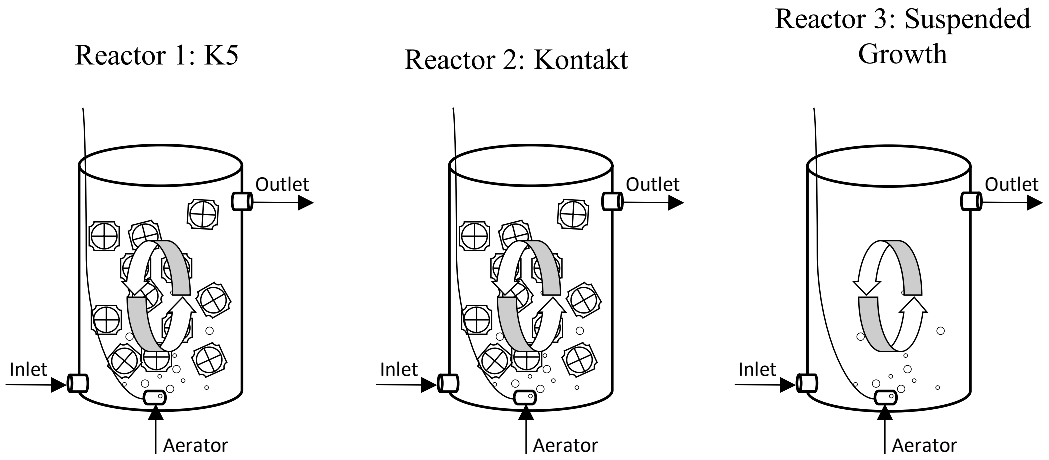

Figure 3.1: Laboratory schematic of MBBR and suspended growth reactors.

A sample of high strength wastewater was collected from the Beau's All Natural Brewing Company in Vankleek Hill, Ontario, stored at 4°C, and used as the wastewater source for the lab experiments (Table 3.1).

Table 3.1: Beau's All Natural Brewing Company wastewater sample taken to conduct the laboratory analyses.

Constituent	Before Dilution	After Dilution
Soluble COD (mg-sCOD/L)	37500±1020	2034±63
Total Nitrogen (mgN/L)	117±6	6.3±2.2
Ammonia (mgNH$_3$-N/L)	105±4	6.1±2.7
Nitrate (mgNO$_3$-N/L)	n.d.	n.d.
Nitrite (mgNO$_2$-N/L)	n.d.	n.d.
Total Phosphorus (mgP/L)	1328±28	69.9±1.2
Orthophosphate (mgPO$_3$^{4}-P/L)	951±15	50.7±4.1
TSS (mgTSS/L)	1620±102	88±49
VSS (mgVSS/L)	1459±89	76±16

n.d. = non detect (Nitrate < 0.01mg/L, Nitrite < 0.01mg/L)

The objective of the laboratory experiment was to compare the efficiencies of three separate systems for the removal of organic matter from raw brewery wastewater. Two distinct trials were conducted wherein four SALRs and three HRTs were tested on each of the three reactors (Table 3.2). The SALRs were chosen based on the information obtained from food and beverage MBBR studies (SALRs 25-45 g-sCOD/m^2/d) (Rusten et al., 1992; Rusten et al., 1996; Johnson et al., 2000), as well as what is typically seen in carbon-based MBBRs. Typical SALR values found for carbon-based MBBRs range between 1-45 g-sCOD/m^2/d (Rusten et al., 1992; Ødegaard, 1999; Ødegaard et al., 2000; Cao et al., 2016). Above 20 g-sCOD/m^2/d is considered to be a high loading rate, 5-15 g-sCOD/m^2/d are moderate loading rates and anything less than 5 g-sCOD/m^2/d are low loading rates (Ødegaard, 1999; Ødegaard et al., 2000).

During each of the four SALRs an HRT of 12hr was maintained and during the three HRTs an SALR of 40 g-sCOD/m^2/d was maintained. The corresponding carrier percent fill for

K5 and Kontakt carriers is demonstrated for each loading rate in Table 3.2. The first experiment was operated at 12 hr HRT to replicate the full-scale system at the participating brewery.

Table 3.2: Operational conditions applied to each reactor during two separate trials.

Trial 1 – SALR Effects				**Trial 2 – HRT Effects**			
SALR (g-sCOD/m^2/d)	HRT (hr)	K5 %fill	Kontakt %fill	SALR (g-sCOD/m^2/d)	HRT (hr)	K5 %fill	Kontakt %fill
10	12	48	76	40	3	48	76
25	12	20	32	40	4	38	60
40	12	13	20	40	12	13	20
55	12	9	15				

The reactors were fed with the same effluent for the duration of the testing. 400 L of highly concentrated wastewater (approximately 38,000 mg-sCOD/L) was retrieved from Beau's All Natural Brewing Company and diluted to produce the feed (2000 mg-sCOD/L) for the lab-scale reactors to simulate MBBR 2 at the full-scale. The reactors were also dosed with concentrated ammonium sulfate to achieve a 10:1 ratio of carbon to nitrogen (with phosphorus already in excess). The feed tank consisted of a 100 L container with an attached mixer. The reactors were run for two months during the initial start-up phase and then run for three weeks between each condition to allow for acclimatization. The SALR effects were run starting with SALRs of 25, 40 and 55 g-sCOD/m^2/d and then 10 g-sCOD/m^2/d at the end. The change in SALR from 55 to 10 g-sCOD/m^2/d required an acclimatization period of five weeks. The HRT effects were run in decreasing order from 12 hr to 3 hr. Once steady state was reached for each condition, the reactors were sampled daily to obtain ten consecutive data points. Steady state was defined as the period during which the carbon removal rate and effluent concentration varied less than 10% in terms of sCOD. Reactor operation was maintained for a total of 302 days.

3.4.2 Biofilm Thickness

Five replicate carriers were harvested from both reactors for each loading rate and change in HRT. The carriers were imaged using a Zeiss Stereomicroscope Stemi 305 (Zeiss, US, VA) within 20 min of removal to minimize the potential effects of biofilm dehydration (Figure S3.2). During this time period, five images across each carrier were captured corresponding to 25 total images per condition (Young et al., 2016). The acquired images were analysed for thickness on Fiji Image J software resulting in a total of 250 thickness measurements per condition.

3.4.3 Biofilm Mass

Five replicate carriers were harvested from both reactors for each loading rate and change in HRT. Within 20 min the carriers were placed in a drying oven at 105°C for twelve hours. After twelve hours, the carriers were removed and placed in a desiccator for fifteen minutes to cool. Once cooled, the carriers were weighed (*W1*). The dehydrated carriers were then cleaned with warm water and a stiff-bristled brush and placed back in the drying oven at 105°C for twelve hours. After twelve hours, the cleaned and dried carriers were placed in a desiccator for fifteen minutes and then weighed (*W2*). The difference between *W1* and *W2* resulted in the measured mass of the biofilm (Young et al., 2016; Schopf et al., 2018).

3.4.4 Cell Viability

Five replicate carriers were harvested from both reactors for each loading rate and each HRT investigated in this study. Each carrier was cut with a scalpel to expose the inner biofilm surfaces and then stained using Propium Iodide (PI) and SYTO 9 from the film Tracer™ LIVE/DEAD® biofilm viability kit (Life Technologies, US, CA). SYTO 9 is a green nucleic acid stain, which is membrane-permeant. It is administered for the illumination of all cells in the biofilm. PI is a cell-membrane impermeable stain and will only stain the cells with a compromised cell membrane. SYTO 9 and PI are combined into a working solution and added dropwise to the exposed biofilm and left for 30 min protected from light sources. The working solution is then gently washed with distilled water. The biofilm is then analysed using Confocal Laser Scanning Microscopy (CLSM), specifically a 510/Axiolmager confocal laser scanning microscope with a x63 water objective (Zeiss, US, VA). Five stacks of five images (25 total images) were acquired for each sample (Figure S3.3-4).

Cell viability analytical quantification of the microscopic images was performed using Nikon NI Vision Assistant (National Instruments, LabView, 8.0). Viable cells were illuminated green, whereas non-viable cells were illuminated red at each of the five distinct depths in the biofilm. The biofilm area was outlined and exported by tracing the SYTO 9 staining and the image threshold function was used to calibrate the area of a single cell. Extraneous portions of the biofilm, such as worms and other organisms, were filtered out and not included in the analysis. Once calibrated, the cell viability and non-viability as well as the biofilm area and total cell coverage could be determined from the images. The live cell fraction was determined by dividing the viable cells by the total number of cells and the dead cell fraction was determined by dividing the non-viable cells by the total number of cells (Hoang et al., 2013; Young et al., 2016).

3.4.5 Statistical Methods

Statistical significance for biofilm thickness, mass and density were tested using single-factor ANOVA with p values less than 0.05 considered significant. CLSM data, including cell viability, biofilm area and total cell coverage were also tested using single-factor ANOVA with p values less than 0.05 considered significant.

3.5 RESULTS AND DISCUSSION

3.5.1 SALR Effects

As described in Boyle et al. (20xx), the sCOD removal attributed to the AG was minimal at numerous operational conditions when compared to the removal attributed to the SG (Table 3.3). The AG removal rates for R1 were 756, 667 and 756 g-sCOD/m^3/d at SALRs of 10, 25 and 40 g-sCOD/m^2/d and decreased to zero at 55 g-sCOD/m^2/d, whereas the SG removal rates ranged from 2923 to 4239 g-sCOD/m^3/d. Similar results were obtained for R2, which demonstrated AG removal rates of 921, 1016 and 269 g-sCOD/m^3/d at SALRs of 10, 25 and 40 g-sCOD/m^2/d and zero at 55 g-sCOD/m^2/d, whereas the SG removal rates ranged from 2644 to 3849 g-sCOD/m^3/d. As such, the reactors were shown to operate as hybrid SG and AG systems in the study, with a higher dependence on SG.

The MLSS concentrations in R1 ranged from 697-731 mg/L from 10-40 g-sCOD/m^2/d and increased to 1089 mg/L at 55 g-sCOD/m^2/d. R2 ranged from 692-698 mg/L at 10 and 25 g-sCOD/m^2/d and saw a significant increase to 874 mg/L and 989 mg/L at 40 and 55 g-sCOD/m^2/d, respectively. An increase in MLSS concentrations resulted in a decrease in the AG removal rates; however, the sCOD removal for each SALR remained consistent between 92-96% for R1, R2 and the control reactor, R3. These results indicated that all three reactors were SG dependent and operating under conditions of substrate limitation.

Table 3.3: Volumetric and surficial removal rates, sCOD removal (%) and MLSS (mg/L) for R1 (K5), R2 (Kontakt) and R3 (control) at SALRs from 10-55 g-sCOD/m^2/d at an HRT of 12hr and DO in excess.

SALR (g-sCOD/m^2/d)	**R3 (Control)**			**R1 (K5)**					
	SG (g-sCOD/m^3/d)	**MLSS** (mg/L)	**sCOD Removal** (%)	**Total** (g-sCOD/m^3/d)	**SG**[a] (g-sCOD/m^3/d)	**AG**[b] (g-sCOD/m^3/d)	**SARR**[c] (g-sCOD/m^2/d)	**MLSS** (mg/L)	**sCOD Removal** (%)
10	3888±79	869±164	95.5±0.9	3898±75	3142±516	756±330	2.0±0.9	703±105	95.3±0.9
25	2778±151	330±91	72.0±4.6	3629±135	2961±404*	667±420	4.2±2.6*	697±90	92.3±1.5
40	3746±58	937±178	94.3±1.2	3679±73	2923±106	756±130	7.3±2.4	731±81	92.8±1.1
55	3768±59	972±200	95.0±0.6	3800±60	4239±509	0	0	1089±99	95.1±0.5

SALR (g-sCOD/m^2/d)	**R3 (Control)**			**R2 (Kontakt)**					
	SG (g-sCOD/m^3/d)	**MLSS** (mg/L)	**sCOD Removal** (%)	**Total** (g-sCOD/m^3/d)	**SG**[a] (g-sCOD/m^3/d)	**AG**[b] (g-sCOD/m^3/d)	**SARR**[c] (g-sCOD/m^2/d)	**MLSS** (mg/L)	**sCOD Removal** (%)
10	3888±79	869±164	95.5±0.9	3891±69	2971±370	921±385	2.3±0.9	665±83	95.1±1.1
25	2778±151	330±91	72.0±4.6	3660±105	2644±436*	1016±411	5.9±2.6*	622±95	93.3±1.2
40	3746±58	937±178	94.3±1.2	3765±72	3496±434	269±116	2.5±0.8	874±99	94.8±0.7
55	3768±59	972±200	95.0±0.6	3774±64	3849±396	0	0	989±96	95.1±0.6

[a] The removal rate attributed to SG in R1 and R2 was calculated by multiplying R3 (g-sCOD consumed/g-MLSS produced/d) rate by the MLSS concentrations found in R1 and R2, respectively.
[b] The removal rate attributed to AG in R1 and R2 was calculated by subtracting the SG removal rates from the total removal rates observed in R1 and R2, respectively.
[c] The SARR was calculated by dividing the AG removal rate by the carrier percent fill and the specific surface area of the carrier.
*Calculated based on the data obtained in SALRs of 10 and 40 (value used to estimate the SG and SARR at an SALR of 25 for R1 and R2).

3.5.1.1 Biofilm Characterization

The biofilm thickness, mass and density were characterized for both reactors at four SALRs (Figure 3.3). The biofilm thickness for R1 (K5) and R2 (Kontakt) followed the same trend of increasing thickness with increasing SALR and ranged from 23 to 237 μm and 44 to 244 μm, respectively. The biofilm mass for K5 and Kontakt carriers also demonstrated an increasing trend with increasing SALR and ranged from 11.6 to 25.3 mg/carrier and from 8.0 to 22.2 mg/carrier, respectively. Municipal AG processes utilizing carriers have found thicknesses ranging from 139 to 253 μm and 186 to 300 μm (Liang, 2005; Mahendran et al., 2012). The biofilm thicknesses at the higher SALRs showed to be within the range of literature; however, the thicknesses at lower SALR were well below the reported values. In substrate limited conditions as more carriers are added the lack of food could limit the biofilm development, thus resulting in lower mass and thickness at low SALR.

The biofilm thickness for both carriers exhibited a clear trend of increasing thickness with SALR from 10-40 g-sCOD/m^2/d for K5 and from 10-55 g-sCOD/m^2/d for Kontakt with significant increases ($p<0.05$) observed except from 10 to 25 g-sCOD/m^2/d for K5 and from 40 to 55 g-sCOD/m^2/d for Kontakt. When comparing the two carriers, Kontakt had significantly larger thicknesses than K5 for loadings of 10, 25 and 40 g-sCOD/m^2/d, while K5 had a significantly larger thickness than Kontakt at 55 g-sCOD/m^2/d.

The biofilm mass for both carriers exhibited a trend of increasing mass with increased SALR, although there were no significant differences observed from 10 to 25 g-sCOD/m^2/d in K5 carriers and from 40 to 55 g-sCOD/m^2/d in Kontakt carriers. When comparing the two carriers, significant differences were observed at each loading rate; however, no trend of effect of carrier type on mass was observed. One of the more interesting observations was biofilm development on the external surface of the Kontakt carriers. The larger depth seen with the Kontakt carriers is thought to be the reason there was significant growth within the divots on the external surface (Figure S3.1). This may have been the reason why there was a constant mass per carrier at lower carrier percent fills; the abrasion caused by 76% fill at an SALR of 10 g-sCOD/m^2/d would have limited the external growth; however, the large decrease in percent fill at SALRs of 25-55 g-sCOD/m^2/d allowed for stable biofilm development on the external surface due to the limited carrier-carrier contact.

The resultant densities for K5 carriers ranged between 40 to 210 kg/m^3; however, the density range for Kontakt carriers was much smaller ranging from 35 to 76 kg/m^3. At SALRs of 10 and 25 g-sCOD/m^2/d K5 carriers had significantly larger densities than Kontakt carriers ($p<0.05$); however, at SALRs of 40 and 55 g-sCOD/m^2/d there was no significant difference between the two carriers. According to literature, densities are typically within 34 to 76 kg/m^3 for heterotrophic biofilm (Hoehn & Ray, 1973; Tanyolaç & Beyenal, 1997), which directly corresponds to the densities seen for Kontakt carriers and for the densities seen at the higher SALRs for K5 carriers. High densities for K5 carriers were found at the lower loading rates of 10 and 25 g-sCOD/m^2/d. A possible explanation for this was described by Zhang and Bishop (1994) where biofilm density increased the closer the biofilm was to the surface of the carrier. They observed that the inner layers were 5-10 times more dense than the outer biofilm layers. This relationship can explain the higher densities observed with low biofilm thickness.

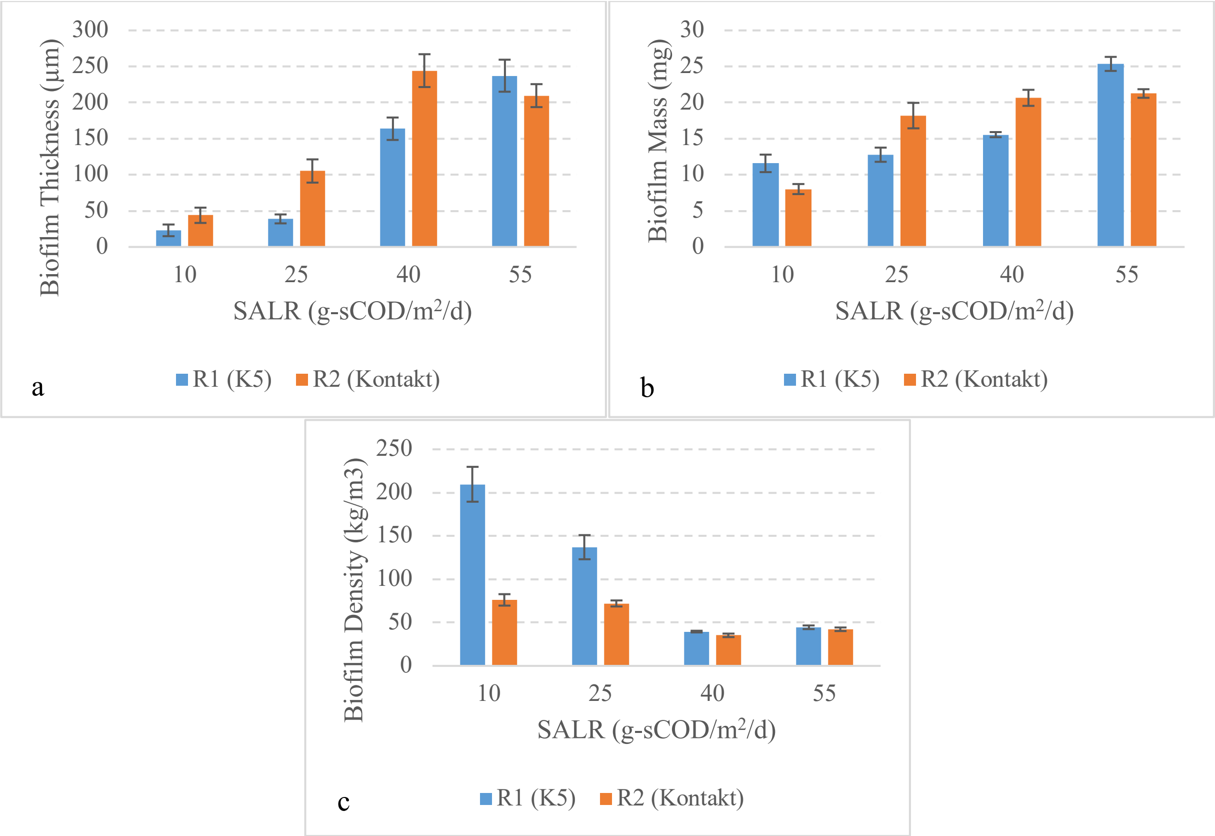

Figure 3.3: Biofilm thickness (a), mass (b) and density (c) for K5 and Kontakt carriers at SALRs of 10-55 g-sCOD/m^2*/d.*

The total SG dry biomass and total AG dry biofilm mass were calculated for each reactor with each change in SALR (Table 3.4). During each condition there was more total biological mass per reactor present in R1 and R2 in comparison to R3 due to the presence of AG in R1 and R2. Interestingly, as the carrier fill was decreased below 30% for both K5 and Kontakt carriers the total AG significantly decreased ($p<0.05$) and the SG mass increased ($p<0.05$). Higher carrier fill, corresponding to lower SALR, resulted in an increase in total AG mass and a decrease in total SG mass, which would lower sludge management costs. Therefore, it appears that K5 and Kontakt carrier fills of 30% or higher provide the best conditions for MBBR technology treating brewery wastewater. Comparing the total AG mass in the reactors to the thickness, mass and density per carrier data suggests that at low SALR and under conditions of substrate limitation it was possible that biofilm development was limited; thus, explaining the observed trend of lower biofilm thickness and mass per carrier at low SALR.

Table 3.4: Suspended and attached growth dry biomass (mg) in R1 (K5), R2 (Kontakt) and R3 (SG) at SALRs of 10-55 g-sCOD/m²/d with an HRT of 12 hr.

	Reactor 3: SG Control	**Reactor 1: K5**			**Reactor 2: Kontakt**		
SALR (HRT) (g-sCOD/m²/d)	Suspended Growth (mg)	Carrier Fill (%)	Suspended Growth (mg)	Attached Growth (mg)	Carrier Fill (%)	Suspended Growth (mg)	Attached Growth (mg)
10 (12hr)	1737±328	48	1304±196	1737±180	76	1287±160	1323±115
25 (12hr)	659±181	20	1346±174	767±60	32	1227±188	1201±118
40 (12hr)	1873±356	13	1420±169	607±16	20	1722±195	868±46
55 (12hr)	1945±400	9	2135±194	685±27	15	1952±191	659±19

Although the thickness, mass and density provide important data, ultimately, it is the biomass within the biofilm that is responsible for reactor performance. The percent of the viable and non-viable cells were quantified for both K5 and Kontakt carriers (Figure 3.4). The purpose of the analysis was to compare the effect of SALR and carrier type on biofilm viability. Unfortunately, at an SALR of 10 g-sCOD/m^2/d, there was not a sufficient amount of biofilm present on the carriers for effective analysis, thus it was not included in the following discussion.

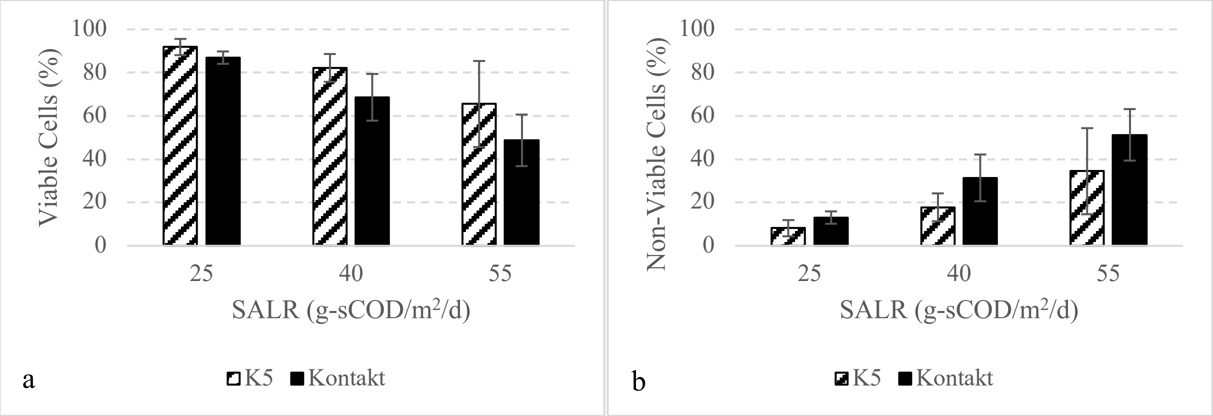

Figure 3.4: K5 and Kontakt carriers at SALRs of 25-55 g-sCOD/m^2/d, (a) biofilm embedded viable cells (%), (b) biofilm embedded non-viable cells (%).

Viable cell percentage for K5 carriers showed a significant difference between an SALR of 25 and both 40 and 55 g-sCOD/m^2/d ($p<0.05$). However, when the loading was increased from 40 to 55 g-sCOD/m^2/d there was no difference seen in the percentage of viable cells. K5 cell viability was 92%, 82% and 66% for loadings of 25, 40 and 55 g-sCOD/m^2/d, respectively. The viable cell percentage became more variant at a loading of 55 g-sCOD/m^2/d resulting in an average of 66%, but a standard deviation of 27%. This phenomenon can be explained because as biofilm matures, its density, and thus the cells, become less uniformly distributed throughout the biofilm (Laspidou & Rittmann, 2004). De Beer et al. (1994) demonstrated that void space in conjunction with large cell clusters allows for better substrate flux from the bulk liquid through the biofilm, so non-uniform density can be very good for the health of biofilm. K5 carriers showed an increasing trend with increasing loading rate for non-viable cell percentage for loadings of 25 and 40 g-sCOD/m^2/d ($p<0.05$), but the loading of 55 g-sCOD/m^2/d, again due to the large standard deviations were not statistically different than 40 g-sCOD/m^2/d. Non-viable cell fractions of 8%, 18% and 34% for loadings of 25, 40 and 55 g-sCOD/m^2/d, respectively, were found for K5 carriers. The viable cell percentage for the K5 carriers was statistically greater than the non-viable cell percentage for each of the three SALRs ($p<0.05$).

The Kontakt carriers viable cell fraction shows a steady decrease with increasing loading rate, as well as higher variability in cell density with higher loading, similar to the K5 carriers. Kontakt cell viability was 87%, 69% and 49% at SALRs of 25, 40 and 55 g-sCOD/m^2/d, respectively. Non-viable cell percentage for Kontakt carriers showed statistically significant differences between each loading rate ($p<0.05$). The non-viable cell fraction increased with increasing loading rate: 13%, 31% and 51% for loadings of 25, 40 and 55 g-sCOD/m^2/d, respectively. The Kontakt carriers were similar to K5 carriers with the viable cell fraction being greater than the non-viable cell fraction for SALRs 25 and 40 g-sCOD/m^2/d ($p<0.05$); however, at an SALR of 55 g-sCOD/m^2/d there was no significant difference. Overall, the same trend can be seen for both carrier types. Viable cells decrease and non-viable cells increase with increasing SALR, which corresponds to increasing biofilm thickness and mass as well as decreasing biofilm density.

The AG removal rates for the K5 carriers remained consistent for SALRs of 10-40 g-sCOD/m^2/d; however, at 55 g-sCOD/m^{2}d there was no AG removal. Kontakt carriers were similar in that the AG removal remained consistent for SALRs of 10 and 25 g-sCOD/m^2/d; however, the AG removal dropped to negligible at 40 and 55 g-sCOD/m^{2}d. The AG removal rates for both carriers do not show a significant relationship to the thickness, mass, density or viability of the biofilm. When comparing the AG mass to the AG removal rates for R1, there is also no direct correlation; however, R2 showed a consistent quantity of AG mass at 10-25 g-sCOD/m^2/d and a drop in AG mass at 40 and 55 g-sCOD/m^2/d, following the same trend as the AG removal rates. However, due to the concentration of SG present in the reactors, AG did not play a significant role in the sCOD removal; the SG removal rates were significantly higher at all

SALRs. Additionally, both reactors still had over 92% removal under all four loadings, as did the control reactor, which is indicative of the system being underloaded and SG dependent.

Overall, it can be concluded that although there were differences in the biofilm growth and viability between K5 and Kontakt carriers at each loading, the fact that the systems were SG dependent masks any carrier effect. In this trial, neither carrier could be definitively chosen as being more effective despite some differences seen in the biofilm.

3.5.2 HRT Effects

The AG and SG removal rates for each HRT are compared for each reactor as described in Boyle et al. (20xx) (Table 3.5). The total removal rates for R1 and R2 were significantly higher than R3 at HRTs of 3 and 4 hr ($p<0.05$) and the AG removal rates for R1 and R2 were the highest at 3 and 4 hr HRT. These results indicated that at low HRT there was a shift from SG to AG removal. The sCOD removal percentages in R1 and R2 also reflected the same microbial shift at lower HRT. The sCOD removal for both R1 and R2 at HRTs of 4 hr and 12 hr remained constant ranging from 92.8-94.8%; however, R3 had a significant difference in sCOD removal at a 4 hr HRT with 88% ($p<0.05$). All three reactors had significant reductions in sCOD removal when the HRT was changed from 4 hr to 3 hr; however, R1 and R2 still had significantly higher removal than R3 at 74% and 73% in comparison to 61%, respectively ($p<0.05$).

R1 MLSS values at 4 hr and 3 hr HRT were 849 and 947 mg/L and R2 MLSS values at 4hr and 3 hr HRT were 748 and 929 mg/L. R3 remained constant ranging between 929 to 986 mg/L. Although the MLSS concentrations were quite high for R1 and R2, so much so that there was no significant difference when compared to R3 ($p<0.05$), there was still significantly higher removal obtained in R1 and R2 at lower HRT. This further indicates that the AG was having a significant, positive impact on the biodegradation of the constituents in the wastewater, demonstrating that AG systems are more effective at lower HRT than SG systems.

Table 3.5: Volumetric and surficial removal rates, sCOD removal (%) and MLSS (mg/L) for R1 (K5), R2 (Kontakt) and R3 (control) at HRTs from 3-12 hr with an SALR of 40 g-sCOD/m^2/d and DO in excess.

HRT (h)	**R3 (Control)**			**R1 (K5)**					
	Total (g-sCOD/m^3/d)	**MLSS** (mg/L)	**sCOD Removal** (%)	**Total** (g-sCOD/m^3/d)	**SG**[a] (g-sCOD/m^3/d)	**AG**[b] (g-sCOD/m^3/d)	**SARR**[c] (g-sCOD/m^2/d)	**MLSS** (mg/L)	**sCOD Removal** (%)
3	9964±592	987±76	61.1±3.4	11722±332	9560±503	2162±642	5.4±1.6	947±83	71.9±1.9
4	10749±277	929±116	88.2±2.2	11348±241	9441±923	1907±926	6.4±3.1	849±87	93.9±0.8
12	3746±58	937±178	94.3±1.2	3679±73	2923±106	756±130	7.3±2.4	731±81	92.8±1.1

HRT (h)	**R3 (Control)**			**R2 (Kontakt)**					
	Total (g-sCOD/m^3/d)	**MLSS** (mg/L)	**sCOD Removal** (%)	**Total** (g-sCOD/m^3/d)	**SG**[a] (g-sCOD/m^3/d)	**AG**[b] (g-sCOD/m^3/d)	**SARR**[c] (g-sCOD/m^2/d)	**MLSS** (mg/L)	**sCOD Removal** (%)
3	9964±592	987±76	61.1±3.4	11787±352	9380±781	2407±643	5.6±1.5	929±73	72.3±2.4
4	10749±277	929±116	88.2±2.2	11434±134	8399±795	3035±770	9.5±2.4	738±73	94.6±0.5
12	3746±58	937±178	94.3±1.2	3765±72	3496±434	269±116	2.5±0.8	874±99	94.8±0.7

[a] The removal rate attributed to SG in R1 and R2 was calculated by multiplying R3 (g-sCOD consumed/g-MLSS produced/d) rate by the MLSS concentrations found in R1 and R2, respectively.
[b] The removal rate attributed to AG in R1 and R2 was calculated by subtracting the SG removal rates from the total removal rates observed in R1 and R2, respectively.
[c] The SARR was calculated by dividing the AG removal rate by the carrier percent fill and the specific surface area of the carrier.

3.5.2.1 Biofilm Characterization

The thickness, mass and density were characterized for both carriers at three different HRTs (Figure 3.5). The biofilm thickness for K5 carriers ranged from 164 to 222 μm and the biofilm mass ranged from 15.6 to 29.9 mg/carrier. The biofilm thickness for Kontakt carriers ranged from 244 to 361 μm and the biofilm mass ranged from 20.7 to 29.6 mg/carrier. The resultant densities for K5 carriers ranged between 40 to 56 kg/m^3; however, the densities for Kontakt carriers were lower ranging from 29 to 35 kg/m^3. The densities fall within literature values for both carriers at all three HRT (Hoehn & Ray, 1973; Tanyolaç & Beyenal, 1997).

At 3 hr and 4 hr HRT the K5 carriers' thicknesses showed no statistical significance with values of 214 μm and 222 μm, respectively; however, at a 12 hr HRT the thickness was significantly lower at 164 μm ($p<0.05$). Kontakt carriers showed the same trend as K5 carriers. Thicknesses at 3 hr and 4 hr were 351μm and 361μm, respectively, whereas at a 12 hr HRT the thickness was 244μm ($p<0.05$). Overall it can be seen that biofilm thickness increased with decreasing HRT. Interestingly, at lower HRTs K5 carriers had higher biofilm densities than Kontakt carriers, but at a 12 hr HRT the two carriers showed similar densities. K5 carriers developed larger biofilm thickness and higher density at lower HRT, whereas Kontakt carriers developed larger thickness, but lower density.

Although K5 and Kontakt carriers show the same trends for biofilm thickness, the thicknesses are very different at each HRT when compared to one another. Kontakt carriers have significantly higher thicknesses at each of the HRTs ($p<0.05$). The difference in thickness may be explained by the different shape and dimension of each of the carriers. The number of arms, as well as the lengths and corners of the arms, within the carriers will provide different surfaces for the biofilm to attach to and aid or hinder the biofilm growth when exposed to sheering forces (Ødegaard et al., 2000). The more compact the area and the larger the number of corners present in the carrier (as is seen in K5) seems to create a more dense biofilm resulting in a lower thickness, whereas larger more open areas with less corners (as is seen in Kontakt) create thicker, but less dense, biofilm. The structure of the K5 carriers may make biofilm migration easier due to the proximity of the numerous corners and spokes. The large gaps and lack of corners on the Kontakt carriers make it harder for the biofilm to migrate and spread out.

The measured biological masses of the K5 and Kontakt carriers show that at an HRT of 3 hr and 4 hr there is no statistical significance. At 3 hr the K5 and Kontakt carriers are 25.2 and

24.2 mg and at 4 hr they are 29.9 and 29.6 mg, respectively. However, there is a significant difference between the two at an HRT of 12 hr ($p<0.05$) with Kontakt carriers having an average mass of 21 mg and K5 carriers having an average mass of 16 mg, respectively.

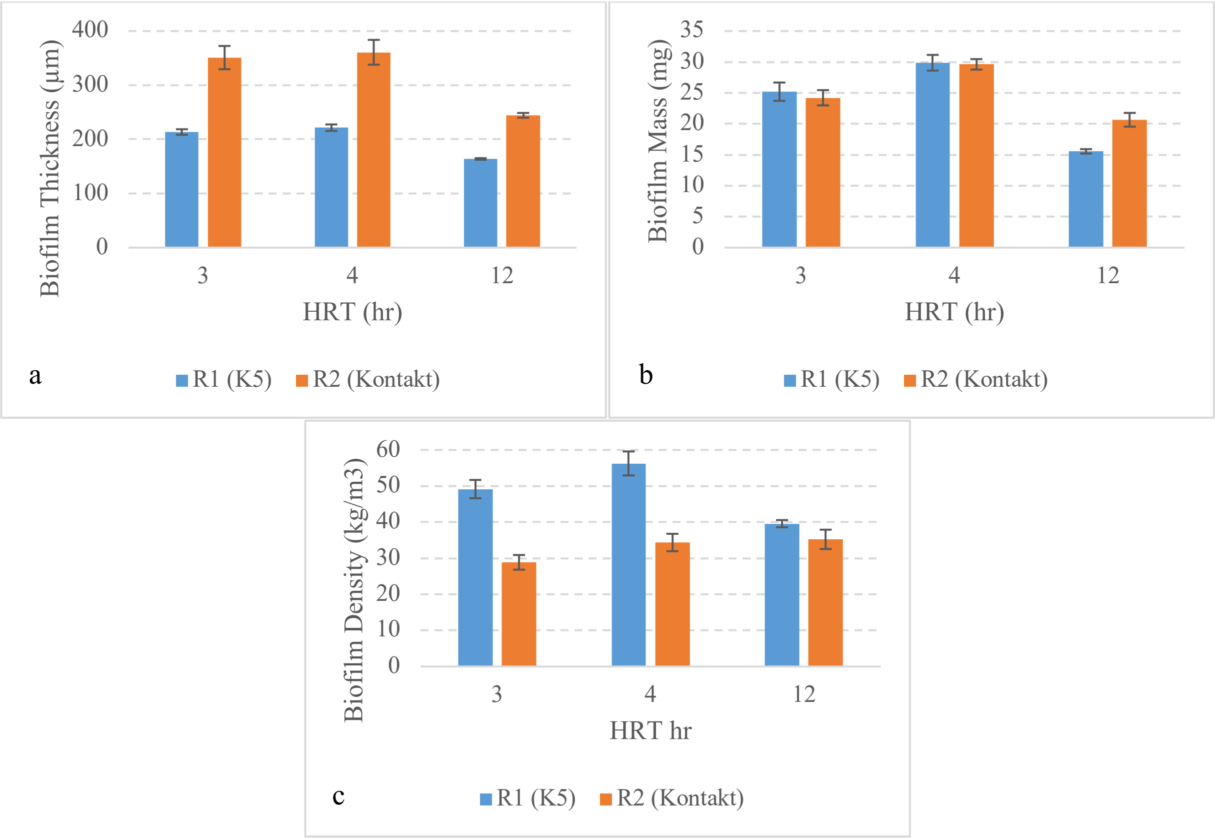

Figure 3.5: Biofilm thickness (a), mass (b) and density (c) for K5 and Kontakt carriers at an HRT of 3, 4 and 12 hr.

The total SG dry biomass and total AG dry biofilm mass were calculated for each reactor with each change in HRT (Table 3.6). Similarly to trial 1, there was more total biomass present in R1 and R2 in comparison to R3 due to the combination of SG and AG and as the carrier fill was decreased below 30% both K5 and Kontakt carriers had significant decreases in the total AG mass ($p<0.05$). Higher carrier fill, corresponding to lower HRT, resulted in an increase in total AG mass up to a 4 hr HRT; however, at an HRT of 3 hr, the AG mass decreased and the SG mass increased for both MBBR systems. Comparing a 12 hr HRT, where there is substrate limitation, to HRTs of 3 and 4 hr, where there is no substrate limitation, it was observed that thickness and mass per carrier increased at low HRT corresponding to the increase observed in total AG mass due to the lack of bacterial competition.

Table 3.6: Suspended and attached growth dry biomass (mg) in R1 (K5), R2 (Kontakt) and R3 (SG) at HRTs from 3-12 hr and an SALR of 40 g-sCOD/m^2/d.

	Reactor 3: SG Control	**Reactor 1: K5**			**Reactor 2: Kontakt**		
SALR (HRT) (g-sCOD/m^2/d)	Suspended Growth (mg)	Carrier Fill (%)	Suspended Growth (mg)	Attached Growth (mg)	Carrier Fill (%)	Suspended Growth (mg)	Attached Growth (mg)
40 (3 hr)	1973±152	48	1828±159	983±51	76	1825±143	1017±34
40 (4 hr)	1858±233	38	1640±160	1165±59	60	1451±144	1244±50
40 (12 hr)	1873±356	13	1420±169	607±16	20	1722±195	868±46

When HRT was manipulated, the SG mass had an increasing trend with lower HRT, with 3 hr and 12 hr being statistically different ($p<0.05$). The AG; however, saw a different trend by peaking at a 4 hr HRT. When the HRT was decreased from 12 hr to 4 hr the AG increased significantly ($p<0.05$); however, when the HRT was decreased from 4 hr to 3 hr the AG decreased significantly ($p<0.05$). At an HRT of 12 hr, R2 had significantly higher AG and SG when compared to R1 ($p<0.05$). However, when the AG mechanisms became more prominent, indicating that the SG was no longer the only factor in the removal of sCOD, at 3 and 4 hr HRT, both reactors had statistically similar amounts of AG ($p<0.05$). The AG was still significantly larger at 3 and 4 hr HRT than 12 hr HRT ($p<0.05$), suggesting that AG is stimulated at lower HRT, with 4 hr being the most optimal HRT at an SALR of 40 g-sCOD/m^2/d.

The percent of the viable and non-viable cells within the biofilm were quantified for both the K5 and Kontakt carriers at three different HRTs (Figure 3.6). The percentage of viable and non-viable cells for Kontakt carriers were very stable with changing HRT. The viable cell percentage was significantly greater than the non-viable cell percentage at each HRT ($p<0.05$), fluctuating between 67-68%, as opposed to the non-viable cells from 31-33%. Overall, it can be seen that the HRT did not have a significant impact on either the viable or non-viable cell fractions for Kontakt carriers. The viable and non-viable cell fractions were stable at a 3 hr and 4 hr HRT for K5 carriers with viable cell percentages of 72% and 64% and non-viable cell percentages of 29% and 36%, respectively. However, there was a difference in cell viability between 3 and 4 hr to a 12 hr HRT. K5 carriers saw a significant decrease of non-viable cells to 18% ($p<0.05$) and an increase in viable cells to 82% ($p<0.05$). However, overall both carriers had significantly more viable cells than non-viable cells ($p<0.05$).

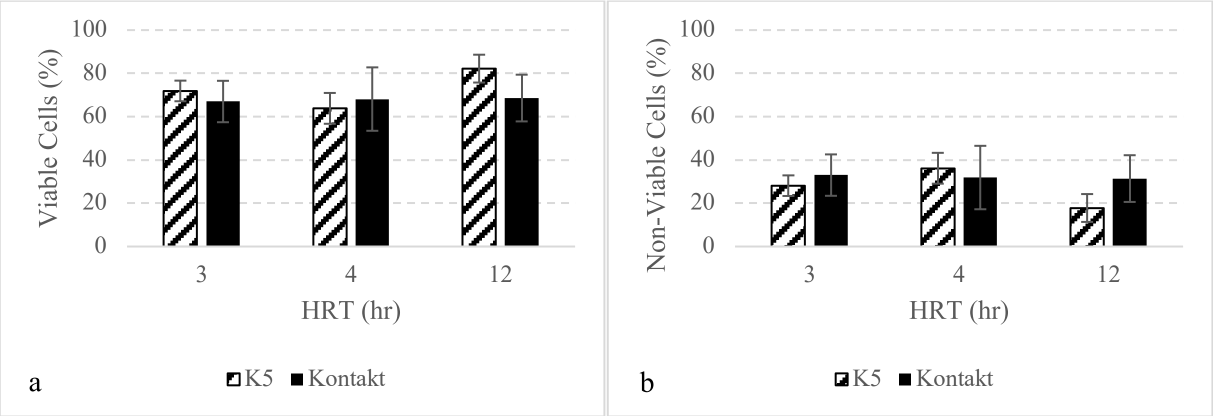

Figure 3.6: K5 and Kontakt carriers at HRTs of 3-12 hr, (a) biofilm embedded viable cells (%), (b) biofilm embedded non-viable cells (%).

At HRTs of 3 and 4 hrs there was no significant difference between the viable and non-viable cell percentages on the K5 and Kontakt carriers; viable cells ranged between 63-71% and non-viable cells ranged between 28-36%, respectively. However, there was a significant difference between the two carriers at an HRT of 12 hrs. Kontakt carriers developed higher percentages of non-viable cells and K5 carriers developed a higher percentage of viable cells ($p<0.05$).

Overall, the differences demonstrated by the K5 and Kontakt carrier biofilms with change in HRT were similar to the changes seen in the AG volumetric removal rates. The AG removal rates increased significantly from a 12 hr to 4 hr HRT and were statistically the same from a 4 hr to 3 hr HRT for both K5 and Kontakt carriers. The thickness, mass and total biomass for K5 and Kontakt carriers, as well as the density for the K5 carriers, showed similar trends as the AG removal rates. Despite the differences in thickness, mass, density and AG removal rates, the sCOD removal remained statistically the same for R1 and R2 at each HRT. At 3 and 4 hr, when AG became significant for the sCOD removal, both carriers had appropriate thickness, mass and density to achieve the same removal.

3.6 CONCLUSION

In the SALR trial, the impact of increasing SALR on AG and SG removal rates and biomass growth and viability was studied. The thickness, mass and density of the K5 and Kontakt carriers both showed the same trends: larger masses and thicker biofilm at higher SALR and larger densities at lower SALR. The cell viability between each carrier was also similar: viability decreased with increasing SALR. The attributed AG removal rates ultimately decreased to zero with increasing SALR and did not have a direct correlation to the differences in thickness, mass, density or cell viability of either the K5 or Kontakt carriers at any of the SALRs. All three reactors had statistically similar total volumetric removal rates and over 92% sCOD removal for all experimental runs, demonstrating that the AG was not a significant factor in the sCOD removal. Increase in SALR was found to have no impact on the sCOD removal due to substrate limited conditions. The results of this study suggest that increasing SALR of brewery MBBR systems effects the characteristics of the biofilm and has the potential to modify the performance of systems that are not underloaded.

During the HRT trial, the impact of decreasing HRT on AG and SG removal rates and biomass growth and viability was studied. The thickness and density varied between the two carriers: Kontakt carriers developed thicker, less dense biofilm, whereas K5 carriers developed thinner, more dense biofilm at low HRT. However, both carriers showed the same mass per carrier with the highest mass observed at a 4 hr HRT. The total volumetric removal rates in R1 and R2 were significantly higher than R3 at HRTs of 3 and 4 hr and resulted in significantly higher sCOD removal efficiencies. The AG removal rates increased significantly from a 12 hr to 4 hr HRT and were statistically the same from a 4 hr to 3 hr HRT for both K5 and Kontakt carriers. The thickness, mass and total biomass for K5 and Kontakt carriers, as well as the density for the K5 carriers, showed similar trends as the AG removal rates. The cell viability was stable for Kontakt carriers showing no statistical differences at each HRT; however, K5 carriers had significantly larger cell viability at an HRT of 12 hr. Throughout this trial there was more total biomass found in R1 and R2 than R3 due to the AG on the carriers. MLSS concentrations were not impacted with similar concentrations in all three reactors at 3, 4 and 12 hrs. Overall, the AG had an observed effect on treatment at lower HRT.

Ultimately, the characteristics of the biofilm were significantly altered with varying SALR and HRT. It appears that under conditions of substrate limitation the biofilm mass and thickness per carrier were constrained at low SALR due to the higher amount of total AG mass in the reactors; however, while under non-limiting conditions (HRT 3-4 hr) it was observed that both biofilm thickness and mass per carrier and total AG mass increased. Despite the differences in biofilm morphology and viability, both MBBR reactors demonstrated an equal aptitude for sCOD removal. Both carriers demonstrated that carrier fills of 30% or higher provided the best conditions for the development of AG. The optimal conditions in terms of total AG mass and AG removal rates were at a 4 hr HRT and SALR of 40 g-sCOD/m^2/d.

3.7 SUPPLEMENTAL MATERIAL

Two types of carriers were utilized in the study to evaluate the impact of carrier surface area and dimensions on treatment effectiveness and biofilm morphology: Kaldness K5 and Kontakt (Figure S3.1). K5 carriers have a specific surface area of 800 m^2/m^3 and Kontakt carriers have a specific surface area of 500 m^2/m^3. K5 carriers have a diameter of 25mm and a depth of 3.5mm. Kontakt carriers have a diameter of 20mm and a depth of 10mm.

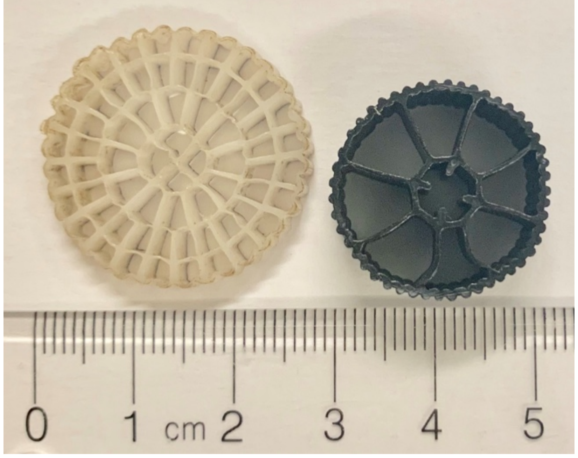

Figure S3.1: Example of a clean Kaldnes K5 carrier (left) and a Kontakt carrier (right).

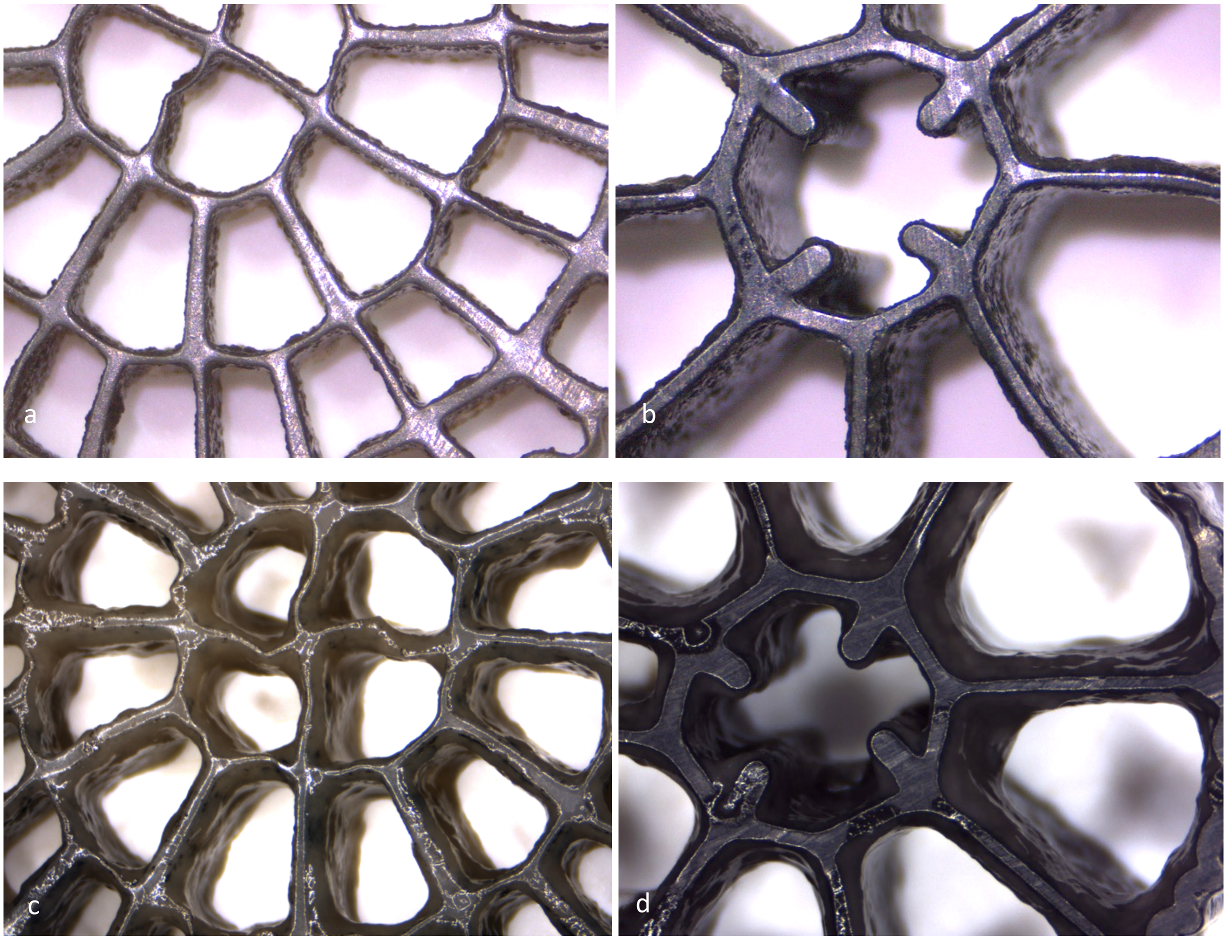

Figure S3.2: Stereoscopic images of K5 (a,c) and Kontakt carriers (b,d) at 12 hr and 3-4 hr HRTs under a SALR of 40 g-sCOD/m^2/d and excess DO conditions. At HRTs of 3 and 4 hrs there was no significant differences seen in the images taken, so only one set of images from those trials is shown.

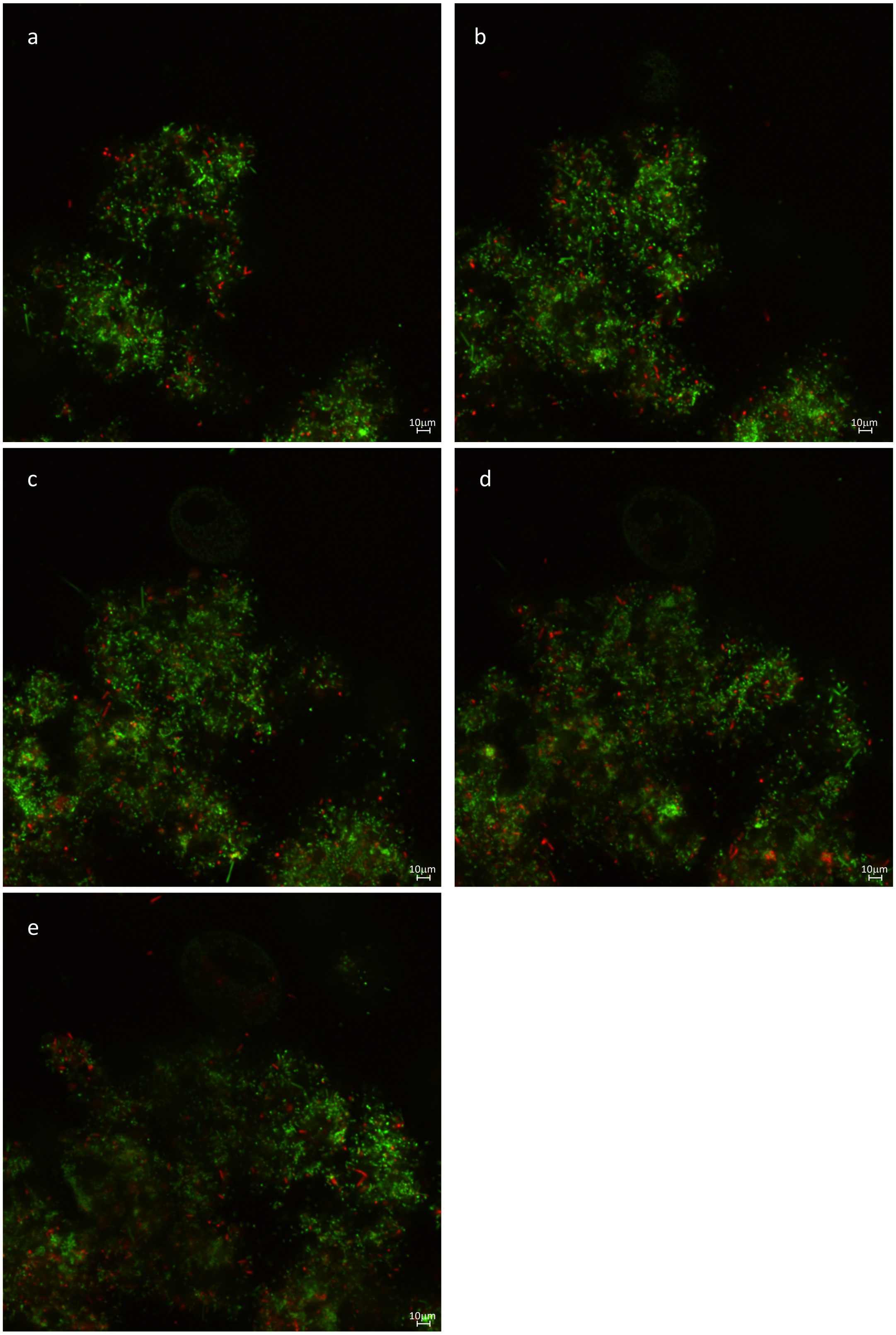

Figure S3.3: CLSM stack images of heterotrophic biofilm on a K5 carrier, live cells are illuminated green and dead cells are illuminated red. Image a is the biofilm farthest from the *carrier surface, image b is at a depth of 5μm, image c is at a depth of 10μm, image d is at a depth of 15μm and image e is the biofilm layer closest to the carrier.*

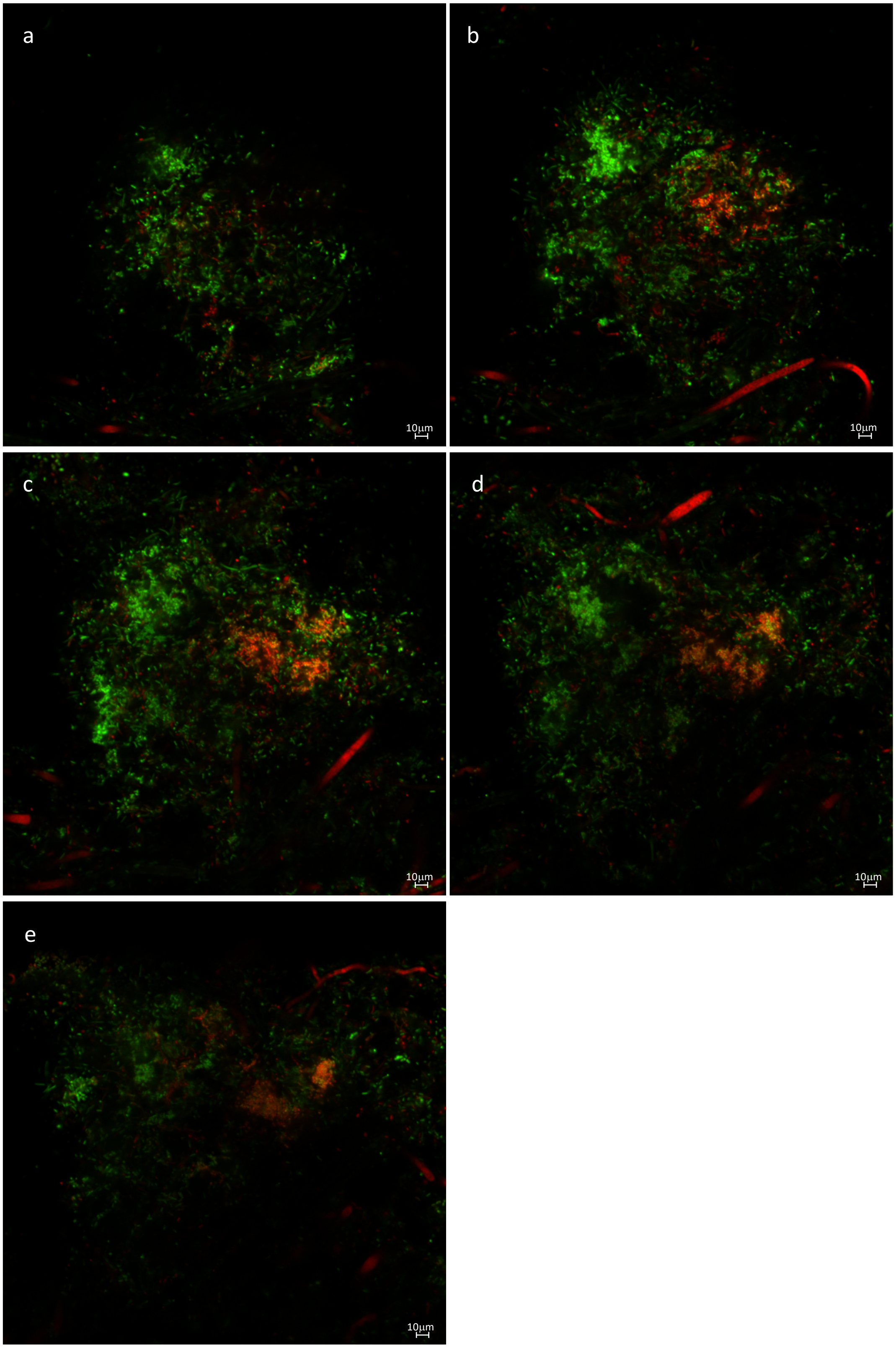

Figure S3.4: CLSM stack images of heterotrophic biofilm on a Kontakt carrier, live cells are illuminated green and dead cells are illuminated red. Image a is the biofilm farthest from the *carrier surface, image b is at a depth of 5μm, image c is at a depth of 10μm, image d is at a depth of 15μm and image e is the biofilm layer closest to the carrier.*

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CHAPTER 4: SYNTHESIS AND CONCLUSION

Moving bed biofilm reactor (MBBR) system kinetics, attached growth (AG) and suspended growth (SG), biologically produced solids, two different carriers (Kaldnes K5 and Kontakt), and subsequent biofilm thickness, mass, density and embedded cell viability were investigated in response to changing surface area loading rates (SALR) (10-55 g-sCOD/m^2/d) and hydraulic retention times (HRT) (3-12 hr) when treating brewery wastewater during the course of this research. Multiple conclusions were drawn from this work and are listed as follows:

- At a 12 hr HRT, change in SALR from 10-55 g-sCOD/m^2/d had no effect on the total volumetric removal rates for both MBBR systems, as well as the SG control reactor, indicating a dependency on SG as well as substrate limited conditions.
- At a 12 hr HRT, AG removal rates decreased to zero at a SALR of 55 g-sCOD/m^2/d, while AG activity was attributed to MBBR systems at SALRs of 10-40 g-sCOD/m^2/d, as determined by lower MLSS concentrations in the MBBR systems compared with the SG control. The reduction in MLSS with the same total volumetric removal rates would result in the same performance, but reduced sludge management costs for the MBBR systems at the full-scale.
- At a 12 hr HRT, sCOD removal percentages of 93-96% were maintained at each SALR for each of the three reactors, indicating substrate limited conditions and a high level of biodegradability of the brewery wastewater.
- At HRTs of 3 and 4 hr and a SALR of 40 g-sCOD/m^2/d, the total volumetric removal rates for the MBBR systems were significantly higher than the SG control, indicating a shift from SG to AG dependency. AG removal rates were highest in both MBBR systems at a 3 and 4 hr HRT and SALR of 40 g-sCOD/m^2/d.

- Both MBBR systems showed an increasing trend in MLSS concentrations with increasing SALR. MLSS concentrations in MBBR R1 (K5) showed an increasing trend with decreasing HRT; however, there was no trend obserevd for MBBR R2 (Kontakt) with decreasing HRT.
- There were no significant differences in total volumetric removal rate by carrier type with change in SALR and HRT.
- At HRTs of 3 and 4 hr and a SALR of 40 g-sCOD/m^2/d, the MBBR systems had significantly higher sCOD removal efficiencies than the SG control. At a 4 hr HRT, the MBBR systems had 95% sCOD removal, whereas the SG control had 88% sCOD removal. At a 3 hr HRT, the MBBR systems had 73% sCOD removal, whereas the SG control had 61% sCOD removal.
- Full-scale implementation validated the results found at the lab-scale in that the total volumetric removal rates and MLSS concentrations were the same for both the lab and full-scale MBBR reactors when operated at a 12 hr HRT and SALR of 10 g-sCOD/m^2/d.
- The thickness, mass and density per carrier of both K5 and Kontakt carriers demonstrated the same trends at a 12 hr HRT and SALRs of 10-55 g-sCOD/m^2/d: thickness and mass increased and density decreased with increasing SALR.
- Comparing the total AG mass in the reactors (which increased at low SALR) to the thickness, mass and density per carrier data at a 12 hr HRT it suggests that at low SALR and under conditions of substrate limitation it was possible that biofilm development was limited due to bacterial competition; thus, explaining the observed trend of lower biofilm thickness and mass per carrier.
- Both carriers showed the same mass per carrier from 3-12 hr HRT at an SALR of 40 g-sCOD/m^2/d with the highest mass observed at a 4 hr HRT. However, the thickness and

density of the two carriers differed at HRTs of 3 and 4 hr. K5 carriers demonstrated thinner, but more dense biofilm, whereas Kontakt carriers demonstrated thicker, but less dense biofilms.

- Comparing a 12 hr HRT, where there is substrate limitation, to HRTs of 3 and 4 hr, where there is no substrate limitation, at an SALR of 40 g-sCOD/m^2/d it was observed that thickness and mass per carrier increased at low HRT corresponding to the increase observed in total AG mass due to the lack of bacterial competition.
- At a carrier percent fill of less than 30%, the total amount of AG within the reactors decreased significantly for both MBBR systems, indicating that optimal AG performance would likely be at a carrier percent fill of 30% or higher for MBBR technology treating brewery wastewater under similar conditions.
- The viability of the biomass embedded in the biofilm for both carriers resulted in similar trends: viability decreased with increasing SALR and viability remained constant with decreasing HRT.

Overall, the microscale characteristics (biofilm) were difficult to correlate to the performance at the macroscale (kinetics). The SG dominance throughout the experiment impeded the ability to accurately determine the true contribution of the AG in the reactors. Further research into MBBR technology treating brewery wastewater will need to be undergone to isolate the reason for the surplus of SG present in an otherwise AG dependent application. Varying the influent concentration and investigating the bacterial taxa in the SG and AG biomass could be the next steps to further understanding the mechanisms at work for MBBR technology treating brewery wastewater.

CHAPTER 5: FULL-SCALE RECOMMENDATIONS

There are three fundamental recommendations that can be made concerning the full-scale moving bed biofilm reactor (MBBR) on-site wastewater treatment system at Beau's All Natural Brewing Company:

I. The full-scale MBBR system is operating at a 12 hr HRT, which is not optimal for attached growth (AG); the system is currently suspended growth (SG) dominated. If the hydraulic retention time (HRT) was lowered to 4 hr, Beau's All Natural Brewing Company could triple their operational capacity, while also stimulating AG.

II. Despite AG stimulation, there will always be SG present in the MBBR systems, thus sludge management will also be a necessity for MBBR systems treating brewery effluent.

III. Accurate design and operation are critical to the performance of the system. MBBR systems treating brewery effluent require constant monitoring to ensure that dissolved oxygen (DO), pH and temperature are all within acceptable ranges. It is also essential that volume equalization tanks are sized correctly to maintain a consistent flow through the system. Effluent changes due to batch production, clean in place (CIP) procedures and downtime at the brewery will not cause adverse effects to the MBBR system if there is an appropriately sized equalization tank.