Influence of carrier surface area and surface area loading rate on moving bed biofilm reactor performance in COD removal from municipal and industrial wastewater

by

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To my family and friends:

Thank you for your support.

ممنون که همیشه حامی من بودید.

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Abstract

Moving bed biofilm reactor (MBBR) is a biological process providing wastewater treatment. Compared to conventional activated sludge (CAS) systems, MBBR includes remarkable advantages such as handling higher volumetric loading rates while employing smaller reactors, including more biomass concentration, lower hydraulic retention time (HRT), and higher solids retention time (SRT) without recycling requirements. Nowadays, a large number of plants are using MBBRs to provide an efficient Wastewater treatment plant (WWTP). However, the number of studies on substrate removal, especially at high loading rates, from municipal and industrial wastewater using these technologies is limited compared to the other conventional systems.

Therefore, this study was conducted to evaluate the organic matter removal performance of aerobic MBBRs, containing biocarriers with various surface areas, operating at different surface area loading rates (SALRs). To fulfill this goal, the experimental research was defined in two phases: 1) assessing the MBBRs' performance in treating low-strength, real municipal wastewater; 2) investigating the MBBRs' efficiency in treating very high-strength, synthetic industrial wastewater. In both phases, the performance of MBBRs containing different types of media and working under various SALRs was evaluated and compared. The second phase also included assessing the recovery time and reactors' efficiency by decreasing the SALR after an almost complete media clogging. The optimal operational parameters were found for each phase, and it was confirmed that the MBBRs are able to handle SALRs as high as 18 g BOD $m^{-2}d^{-1}$ in treating municipal wastewater with >70% sCOD removal and SALRs as high as 28 g BOD $m^{-2}d^{-1}$ in treating synthetic industrial wastewater with ~87% sCOD removal.

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Nomenclature

Symbol	Description	Unit
AMBBR	Anaerobic Moving Bed Biofilm Reactor	
AOB	Ammonium Oxidizing Bacteria	
bCOD	Biodegradable Chemical Oxygen Demand	$g\ m^{-3}$
BNR	Biological Nutrient Removal	
BOD	Biological Oxygen Demand	$g\ m^{-3}$
CAS	Conventional Activated Sludge	
CBOD	Carbonaceous Biochemical Oxygen Demand	$g\ m^{-3}$
COD	Chemical Oxygen Demand	$g\ m^{-3}$
DAF	Dissolved Air Floatation	
DO	Dissolved Oxygen	$mg\ L^{-1}$
EPS	Extracellular Polymeric Substance	
FF	Filling Fraction	%
HRT	Hydraulic Retention Time	d
LoT	Limit of Treatment	$g\ m^{-3}$
MBBR	Moving Bed Biofilm Reactor	
MLSS	Mixed Liquor Suspended Solids	$mg\ L^{-1}$
NOB	Nitrite Oxidizing Bacteria	
PE	Primary Effluent	
R	Reactor	
RAS	Return Activated Sludge	
SALR	Surface Area Loading Rate	$g\ m^{-2}d^{-1}$
sBOD	Soluble Biological Oxygen Demand	$g\ m^{-3}$
sCOD	Soluble Chemical Oxygen Demand	$g\ m^{-3}$
SRR	Surface Removal Rate	$g\ m^{-2}d^{-1}$

Symbol	Description	Unit
SRT	Solids Retention Time	d
SS	Suspended Solids	mg L^{-1}
TCOD	Total Chemical Oxygen Demand	g m^{-3}
TS	Total Solids	mg L^{-1}
TSS	Total Suspended Solids	mg L^{-1}
VLR	Volumetric Loading Rate	g $m^{-3}d^{-1}$
VS	Volatile Solids	mg L^{-1}
VSS	Volatile Suspended Solids	mg L^{-1}
WAS	Waste Activated Sludge	
WEWPCC	West End Water Pollution Control Center	
WWTP	Wastewater Treatment Plant	

Chapter 1: Introduction

1.1. Problem Description

The vast increase in population has led global urbanization to grow at a very high speed. It has been predicted that more than 6.5 billion people would occupy the world's urban areas by 2050, which would be about 68 percent of the total world's population. Globally, more people live in urban areas than in rural areas; however, Northern America is the most urbanized geographic region, with 82% living in urban areas in 2018 [1]. The continuous urbanization of the world accompanies by industrialization, which provides severe environmental problems, including water scarcity and contamination. Therefore, sufficient municipal and industrial wastewater management and advanced treatment technologies must preserve the water quality.

Enrichment of organic matter and nutrients (i.e. Phosphorus and Nitrogen) in water bodies, as a result of wastewater discharge, is one of the critical factors affecting water quality and harming the environment by causing toxicity, oxygen consumption, eutrophication, and finally, death of many aquatic species [2], [3]. It is, therefore, necessary to implement sustainable treatment processes to control these hazardous contents before returning the water to the source.

The overall goal of each Wastewater Treatment Plant (WWTP) is to minimize the concentration of the contaminants in its effluent to not only meet the regional restrictions on wastewater discharge but also reach the Limit of Treatment (LoT) [4]. Yet, plenty of other aspects are being considered along with this main target, which all together introduces an efficient WWTP. Treating the wastewater with minimal capital costs, having the least possible sludge production, which reduces the need for sludge treatment, improving the plant's capacity as well as compacting the facilities, and increasing the reaction rates are such examples.

The methods for removing constituents from wastewater fall into three categories: Physical means, including screening, sedimentation, adsorption, flocculation, flotation, and filtration; Chemical means, such as adsorption, gas transfer, precipitation, and disinfection; and Biological means, by which the biodegradable organic substances, as well as nutrients, can be removed by aerobic, anaerobic, and anoxic modes. Although treatment sets may include a variety of combinations of these systems, it is necessary to study the basics of each part to optimize the system.

Biological treatment is an approved method that can be used for organic matter and nutrient removal under proper environmental control. Currently, biological processes based upon suspended biomass, Conventional Activated Sludge (CAS) systems, are being employed by many WWTPs. Although these systems can reach adequate effluent quality, especially when combined with Biological Nutrient Removal (BNR) facilities (i.e. additional anaerobic and anoxic sections), they still need to be upgraded to provide an efficient WWTP [5]. The most highlighted challenges associated with these systems are sludge settleability difficulties, biomass recycling requirement, the need for large reactors and settling tanks, and the necessity of chemical addition and filtration facilities in regions with lower LoT [6]–[9].

Nowadays, a large number of plants are using relatively new technologies such as Moving Bed Biofilm Reactors (MBBRs) to address the mentioned challenges and provide an efficient WWTP. However, the number of organic and nutrient removal studies from municipal and industrial wastewater using these new technologies is limited compared to the other conventional systems [10]. Therefore, more research over the efficiency of these processes, the associated costs, the amount of sludge production, and their ability to increase the plant capacity are still sought and required by municipalities.

1.2. Aims and Objectives

The present work proposes to evaluate the efficiency of a biological wastewater treatment system, MBBR, in organic matter removal from actual municipal wastewater with a moderate concentration of organics (i.e. ~350 mg COD L^{-1}); as well as synthetic industrial wastewater as very strong and concentrated wastewater (i.e. ~10000 mg COD L^{-1}). The optimization of the system in each situation is the goal of this thesis. The functionality of BioPorts media with different surface areas under various Surface Area Loading Rates (SALRs) is analyzed to find both optimum media surface area and SALR in order to simulate the desired future conditions in using MBBR. The objectives of both research phases (i.e., municipal and industrial wastewater treatments) are as follows:

- Investigating the optimum SALR which causes maximum COD removal;
- Generation of correlation curves describing the percentage of substrate removal vs. surface area loading rate for the municipal wastewater treatment phase, where three SALRs were investigated;
- Evaluation of COD removal dependency on biocarriers shape and specific surface area at different loading rates;
- Investigating the most efficient media type (in case of the surface area) in terms of both COD removal and maintaining a thin biofilm;
- Assessing the amount of solid production at each operational condition;
- Finding the most active attached biofilm with the lowest mass transfer limitation;
- Studying the kinetics of the COD removal by the attached biomass to investigate the role of suspended biomass in COD removal.

In addition to the mentioned points, the following specific objectives were also considered for the second research phase (i.e., industrial wastewater treatment):

- Assessing the carriers resistance against getting clogged at the SALR beyond the system's limit and the possibility of applying intermittent high loads;
- Studying the recovery time of clogged carriers and their biofilm performance in COD removal after recovery.

1.3. Scope and Overview

A literature review and background information about the biological wastewater treatment, biofilm, and MBBR processes are presented in Chapter 2. Detailed knowledge about biocarriers function in the MBBR process as well as system design and operational parameters are also provided in this chapter.

To achieve the objectives, the experimental studies have been done in two phases: 1) The research project started with organic matter removal from municipal wastewater, presented in Chapter 3. The carbon removal performances of biofilm formed on different carriers under different SALRs were compared; 2) During the next phase, discussed in Chapter 4, the carbon removal from synthetic industrial wastewater by MBBR system under the same conditions as the previous phase was investigated.

Chapter 2: Moving Bed Biofilm Reactor (MBBR)

2.1. Biological Wastewater Treatment

The components present in wastewater are removed by physical, chemical, and biological means in a variety of combinations known as primary, secondary, tertiary, and advanced treatment. In preliminary and primary treatment, Physical unit processes such as screening, sedimentation, floatation, filtration, and adsorption are used to remove constituents such as rags, grit, and suspended solids (SS) from wastewater. Chemical and biological processes are being applied as secondary treatment units for disinfection and removing SS, biodegradable organic matter, and nutrients. Further, a combination of all three (i.e., physical, chemical, and biological processes) introduces a tertiary treatment that is beneficial for removing residual SS and disinfection. Nutrient removal is often considered a part of this definition. And finally, advanced treatment completes the removal of dissolved and suspended materials that remained after previous steps [4]. This should be noted that the mentioned terms and classifications are arbitrary and may vary in different references.

Biological units are mainly applied for Biological Oxygen Demand (BOD) and nutrient (i.e., nitrogen and phosphorous) removal as well as water stabilization. During the biological treatment, microorganisms consume and transform particulate and soluble biodegradable pollutants to either innocuous form, such as gases that escape to the atmosphere, or new microbial biomass [11]. Additionally, colloidal solids are captured into a biological floc or biofilm and can be removed by solid separation processes [4]. This method is widely being used for municipal, agricultural, and industrial wastewater treatment due to its relatively low energy and chemical matter requirements that make it a cost-effective process. Besides, ease of handling and

comparatively less harmful impacts on the corresponding environment have made this method become a suitable choice [12].

Based on biomass structure, biological wastewater treatment processes can be divided into two primary configurations: suspended growth and attached growth (biofilm) systems. In suspended growth processes, continuous mixing keeps microorganisms in liquid suspension. These processes are being used for the treatment of both municipal and industrial wastewater for organic substances and ammonia biodegradation in the presence of oxygen (aerobic), nitrate/nitrite utilization (anoxic), and phosphorous removal in the absence of oxygen (anaerobic). Activated sludge processes, aerated lagoons, aerobic digestion, membrane bioreactors, suspended-growth denitrification, anaerobic contact processes, and anaerobic digestion are major suspended growth biological treatment practices used for carbonaceous matter removal, nitrification, stabilization, and denitrification [4].

Currently, biological processes based upon suspended biomass, a sample depicted in Figure 1, are being employed by a significant number of municipal WWTPs. Nevertheless, the challenges associated with these systems, such as sludge settleability difficulties, biomass recycling requirement, the need for large reactors and settling tanks, and the necessity of chemical addition and filtration facilities in regions with lower LoT, have led to the increased interests in substitutional processes with minimal costs and environmental impacts [6]–[9]. To overcome the mentioned disadvantages, the application of fixed biofilm systems to remove organic and inorganic matters from wastewater is becoming more popular.

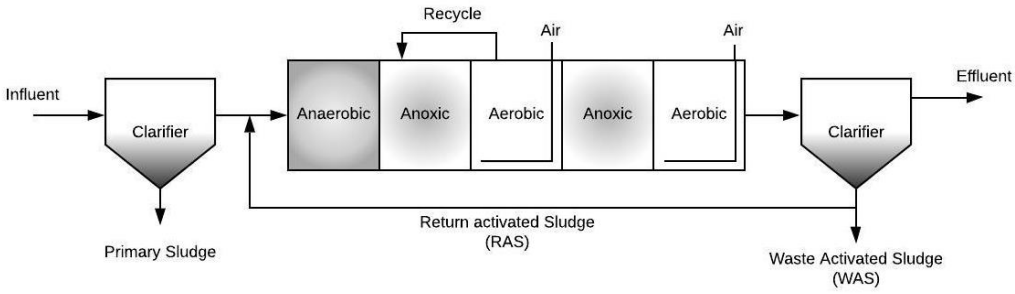


Figure 1 – Suspended growth system for organic matter and nutrient removal [4].

2.2. Biofilm and Attached Growth Systems

The term "biofilm" was described in 1978 for the first time by Costerton et al. [13]. In the early 1980s, however, the advantages of biofilm processes became a focus of interest for a considerable number of researchers due to their potential advantages over the suspended growth systems [14].

Fixed biofilms are complex clusters of microbial cells attached to a solid surface, which provides a large surface area per unit volume for biofilm development. It has been illustrated that the attachment provides protection and resistance against some environmental dangers and stressing conditions such as predation, toxicants, dehydration, and lack of nutrients [15]. Rock, stone, sand, soil, wood, and plastic are examples of support mediums for biofilm growth.

For the biofilm formation, first, planktonic microorganisms adhere to the carrier surface. At the early stage, the possibility of detaching is high due to the loose connection. However, a tight connection (i.e., hydrogen bonding) between the living species and the support surface is gradually produced due to bacterial self-generated extracellular polymeric substances (EPSs). EPSs are a mixture of polysaccharides, proteins, and extracellular DNA produced by microbes [16], [17]. Then, due to the replication of the attached bacteria, a monolayer of microcolonies is formed on the surface, and biofilm maturation into 3D arrangement happens. These phenomena cause the

overall density and complexity of the biofilm to increase [12]. The availability of organic matter and nutrients in the environment, carbon source, oxygen perfusion, and internal pH are environmental factors that limit the growth potential of bacterial species and control biofilm maturation [18]. Releasement of the biomass into the liquid, detachment, is a natural mechanism that happens with the bacteria decay. Also, hydrodynamic shear forces and mentioned environmental conditions substantially impact the detachment process, limiting biofilm accumulation and thickness [19].

Figure 2 shows the process of adhesion and biofilm formation of a sample bacteria (P. aeruginosa ATCC 27853) and the progressive production of EPS.

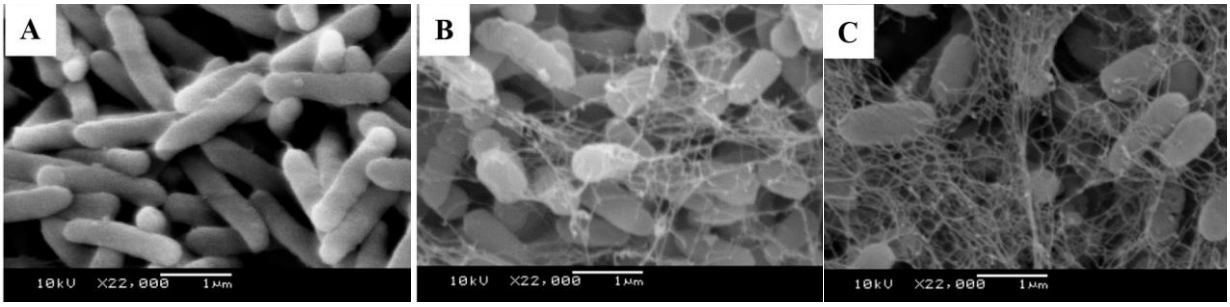


Figure 2 - Scanning electron microscopy images of adhesion and biofilm formation of P. aeruginosa at 37° C with a magnification of 22,000X. A, B, and C represent the incubation times of 6, 24, and 48 hours, respectively [20].

As the substrates (i.e., carbon, nitrogen, phosphorous, and oxygen) have to pass the biofilm-liquid interface and penetrate or diffuse through the formed microbial population to be consumed, a concentration gradient is formed within the aggregate, which leads to a biofilm with a layered structure [21]. The diffusion limiter factors such as thickness, density, and porosity of the biofilm, substrate concentration in the bulk liquid, mass transfer and reaction rate are parameters that determine the penetration depth of substrates [21]–[23]. The diffusion-limited

concept is fundamental when considering Dissolved Oxygen (DO) diffusion since it determines the biofilm layers properties. The oxygen penetration depth has been illustrated to be shallow (100-150 µm) [24]. Therefore, the microorganisms which consume oxygen will be placed at the outer layer of the biofilm and forms an aerobic zone. These organisms, including aerobic heterotrophic and autotrophic bacteria, are responsible for carbon and ammonia oxidation, respectively. As a result of nitrification occurs in this layer, the anoxic zone where there is oxygen in the forms of nitrite (NO_2^-) and nitrate (NO_3^-), but no free oxygen (O_2) will be formed in the next layer. Denitrifying bacteria are present in this zone and convert the nitrite/nitrate to nitrogen gas. The most interior layer (i.e., attached to the surface), thus, is the anaerobic zone where there is no oxygen and includes bacteria responsible for phosphorous removal. A schematic of a multilayer biofilm is shown in Figure 3.

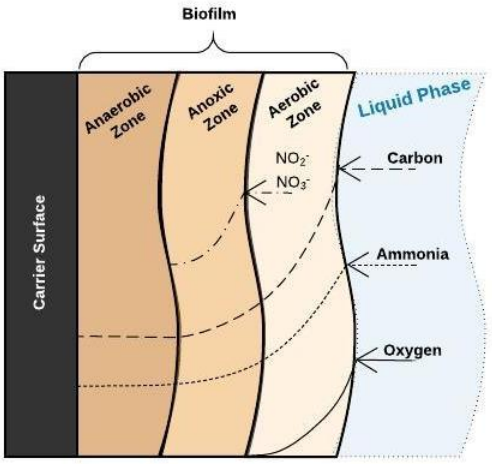


Figure 3 - A steady-state biofilm and mass transfer model

The performance of the biofilm depends on the protected surface of the carriers (i.e., the internal surface area of the media to which microorganisms can attach and do not be intercepted by the high shear force or the friction), biofilm thickness, biomass density, and porosity due to the diffusion limitations [22], [25]. Therefore, mechanical and environmental conditions, such as type

of biocarrier and carrier filling fraction (FF), SALR, hydraulic retention time (HRT), pH, temperature, DO level, chemical exposure, and hydrodynamic shear forces, affect the biofilm quantity, thickness, density and performance at the steady-state condition [12], [17], [26].

Applying attached growth set in wastewater treatment introduces some privileges, such as lower reactor volume demand, higher active biomass concentration, handling extensive volumetric loading, more flexibility with the environmental changes, lower HRT, higher biomass retention time, and lower sludge production without the need for solid recycling, when comparing to suspended growth systems [12], [27], [28].

Biological aerated filters, packed-bed reactors, rotating biological contactors, trickling filters, moving bed bioreactors, anaerobic packed, and fluidized bed are major attached growth treatment processes used for wastewater treatment [4].

2.3. Introducing the MBBR

2.3.1. Background

Moving Bed Biofilm Reactor (MBBR) is an attached growth system based on the formation of a biofilm on plastic biocarriers with low density and large surface area that moves freely in the biological reactor. It was developed in Norway in 1989 by professor Ødegaard and colleagues at the Norwegian University of Science and Technology. The idea was formed due to the demand for small sewage treatment plants with large capacity in Norway and the motivation to upgrade the existing plants. The basic concept of MBBR was to have a system that operates continuously with no clogging, no requirement for backwashing, low head loss, and a high specific biofilm surface area [29].

The first utilization of this new system was in two existing treatment plants in 1992 [30]. Presently, this technology is being employed in more than 1200 wastewater treatment plants in at least 50 countries [31]. This technology has been successfully used for municipal and various types of industrial wastewater, including pulp and paper industry wastewater [32], phenolic sewage [33], pharmaceutical wastewater [34], poultry processing wastewater [35], and commercial laundry wastewater [36].

Flow schematic of examples of MBBR process configurations for BOD alone and BOD plus nitrogen removal are given in Figure 4.

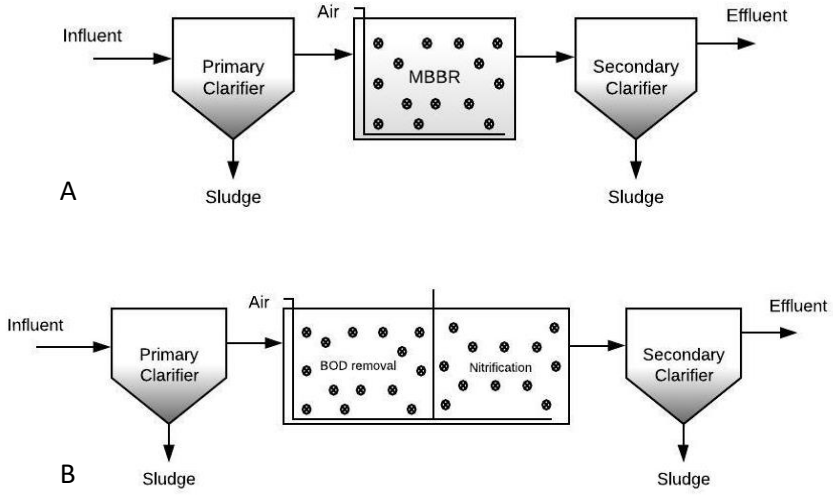


Figure 4 - MBBR system configurations for: A) BOD removal; and B) BOD + Nitrogen removal [4].

Although many plants worldwide use MBBR to address the common challenges associated with conventional systems, the number of studies on organic and nutrient removal from both municipal and industrial wastewater using this system is limited compared to the other methods [10]. Therefore, more research over the efficiency of these processes, the associated costs, the amount of sludge production, and their ability to increase the plant capacity are still sought and required by municipalities.

2.3.2. Biocarriers

The first biocarriers used in MBBR systems were the K-series AnoxKaldnes™, with a density of 0.95 g cm^{-3} and polyethylene as material [10]. Nowadays, several different biocarriers, such as K-series, Biofilm Chips, and Z-biocarriers, with different shapes, densities, and protected surface areas are being used depending on the process (i.e., aerobic, anoxic, or anaerobic). Carrier shape is a factor that affects the carrier's strength, shearing, colliding, and friction condition, as well as the chance of clogging. The density determines the suspension criteria as it usually is lower than water density, at around 0.98 kg L^{-1}, with the aim of energy consumption reduction for mixing even after biofilm attachment. The carriers' protected surface area, typically used in full-scale WWTPs, is in the range of 500 to 1000 m^2 m^{-3}; however, the larger the carrier protected surface area, the more complex carrier structure and the higher production costs [37].

Table 1 presents the main characteristics of some available carrier models in the market as well as three media types utilized in this study, and Figure 5 shows some of the carrier elements described in Table 1.

Table 1 - Characteristics of some commercially available Kaldnes® carriers [10], [38], *and BioPorts™ carriers used in this study.*

	Kaldnes®										**BioPorts™**			
	K-series				**Natrix**		**Biofilm Chip**		**Z-series**					
	K1	**K2**	**K3**	**K5**	**C2**	**F3**	**M**	**P**	**Z-200**	**Z-400**	**600-14**	**800-07**	**900-09**	**Unit**
Nominal diameter	9.1	15	25	25	36	46	48	45	30	30	18.5	17.1	17.1	mm
Nominal height	7.2	15	10	3.5	30	37	2.2	3	-	-	14	7	9	mm
Density	0.95	0.95	0.95	-	-	-	0.96	0.96	0.95	0.95	0.96	0.96	0.96	kg m^{-3}
Protected surface area	500	350	500	800	220	200	1200	900	-	-	589	851	942	m^2 m^{-3}

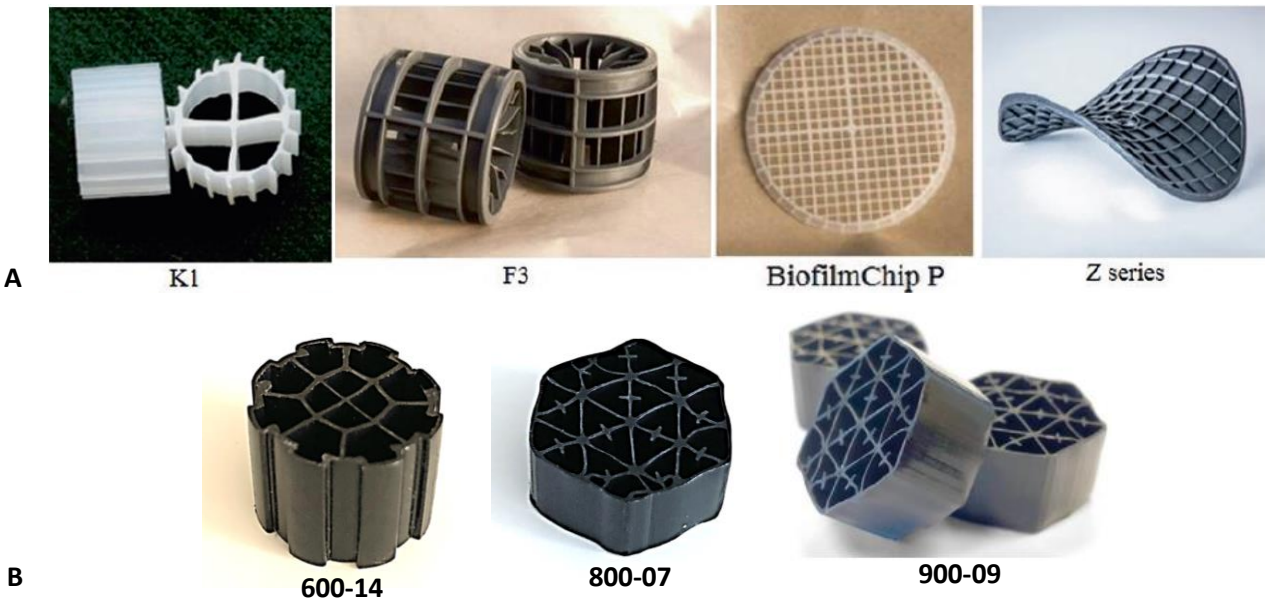


Figure 5 – A) Commercially available Kaldnes® carriers for the MBBR process [10]; B) BioPorts™ carriers used in this study.

The main goal of using biocarriers is to provide a large protected surface area per unit of carriers' volume for the biofilm adhesion to have the best treatment performance. However, other than protected surface area, various carrier characteristics are needed for different processes to have the best efficiency. For example, it has been mentioned that as the aerobic heterotrophic bacteria grows very fast, biocarriers with wider openings perform better for their growth due to avoiding loss of effective surface area caused by clogging. Smaller openings and larger surface areas, on the other hand, are beneficial for the slow-growing autotrophic bacteria [39].

One of the important considerations while using the supports is the number of carriers added to the reactor, defined as the Filling Ratio or Filling Fraction (FF). FF is the ratio of the volume occupied by carriers over the total volume of the reactor (i.e., V_{bc}/V_R). Indeed, this factor inures a sufficient amount of biofilm in the reactor, cooperative movement, appropriate mass transfer, and meeting the system's need. Appropriate FF in a tank depends on the size and design of the biocarriers (i.e., the specific surface area available for biomass growth) as well as the

required available surface area in the reactor. However, FF lower than 70% is recommended for a typical MBBR process where energy consumption, mixing, and mass transfer are reasonable [37], [40].

For a given substrate removal rate required and possible removal flux in an MBBR treatment zone, the required media surface area and FF can be determined by the following equations [4]:

$$A_{BF} = \frac{Q(S_0 - S_e)}{J_s} \qquad \textit{Equation 1}$$

$$FF\ (\%) = \frac{A_{BF}}{V(SS_A)} * 100 \qquad \textit{Equation 2}$$

Where A_{BF} is biofilm surface area (m^2); Q is influent flowrate ($m^3\ d^{-1}$); S_0 and S_e are influent and effluent substrate concentrations ($g\ m^{-3}$), respectively; J_s is substrate removal flux ($g\ m^{-2}d^{-1}$); V is reactor volume (m^3); and SS_A is media specific available surface area ($m^2\ m^{-3}$).

For BOD (or COD) removal, the substrate removal flux (J_s) can be determined based on its relation to substrate loading flux reported by Ødegaard [41] and shown in Figure 6.

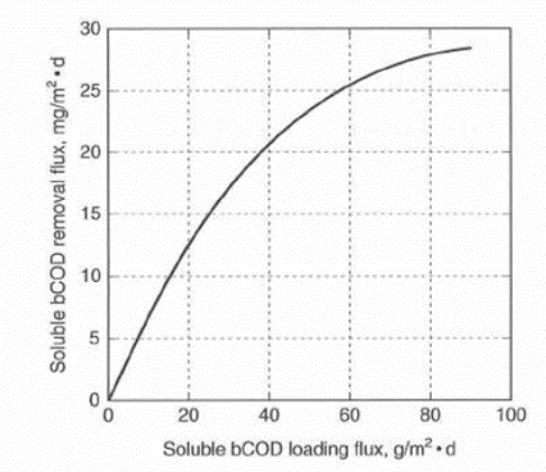


Figure 6 – Biodegradable COD (bCOD) removal flux versus loading flux [42]

2.3.3. Advantages and Disadvantages

MBBR system includes some challenges such as high energy consumption due to mixing and aeration, high required initial investment [39], issues of media removal for diffuser maintenance, the need for improved influent wastewater screening, the limitation of biological phosphorous removal [4], and relatively weak settleability of the effluent suspended solids without additional modifications (e.g. chemically enhanced settling or sludge recycling) [43]. However, despite the mentioned features, the advantages associated with MBBR have made this system be an excellent option to be considered for wastewater treatment.

Applying MBBR with the attached biofilm rather than methods with suspended flocs provides the capability of fitting more active biomass concentration in the reactor. This benefit not only reduces the reactor volume demand but also enables the system to receive a higher Volumetric Loading Rate (VLR) [6]. Concurrently, treatment requires lower HRT due to the higher population of microorganisms than in CAS systems [28]. Also, the system design introduces high SRT and low sludge production without the need for recycling, which reduces both footprint and the chance of clogging. The biofilm formed on the media is more resistant and flexible in environmental changes such as temperature, pH, and change in organic loads, due to microorganisms' symbiotic relationships and increased inclination for survival [10], [39]. This process, furthermore, may have an advantage over granulation-based methods where the fluctuation of industrial wastewater may cause degranulation [44], [45].

Additionally, the free-floatation of the carriers makes this system defeat the diffusional limitations and improve the mass transfer and reaction rate [46]. The main advantages and disadvantages are summarized in Table 2.

Table 2 – Main positive and negative points associated with MBBRs

Advantages	Disadvantages
The capability of including more biomass concentration	High energy consumption
Smaller reactor requirement	High minimum mixing energy
Handling higher VLR	High initial investment requirement
Lower HRT	Challenging media removal
High SRT without recycling requirements	Improved screening requirement
Less footprint and chance of clogging	Limitation of biological phosphorous removal
More resistant biofilm	Relatively weak effluent particle settleability without additional modifications
Avoiding degranulation	
Improved mass transfer and restricted diffusional limitations	

2.3.4. Design and Operational Parameters

Designing an efficient MBBR and calculating some design parameters such as reactor volume (based on Equation 7), required preserved surface area and media FF (based on Equation 1 and Equation 2) need a determination of some primitive operational parameters. The influent wastewater characteristics (such as substrate concentration, temperature, and pH), the required effluent substrate concentration, desired SALR and corresponding influent flowrate, adequate HRT, and sufficient DO level are such factors that should be specified.

The influence of all the mentioned parameters on MBBR design and efficiency can be explained as follows.

2.3.4.1. Influent Substrate Concentration

The source of wastewater is the factor that determines wastewater substrate concentration. Municipal wastewater, discharged from residential, commercial and public facilities, and industrial wastewater, discharged from industries, are two common sources of wastewater that contain a wide variety of constituents with different concentrations. Therefore, it is common to characterize wastewater in terms of its chemical constituents (i.e., inorganic and organic

chemicals). Based on the Canadian Council of Ministers of the Environment report in 2006 [47], a typical strength raw municipal wastewater in Canada consists of about 170 mg BOD L^{-1} and 430 mg COD L^{-1}. Industrial wastewater, however, has very variable quality, which may be highly biodegradable or not at all, based on the type of industry producing it. For instance, cane and beet sugar industry wastewaters include 1.7-6.6 and 4-7 g BOD L^{-1} while their COD ranges are 2.3-8 and up to 10 g COD L^{-1}, respectively [48].

Wastewater substrate concentration can highly affect the system selection and reactor design in terms of the type of treatment system (i.e., physical, chemical, or biological), the reactor type (e.g. CAS or MBBR), the number of the reactors (i.e., single or combined treatment systems), the aeration condition (i.e., aerobic, anoxic, or anaerobic), etc.

2.3.4.2. Temperature

Temperature is a factor that affects bacteria's metabolic activities, biological reaction rate, oxygen solubility, and oxygen transfer rate.

Generally, the optimum temperature for BOD removal is in the range of 25 to 35˚C [4], and temperature drop has a negative impact on bacterial activities and biological reaction rates [49]. The significant effect of temperature on the reaction rate can be modelled by Equation 3.

$$k_T = k_{20}\theta^{(T-20)} \qquad \textit{Equation 3}$$

Where K_T is reaction rate coefficient at temperature T (˚C); k_{20} is the reaction rate coefficient at 20˚C; θ is temperature- activity coefficient; and T is the temperature (˚C).

BOD removal rate is less affected by the temperature as its temperature activity coefficient, θ, is about 1.04 [50], while according to Rusten et al. [51], θ = 1.09 can be established for nitrification in MBBRs. Still, di Biase et al. [52] studied the performance of an anaerobic MBBR

(AMBBR) working at different temperatures (15, 25, and 35˚C) and showed that temperature has a significant impact on an AMBBR efficiency. They showed that the lower the temperature, the higher the biomass concentration. However, despite the higher biomass concentration, biofilm acclimated to 15 and 25˚C performed significantly slower than the one acclimated to 35˚C.

Temperature can influence aerobic MBBR efficiency as well. Although oxygen solubility increases when temperature decreases, the low temperature is not favorable for oxygen transfer due to the high viscosity. The lower the water temperature, the lower the transfer rate, which causes substrate removal rate reduction [53].

2.3.4.3. pH

For organic matter and nitrogen removal, optimal performance occurs when pH is almost neutral, while pH in the range of 6 to 9 is tolerable [4]. For BOD removal, there is no need for an external source of alkalinity. For the nitrification process, however, minimum alkalinity of 70 mg L^{-1} as $CaCO_3$ is needed in the nitrification reactor to assure the pH of at least 6.8 [4].

The attached biofilm in MBBR is more resistant against environmental changes, including pH variation in influent compare to suspended growth systems. However, influent pH adjustment may be needed, especially for some industrial wastewaters, as the wastewater acidic or alkaline condition may harm the microorganisms.

2.3.4.4. Required Effluent Substrate Concentration (Discharge regulation)

Based on each region's environmental regulations, the treated effluent is required to meet specific standards to be discharged. In Canada, for instance, the average carbonaceous biochemical oxygen demand (CBOD) matter, the average concentration of suspended solids, and the maximum concentration of un-ionized ammonia in the effluent must not exceed 25 mg CBOD L^{-1}, 25 mg SS

L^{-1}, and 1.25 mg N L^{-1}, respectively [54]. This regional regulations affect the type of treatment system selection as well as design parameters to meet the mentioned standards.

2.3.4.5. Surface Area Loading Rate (SALR)

The substrate loading rate, which is generally based on the carrier protected surface area, g BOD $m^{-2}d^{-1}$, for instance, is called surface area loading rate (SALR) or loading flux, and it is shown by Equation 4.

$$SALR = \frac{Q.S_0}{A_{BF}} \qquad \textit{Equation 4}$$

Where Q is influent flowrate (m^3 d^{-1}); S_0 is influent substrate concentration (g m^{-3}); and A_{BF} is the available carrier surface area (m^2).

MBBR can be designed under high to low SALR levels. MBBR with high SALR, roughly up to 28 g BOD $m^{-2}d^{-1}$, may be used as a CAS modifier for partial BOD removal before activated sludge treatment to enhance the domestic/industrial wastewater treatment capacity. It also may be used as a reactor before the nitrification process [4]. Under such a high loading rate, however, the biocarriers may clog by excessive biofilm growth, and the removal efficiency may be reduced as the oxygen becomes a limiting factor [55], [56]. The moderate SALR, in the range of 5 to 15 g BOD $m^{-2}d^{-1}$, on the other hand, is usually used for secondary treatment and provides 80-90% BOD removal [39]. Lower BOD SALRs are mainly utilized as prenitrification designs to minimize the effluent bCOD to the nitrification tank in order to control heterotrophic bacteria over the nitrifying bacteria population [57].

For nitrification, nonetheless, both BOD and ammonium loading should be considered as design factors. A high BOD load causes heterotrophic bacteria to grow and compete with nitrifiers for oxygen. Therefore, the organic load should be maintained as low as possible using a MBBR

for BOD removal before the nitrification tank. The impact of BOD SALR on nitrification flux is shown in Figure 7.

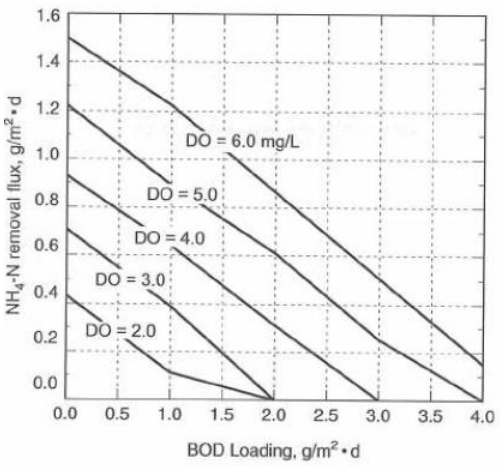


Figure 7 - Effect of DO and BOD SALR on nitrification flux [42]

The ammonium SALR, on the other hand, can be variable from high loading conditions such as about 2 g N $m^{-2}d^{-1}$ to a conventional or normal loading condition of 0.9 g N $m^{-2}d^{-1}$, as mentioned by Young et al. [58].

2.3.4.6. Hydraulic Retention Time (HRT)

Hydraulic retention/residence time (HRT) refers to the average time that liquid remains in the system and can be calculated by the following equation:

$$HRT = \frac{V}{Q} \qquad \textit{Equation 5}$$

Where HRT is hydraulic retention time (d); V is the volume of the reactor (m^{-3}); and Q is the influent flowrate (m^3 d^{-1}).

Based on the equation, it can be concluded that this factor is dependent on the Q and, consequently, on the SALR while the reactor volume and carrier FF are fixed. More substrate loading rate (i.e., higher SALR) would be provided by higher influent flowrate to the reactor,

which consequently reduces the HRT as the volumes of the reactor and carriers are fixed. However, by changing the carrier FF in the system, the SALR and HRT of the system may be designed independently, as shown by Soleimani et al. [26].

Generally, compared to the conventional processes, MBBR requires less HRT for substrate reduction to the optimum level due to the higher population of microorganisms [28]. Short HRT leads to needing a smaller tank for the same substrate amount reduction. For municipal wastewater, depending on the wastewater BOD concentration and SALR, the HRT in a MBBR for carbonaceous matter removal is relatively low as of 15-90 min [42]. However, due to the oxygen and mass transfer limitations, HRT of 45 to 60 min is usually being used to ensure the soluble organic matter degradation as well as particulate organic matter hydrolyzation and degradation even at a relatively cold temperature (10-15˚C) [4], [59]. Contrary to what was mentioned, some researches have shown that increasing the HRT even to more than 20h has a positive effect on organic removal efficiency [60]–[63].

On the other hand, for industrial wastewater with a very high BOD concentration, relatively longer HRT is needed to assure sufficient BOD degradation. For an anaerobic MBBR, for instance, Di Biase et al. [64] have shown that for brewery wastewater with a COD concentration of 5.2±2.1 g COD L^{-1}, HRT of 18 h exhibited the highest COD removal (88±2.5%) compare to HRTs of 6, 8, 10, 12, and 24 h.

In terms of nitrification, the HRT becomes a critical factor, especially at low temperatures where the nitrification rate decreases. A significant efficiency reduction has been observed below 6.5˚C at HRT<5h [61]. At room temperature, on the other hand, it has been noted that increasing the HRT from 4 to 8h has a positive effect on total nitrogen removal while the removal efficiency decreases at 12h HRT [62].

2.3.4.7. Dissolved Oxygen (DO)

Generally, the minimum DO level required for the operation of an aerobic process for BOD removal is 2 to 3 mg L^{-1} [42]. However, as mentioned before, in MBBR, the supply of oxygen by air bubbling is responsible for both aeration and mixing. Therefore, the airflow rate employed in MBBR systems is much higher than in suspended biomass reactors, which provides DO in the range of 4 to 6 mg L^{-1} [4].

For nitrification, on the other hand, a high oxygen concentration is required to have an adequate nitrification rate, especially at high BOD concentrations. This is due to the fact that in the presence of organic matter, the heterotrophic bacteria compete with nitrifying organisms on oxygen consumption. The minimum DO concentration at which nitrification can occur is reported to be 0.3 mg L^{-1}, and the maximum nitrification rate usually happens over the DO range of 0.3 to as much as 4 mg L^{-1} [65]. The effect of BOD and DO concentration on nitrification flux is provided by Ødegaard et al. and shown in Figure 7.

In addition to the DO level, the design of the air diffusers is also an important consideration since the air bubbles should present an appropriate size. Huge bubbles lead to oxygen transfer reduction by significantly decreasing the oxygen transfer coefficient. Tiny bubbles, diversely, favor oxygen transfer but do not provide adequate mixing and carrier movement [10]. As a result, the best air supply must be provided to the reactors to keep the DO at a sufficient level, introduce a suitable carrier suspension with the minor stagnant zones in the reactor, and prevent biofilm dispersion from the carriers.

2.3.5. Removal Performance

High biomass concentration in MBBR, compare to CAS systems, has made this process able to handle much higher loadings with a satisfying removal performance. At SALRs greater

than 20 g BOD $m^{-2}d^{-1}$ (i.e. high rate), a BOD removal in the range of 75-80% is achievable, while at SALRs between 5 and 15 g BOD $m^{-2}d^{-1}$ (i.e. moderate rate), 80-90% removal can be attained. At low loading rates (i.e., less than 5 g BOD $m^{-2}d^{-1}$), on the other hand, it is feasible to reach more than 90% BOD removal [39], [57]. However, a combination of different processes may enhance the removal performance of MBBR under various SALRs. For instance, a combination of a pre-coagulation for suspended particles and colloids with a high rate MBBR may result in 85% of BOD removal [40]. Besides, utilizing multiple MBBRs in series can provide high rate BOD uptake and low soluble BOD concentration in the effluent. This also minimizes the possibility of short-circuiting of the influent flow [4].

In nitrification, a successful ammonia removal from 80% to greater than 90% is achieved by different researchers using MBBR [66], [67].

Typical substrate removal rate values for BOD removal and nitrification are shown in Table 3.

Table 3 - Typical removal rates for BOD removal and nitrification in MBBR [4], [57]

Application	**Substrate**	**SALR**	**Surface removal rate g $m^{-2}d^{-1}$**	**Volumetric removal rate (FF = 60%) kg $m^{-3}d^{-1}$**
Partial BOD removal	BOD	high	15 -20	4.5 – 6
Secondary treatment	BOD	moderate	5 - 15	1.7 – 5
Prenitrification	BOD	low	4 - 5	1.2 - 1.5
Nitrification	NH4-N	variable	0.4 - 1.4	0.1 - 0.4

2.3.6. Solids Production and Removal

The process type for primary and secondary wastewater treatment has a substantial impact on solids removal and the quantity and quality of the sludge produced. For instance, BNR systems produce a relatively low amount of sludge, but the produced sludge is more difficult to be processed by dewatering or digestion [4]. Sludge treatment has been estimated to form 50-60% of

municipal wastewater treatment costs [56]. Therefore, modification of treatment processes to have relatively low sludge with high quality (i.e., readily treatable) produced and efficient solids removal performance (i.e., low solids concentration in reactor effluent) could be an asset for cost reduction.

The MBBR design is in the way that the reactor includes high biomass concentration. The biofilm areal solids concentration may be as high as 28 g TS m^{-2} for a high rate BOD removal operation to a concentration as 12 g TS m^{-2} for combined carbon removal and nitrification process. Assuming using biocarrier with 500 m^2m^{-3} surface area and 60% FF, the volumetric total solids (TS) can be in the range of 3600 to 8400 g m^{-3}, which includes volatile solids (VS) plus trapped and non-biodegradable organic matter and nutrients [4]. Even though the amount of solids produced is high, there is no need for treating this biomass much often as MBBR has a relatively long SRT.

The mixed liquor suspended solids (MLSS) concentration in the effluent of a MBBR, on the other hand, may be in the range of 100 to 250 g $m^{-3,}$ which is about ten times fewer solids compare to CAS [4], [68]. This makes the WWTP not only deal with a low amount of solids but also become independent of solids recycling. The SS part of effluent, which is a composition of bacteria cells detached from the carriers, soluble microbial products, non-biodegradable organic matter (colloidal and soluble), and a portion of influent particulate matter that passes through the system [43], has relatively weak settling characteristics. Due to this spec, several full-scale plants use a combination of sedimentation with coagulation/flocculation for MLSS separation [69]. Yet, dissolved air floatation (DAF) is currently the most common separation method used after MBBR, which can also be combined with coagulation/flocculation for removing very small particles, as reported in some studies [70], [71]. Filtration processes such as membrane filtration, dual media

sand filters, cloth disc filters, and ballast flocculation are such operations that can also be used for solid separation instead of gravity-based clarifiers [43].

2.3.7. Process Modeling

The substrate utilization rate for soluble substrates in biological systems, including MBBR, is commonly modelled by Equation 6, called the Monod equation [4]. This factor can then be used to calculate either the effluent substrate concentration or the required reactor volume using a mass balance of substrate entering and exiting the reactor employing Equation 7.

$$r_{su} = \frac{kXS_e}{K_s+S_e} \qquad \textit{Equation 6}$$

$$r_{su} = \frac{Q(S_0-S_e)}{V} \qquad \textit{Equation 7}$$

Where, r_{su} is substrate utilization rate (g $m^{-3}d^{-1}$); Q is flow rate of feed wastewater (m^3 d^{-1}); V is reactor volume (m^3); S_0, and S_e, are the substrate concentration in feed, and effluent, respectively (g m^{-3}); k is maximum specific substrate utilization rate (gS $gVSS^{-1}d^{-1}$); X is biomass concentration (g VSS m^{-3}); and K_s is half-velocity constant or substrate concentration at one-half the maximum specific substrate utilization rate (g m^{-3}).

Kincannon-Stover is another mathematical model which has been reported that can describe the substrate utilization rate in MBBR very well. The applicability of both Kincannon-Stover and Monod models for organic removal in a MBBR is studied, and the results indicate that the Kincannon-Stover model is more accurate for describing the kinetics of BOD removal in MBBR compare to the Monod model [72], [73]. Equation 8 presents the Kincannon-Stover mathematical model, which also can be used for calculating the effluent substrate concentration or reactor volume using Equation 7.

$$r_{su} = \frac{U_{max}(\frac{QS_0}{V})}{K_B + (\frac{QS_0}{V})} \qquad \textit{Equation 8}$$

Where U_{max} is the maximum substrate utilization rate constant (g $L^{-1}d^{-1}$); and K_B is saturation constant (g $L^{-1}d^{-1}$).

2.3.8. Contribution in Full-Scale Wastewater Treatment Plants

The first full-scale utilization of the MBBR process was in two existing treatment plants in 1992 [30], and in 2014, this technology was being employed in more than 1200 wastewater treatment plants in at least 50 countries [31]. Several full-scale industrial wastewater treatment plants have also upgraded their facilities and started to use MBBR processes [34], [74]–[77].

Figure 8 shows different setups of MBBR used for BOD removal and nitrification.

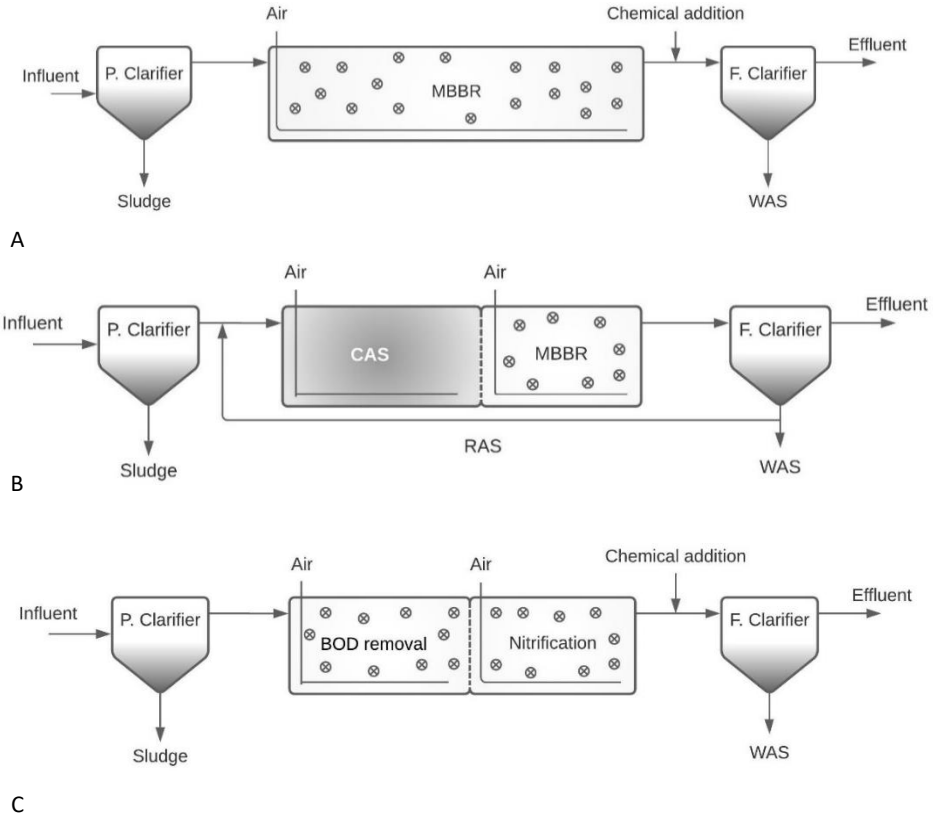


Figure 8 - Typical MBBR processes: A) BOD removal with chemical addition for solid separation and phosphorous removal if needed; B) Combination of CAS and MBBR for nitrification; C) BOD removal + Nitrification with chemical addition for solid separation and phosphorous removal if needed [39]

Nowadays, the need for upgrading the existing WWTPs to enhance the volumetric loading capacity with the least space demand has made the MBBR system attract more attention to be applied in full-scale. Table 4 presents the BOD removal operational details of some full-scale facilities employing MBBR.

Table 4 - BOD removal operational details in MBBR full-scale employments

Wastewater Type	Q (MLD)	SALR (g BOD $m^{-2}d^{-1}$)	T (˚C)	FF (%)	Biocarrier type	Region	Reference
Municipal	71	20 - 100	-	30	Kaldnes K1	Wellington, New Zealand	[57]
Food industry	0.152	8.11	NA	35	AnoxKaldnes	Italy	[74]
Slaughter house	0.64	20	25	50	Kaldnes	Norwalk, US	[75]
Refinery	19.68	10.4	40	30	Kaldnes	Borger, US	[75]
Beverage industry	0.06	6	NA	60	NA	Italy	[76]

It can be predicted that soon, more WWTPs will upgrade their facilities to cover increasing population requirements. MBBR can be a good choice due to its advantages, discussed in section 2.3.3., over other systems.

Chapter 3: Municipal Wastewater treatment

3.1. Introduction

Organic constituents present in municipal wastewater are typically composed of proteins, carbohydrates, oils and fats, and small quantities of different synthetic organic molecules. A low strength untreated domestic wastewater includes an ordinary amount of 133 and 339 mg L^{-1} of BOD and COD, respectively. Regular quantities are 200 mg BOD L^{-1} and 508 mg COD L^{-1} in medium strength and 400 mg BOD L^{-1} and 1016 mg COD L^{-1} in high strength wastewaters. Moreover, untreated domestic wastewaters may include TSS and VSS in the rages of 130-389 and 101-304 mg L^{-1}, respectively, depending on their strength [4].

Regional discharge regulations may vary based on associated sewage collection and treatment systems. In Canada, for instance, the general principle expresses that the average carbonaceous BOD and suspended solids should not exceed 25 mg BOD L^{-1} and 25 mg TSS L^{-1}, respectively [54]. Therefore, it is necessary to significantly reduce the level of organic matter and solids in wastewater using efficient and cost-effective systems to release the effluent into the environment.

Conventional Activated Sludge (CAS) system is an approved biological treatment method currently being employed by a great number of WWTPs for organic matter removal. However, despite its acceptable performance in providing adequate effluent quality, it comes with some associated challenges, such as sludge settleability difficulties, biomass recycling requirement, the need for large reactors and settling tanks, and the necessity of chemical addition and filtration facilities in regions with lower LoT [5]–[9].

Nowadays, a large number of plants are using relatively new technologies such as Moving Bed Biofilm Reactors (MBBRs) to address the mentioned challenges and provide an efficient WWTP. However, the number of studies on organic removal from municipal and industrial wastewater using these new technologies is limited compared to the other conventional systems [10]. Therefore, more studies over the efficiency of these processes, the associated costs, the amount of sludge production, and their ability in increasing the plant capacity are still sought and required by municipalities.

In this study, aerobic moving bed biofilm reactor (MBBR) efficiency in organic matter removal from actual municipal wastewater is evaluated. The functionality of BioPorts media with different surface areas under various Surface Area Loading Rates (SALRs) is analyzed to find both optimum media surface area and SALR for MBBR treating municipal wastewater.

3.2. Material and Methods

3.2.1. MBBR Setup

To assess the influence of two variables of this experiment (i.e. carrier surface area and SALR) on organic matter removal performance of the MBBR, the study was conducted in two phases: 1) Actual municipal wastewater treatment and; 2) Synthetic sugar industry wastewater treatment.

For the first phase, three 20L reactors, each containing 6 L of BioPorts media (FF = 30%) with a surface area of 851 m^2m^{-3}, were established. The operational SALRs for the rectors were 4, 9, and 18 g BOD $m^{-2}d^{-1}$, respectively. Moreover, three other 20L reactors, each containing 8.66 L of BioPorts media (FF = 43.3%) with a surface area of 589 m^2m^{-3}, were established with the same operational condition, SALRs, as the previous ones. The filling fractions were selected, providing

5 m^2 of protected surface area available for biofilm development in each reactor. Two types of biocarriers used in this study are shown in Figure 5. Air was diffused, at a rate of 45.3 L min^{-1}, to supply oxygen to the microorganisms for biological activities and provide mixing. The reactors' design parameters for this phase and their schematic setup are summarized in Table 5 and showed in Figure 9, respectively.

Table 5 - Reactor configurations for BOD removal from municipal wastewater

Parameters	Reactor 1	Reactor 2	Reactor 3	Reactor 4	Reactor 5	Reactor 6	Unit
Biocarrier SA	851	851	851	589	589	589	$m^2 m^{-3}$
FF	30	30	30	43.3	43.3	43.3	%
Available SA	5	5	5	5	5	5	m^2
$V_{reactor}$	20	20	20	20	20	20	L
Airflow	45.3	45.3	45.3	45.3	45.3	45.3	L min^{-1}
SALR	4	9	18	4	9	18	g BOD $m^{-2}d^{-1}$
SALR	10	22.5	45	10	22.5	45	g COD $m^{-2}d^{-1}$
Q	93	233	466	93	233	466	ml min^{-1}
HRT	3.58	1.43	0.72	3.58	1.43	0.72	h

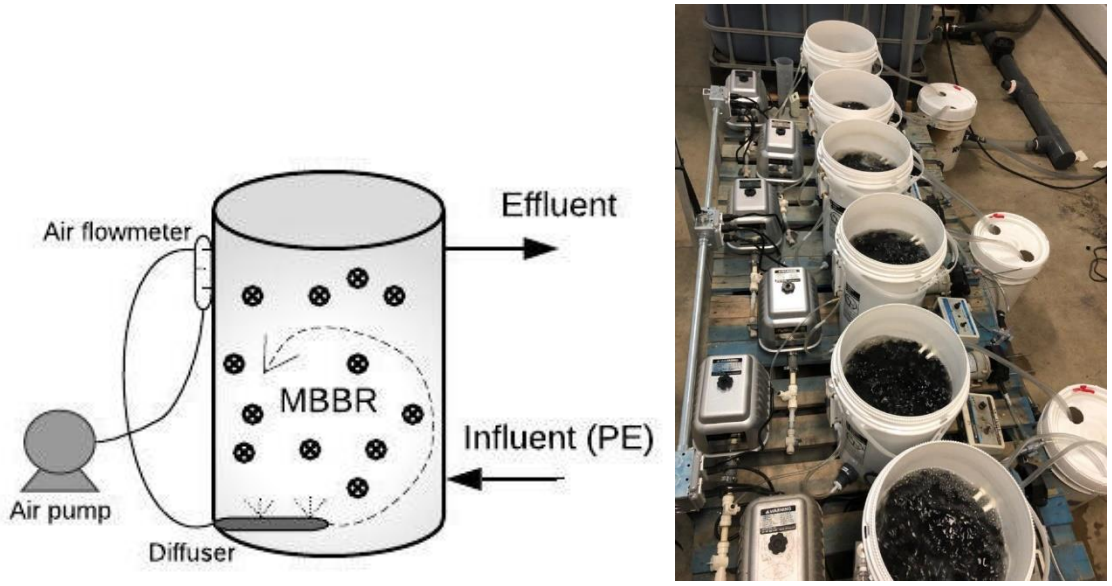


Figure 9 - MBBRs setup

For the heterotrophic bacteria attachment and growth, no seeding was required based on previous lab experiences. The biofilm started to form on the carriers' protected surface area by introducing the wastewater and air to the reactors. Adhesion forces brought microorganisms, which were present in the wastewater, to attach to the carrier surface. At the early stage, the possibility of detachment was high due to the loose connections. By passing the time, however, a tight connection between the living species and the support surface was formed due to the extracellular polymeric substances (EPSs) and the biofilm was gradually developed.

This phase lasted six weeks as the steady-state condition was reached over the first month of operation, and the following two weeks were required for fulfilling the tests on biofilm and SS.

3.2.2. Wastewater Characteristics

During the experiment period, reactors were continuously receiving the actual municipal wastewater from the West End Water Pollution Control Center (WEWPCC), Winnipeg, MB, Canada, primary effluent (PE) (i.e. the effluent of the primary clarifier). The flow diagram of WEWPCC and the line to the feed (PE) tank, providing the MBBRs' influent, are shown in Figure 10.

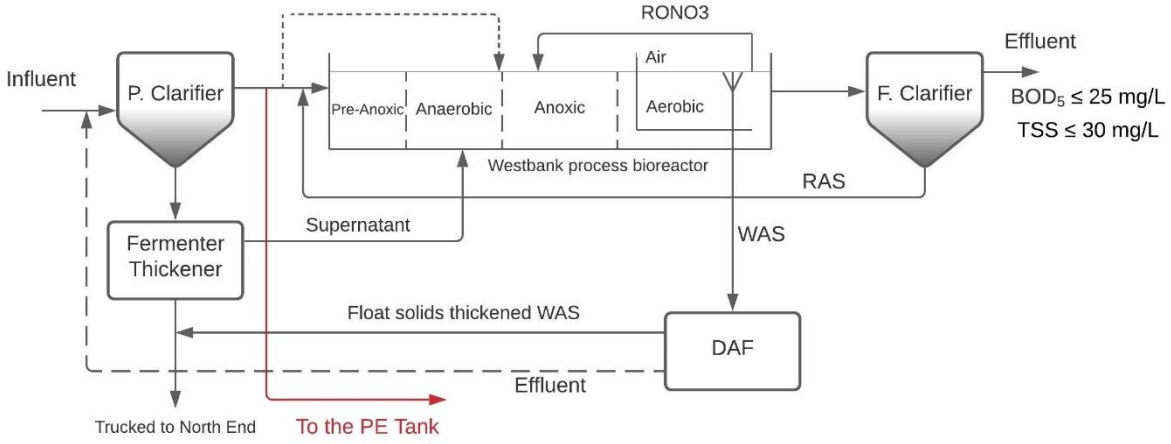


Figure 10 - West End Water Pollution Control Centre (WEWPCC) flow diagram and the line providing the MBBRs' influent

Influent BOD was on the average of 140 mg BOD L^{-1}, based on the years of data collection on primary effluent (PE) from the WEWPCC. Total COD and soluble COD, on the other hand, were in the range of 300-400 mg TCOD L^{-1} and 150-180 mg sCOD L^{-1}, respectively, based on measuring three times a week during the project period. Also, the amount of TSS and VSS were in the range of 25-95 and 20-90 mg L^{-1}, respectively, based on the measurements, and the PH value was 7±0.5. The influent characteristics are summarized in Table 6.

Table 6 - West End Water Pollution Control Center (WEWPCC) primary effluent characteristics

Parameter	Value	Unit
TCOD	350 ± 50	mg L^{-1}
sCOD	165 ± 15	mg L^{-1}
BOD_5	140	mg L^{-1}
TSS	60	mg L^{-1}
VSS	50	mg L^{-1}

3.2.3. Analytical Methods

Operational parameters including DO, temperature, and pH were recorded on-site using a Thermo Scientific STARA2230 DO Meter and Alpha pH200 meter (Eutech Instruments, USA), respectively, three times a week to make sure that reactors are working under the suitable condition. The MBBRs' influent and effluents were tested three times a week for TCOD and sCOD through spectrophotometric methods according to the Standard Methods [78]. The obtained data was then used to calculate the percentages of the removed TCOD and sCOD and the surface removal rate, which demonstrate the system performance. The surface removal rate was calculated using Equation 9 [64].

$$SRR = \frac{Q*(S_0-S_e)}{MSA*FF*V} \qquad \textit{Equation 9}$$

Where SRR is the surface removal rate (g COD $m^{-2}d^{-1}$); Q is the influent flow rate (L d^{-1}); S_0 and S_e are substrate concentration in influent and effluent, respectively, (g L^{-1}); MSA is media surface are (m^2 m^{-3}); FF is filling fraction (%); and V is the reactor volume (m^3).

Influent and effluent TSS and VSS were also measured using 50 ml of PE and effluent samples, filtered into a single crucible equipped with glass microfiber filters (0.45 µm GF/C filters, Whatman, UK) based on the Standard Methods [78]. After filtration, the samples were dewatered at 105˚C for 24 h and weighted for the TSS calculations. Then, they were placed in a 550˚C oven for 2 h and weighted again for the VSS calculations.

To monitor biofilm development, three biocarriers were removed from each reactor as triplicates once a week. Zeiss SteREO Discovery.V8 Microscope was used for observing the biofilm shape, distribution, and thickness. Then, the CMEIAS-IT software was applied for measuring the thickness. The thickness of biofilm was measured at three points with different widths (i.e. at minimum, medium, and maximum thicknesses). This was done for 12 central edges of the carrier with the surface area of 851 m^2m^{-3} and 8 central edges of the carrier with the surface area of 589 m^2m^{-3}. Finally, the averages of achieved 36 and 24 numbers were calculated.

Biocarriers total solids (TS) and volatile solids (VS), representing the attached biomass quantity, were measured by mechanical (i.e., manual) biofilm removal from carriers in triplicates. Technically, the number of biocarriers per conducted test should be defined based on the biofilm thickness observed under stereomicroscope analysis. As the biofilm thickness and concentration get lower, the number of the carrier needed to be examined becomes higher. However, previous lab studies have shown that using two biocarriers in a 100 mL beaker in triplicates is a potential method for biofilm quantification. After manually removing biofilm from biocarriers using a brush and deionized water, the same steps as TSS and VSS measurements were done to achieve

biocarriers TS and VS. The density was then calculated based on the biofilm mass and volume. Biofilm quantification was performed once at steady-state in the last week of the experiment.

To achieve the organic matter removal rate, which represents attached biofilm performance, the kinetic test was done once at steady-state in the last week of the experiment. 0.6 L of media with the surface area of 851 m^2m^{-3} was collected from each reactor and transferred to two 1 L beakers as duplicate batch reactors (i.e. 0.3 L of media in each, which means FF=30%). Moreover, 0.866 L of media with the surface area of 589 m^2m^{-3} was collected from each reactor and transferred to two 1 L beakers as duplicate batch reactors (i.e. 0.433 L of media in each, which means FF=43.3%). Reactors were filled up to 1 L with the PE and started receiving air at a 2.27 L min^{-1} flowrate. Samples were collected every 15 minutes, starting from time zero to 1 hour and every 30 minutes for the next 1 hour. Finally, TCOD and sCOD tests were accomplished to achieve the rate of organic matter removal.

The MBBRs' effluents were tested three times during the last week of the experiment for ammonium, nitrite, and nitrate concentrations assessment by Flow Injection Analysis (FIA) according to the Standard Methods [78]. This test was done to assure that Ammonium Oxidizing Bacteria (AOB) and Nitrite Oxidizing Bacteria (NOB) populations were negligible, and most of the active microorganisms were responsible for BOD removal.

3.3. Results and Discussion

3.3.1. Operational Condition

The averages of monitored pH, temperature and DO of the mixed liquid in the reactors are presented in Table 7 - Operational parameters. Although some instabilities in pH were observed due to daily variations in actual wastewater contents, its variation range was between 7 and 8, which

is sufficient for heterotrophic bacteria growth and survival. Therefore, there was no need to control pH during the experiment time. Unsteady temperature and DO were also observed, even between the reactors working at the same location and under the same condition. Both site atmospheric temperature variation and microorganism activities could be the reasons for the monitored changes.

Table 7 - Operational parameters

Parameter	R1	R2	R3	R4	R5	R6	Unit
pH	7.5 ± 0.5						-
T	17.5 ± 0.5	16.5 ± 2	16.5 ± 2.5	17.5 ± 2	16.5 ± 2	16.5 ± 2.5	˚C
DO	6 ± 0.5	5 ± 0.5	4 ± 1	6 ± 1	4.25 ± 1	4 ± 1	mg L^{-1}

3.3.2. Influent and Effluent Characteristics

The influent and effluent average COD and solids are reported in Table 8 - Influent and Effluent Characteristics. The first month of the experiment was considered an accumulation and stabilization period. Therefore, data obtained during that time was not considered in calculations.

All MBBRs were able to remove a significant amount of TCOD and sCOD even though the removed TCOD was less remarkable than the removed sCOD. The challenges of particulate and colloidal COD removal, as well as inconsistent solids presence in both influent and effluent, were most likely the reasons for relatively low TCOD removal. Highly variable TSS and VSS in influent, ranging from 20 to 100 mg L^{-1}, could be explained by daily variations in actual wastewater contents.

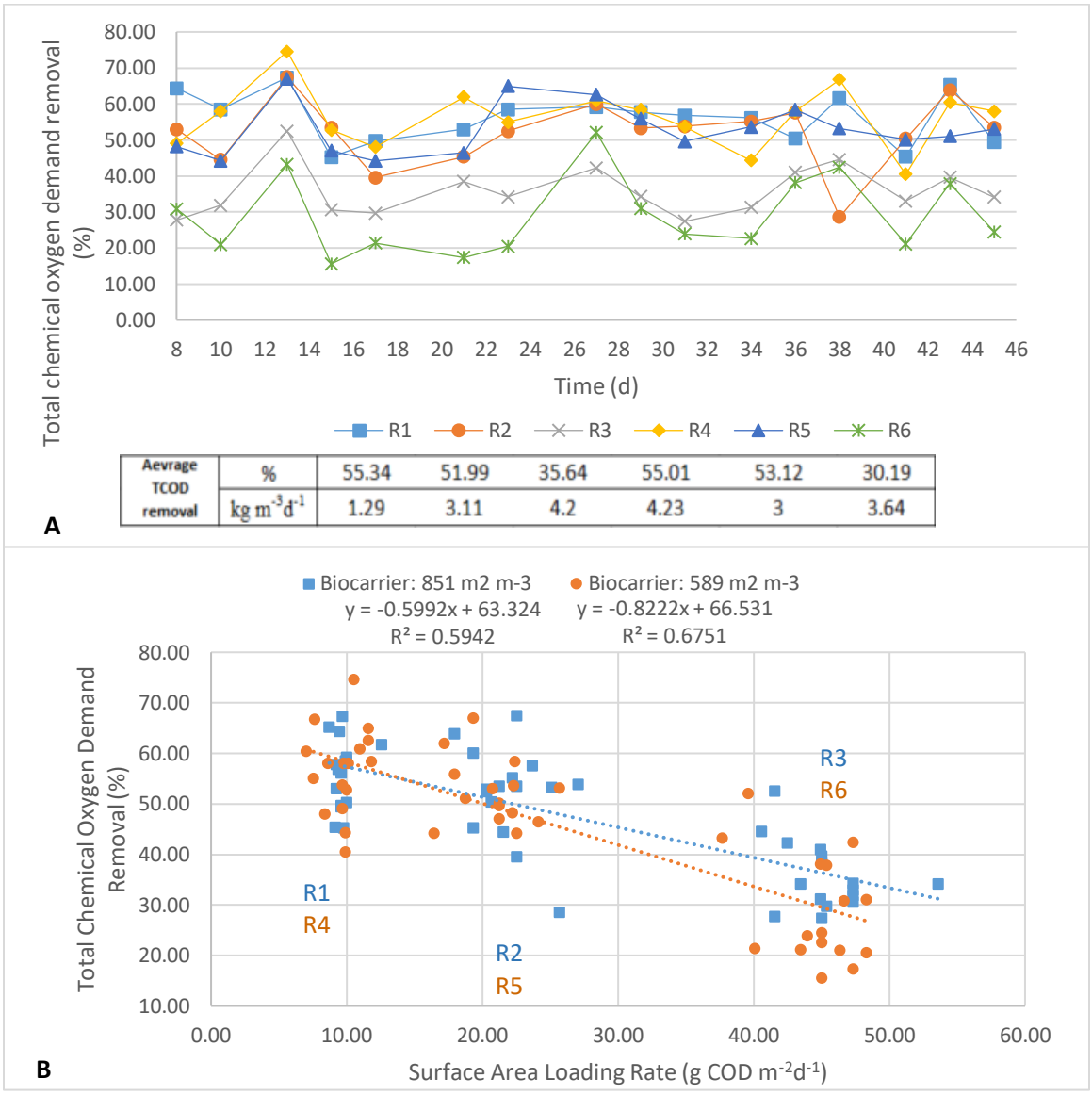
Table 8 - Influent and Effluent Characteristics

Parameter		Influent	Effluent						Unit
			R1	R2	R3	R4	R5	R6	
TCOD	Average	355.8	159.4	172.6	229.1	158.9	167.2	248.8	mg L^{-1}
	Standard Deviation	47.8	34.7	52.2	38.5	30.3	27.3	48.1	
sCOD	Average	172.5	40.7	36.6	51.2	40.2	39.6	54.3	mg L^{-1}
	Standard Deviation	17.7	12.9	14.5	14.2	12.4	14.0	12.9	
TSS	Average	42.0	118.0	119.6	120.8	107.2	123.6	90.4	mg L^{-1}
	Standard Deviation	15.0	26.3	43.4	24.9	20.1	45.1	6.7	
VSS	Average	38.8	108.8	110.8	104.0	93.2	112.4	84.8	mg L^{-1}
	Standard Deviation	15.5	27.0	43.1	14.4	22.7	41.2	9.0	

Considering an average BOD to COD ratio of 0.2 in effluent [4], the average BOD concentrations in R1 to R6 could be calculated as 31.9, 34.5, 45.8, 31.8, 33.6, and 49.8 mg BOD L^{-1}, respectively. As shown by the results, none of the reactors could provide BOD concentration of less than 25 mg BOD L^{-1} to be released directly to the environment based on the Canada regulations. However, the calculated values were close enough (especially in R1 and R4) that could probably easily meet the requirements employing a small high rate secondary MBBR.

In the case of solids concentration in effluent, the reactors could not meet the discharge rules as well. Still, a much lower concentration of TSS in MBBRs' effluent than CAS systems proved that no sludge recycling is needed. Therefore, just a settling tank for sludge removal could be employed to reduce the final effluent TSS content to less than 25 mg TSS L^{-1}.

3.3.3. Total Chemical Oxygen Demand (TCOD) Removal



Aevrage TCOD removal	%	55.34	51.99	35.64	55.01	53.12	30.19
	$kg\ m^{-3}d^{-1}$	1.29	3.11	4.2	4.23	3	3.64

Figure 11 – A) MBBRs performance in Total Chemical Oxygen Demand (TCOD) removal; B) TCOD removal correlated to SALR

TCOD removal was unstable during the first month due to startup and unsteady-state periods. However, after one month, reactors got more stable, and variations in removal percentages decreased, as shown by Figure 11-A. This showed that reactors reached steady-state condition after almost one month.

As has been mentioned by Metcalf and Eddy [4] (adapted from McQuarrie and Boltz [68] and WEF [57]), the typical volumetric BOD removal rate (kg $m^{-3}d^{-1}$) is 1.7-5.0 kg $m^{-3}d^{-1}$ for secondary treatment by MBBR. Considering average BOD/COD ratio of 0.4 in influent (See Table 6) and 0.2 in effluent [4], the average BOD removal in R1 to R6 could be calculated as 0.73, 1.84, 3.21, 0.70, 1.74, and 3.14 kg BOD $m^{-3}d^{-1}$, respectively. The results showed that all reactors worked as an MBBR with a typical volumetric BOD removal rate for secondary treatment, but R1 and R4, of which the removal rates were lower than 1.7 kg BOD $m^{-3}d^{-1}$. This showed that the SALR of 4 g BOD $m^{-2}d^{-1}$ did not provide a sufficient amount of COD removal per cubic meter per day, most probably due to lack of food the condition provided. The food shortage not only caused AOBs and NOBs growth in R1 and R4 (See section 3.3.9) but also made a notable amount of microorganisms be detached and washed out from these reactors as the relatively high VSS in these reactors shows (See section 3.3.6.). The mentioned observation could also be the reason why the SALRs in the range of 5 to 15 g BOD $m^{-2}d^{-1}$ are usually used for secondary treatment [39].

3.3.3.1. SALR influence

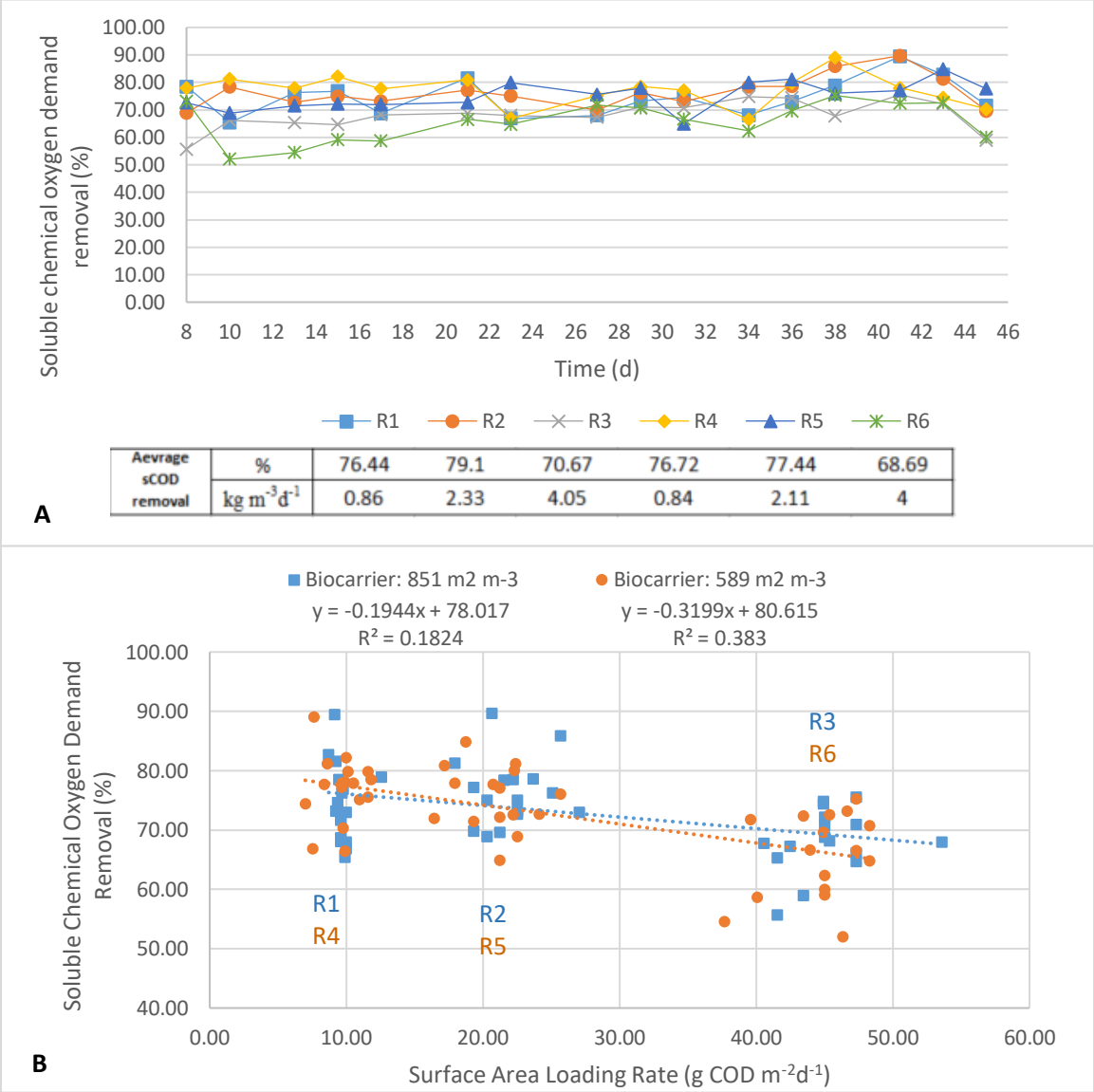
It is noticeable in Figure 11 that by increasing the SALR, the capability of the reactors in removing TCOD decreased. At an HRT of 3.58 h, corresponding to the SALR of 4 g BOD $m^{-2}d^{-1}$, MBBR removed about 55% of TCOD, independent of the media surface area. This removal percentage matched with 1.3 kg $m^{-3}d^{-1}$ of TCOD removal. However, the removal percentage decreased by 20±5% when the HRT decreased to 0.72 h, corresponding to the SALR of 18 g BOD $m^{-2}d^{-1}$. This can be explained by lower mass transfer in denser biofilms in reactors with higher SALR (See section 3.2.7) as well as lower HRT that caused biomass to have less time for uptaking the food.

Different types of equations such as linear, exponential, logarithmic, and power were examined to find the best correlated one. Finally, correlation coefficients of 0.5942 and 0.6751 were found between TCOD removal and SALR for biocarrier with the surface area of 851 and 589 m^2m^{-3}, respectively, for linear equation. The correlated equations provided in Figure 11-B make it possible to estimate the required SALR to have the desired amount of TCOD removal. Based on the results, SALR should be less than 8 g BOD $m^{-2}d^{-1}$, corresponding to 20 g COD $m^{-2}d^{-1}$, for both carrier types to assure TCOD removal percentage greater than 50%.

3.3.3.2. Carrier surface area influence

The removal percentage was almost independent of media surface area at low and medium SALRs (i.e. 4 and 9 g BOD $m^{-2}d^{-1}$). However, the media with the surface area of 851 m^2m^{-3} was able to remove about 5% more TCOD than the media with the surface area of 589 m^2m^{-3} at the highest SALR (i.e. 18 g BOD $m^{-2}d^{-1}$), most probably because of the higher amount of biofilm it included (See section 3.2.7). To determine if the average TCOD removal (kg $m^{-3}d^{-1}$) by the media with the surface area of 851 m^2m^{-3} was significantly greater than the removal by the other media type, hypothesis t-test (at $\alpha = 0.05$) was done. Based on the obtained p-value of 0.257, there was not enough evidence to conclude the difference between averages was greater than zero. This means that it cannot be supposed that one average was significantly greater than the other one. Therefore, the removal percentage could also be considered almost independent of the media surface area at the SALR of 18 g BOD $m^{-2}d^{-1}$.

3.3.4. Soluble Chemical Oxygen Demand (sCOD) Removal



Aevrage sCOD removal	%	76.44	79.1	70.67	76.72	77.44	68.69
	kg $m^{-3}d^{-1}$	0.86	2.33	4.05	0.84	2.11	4

Figure 12 - A) MBBRs performance in Soluble Chemical Oxygen Demand (sCOD) removal; B) sCOD removal correlated to SALR

As shown in Figure 12-A, sCOD removal was almost stable since the beginning; however, after one month, reactors got more stable, and variations in removal percentages decreased. This again showed that reactors reached steady-state condition after almost one month.

3.3.4.1. SALR influence

The results plotted in Figure 12 show that the SALR of 9 g BOD $m^{-2}d^{-1}$, corresponding to HRT of 1.43 h, are the best load and HRT for the sCOD removal by both types of biocarriers. MBBRs working under this load were able to remove 77-79% of sCOD, while the removal percentages were 76% and 68-70% in reactors with SALRs of 4 and 18 g BOD $m^{-2}d^{-1}$, respectively. The highest removal percentage matched with 2.22±0.11 kg $m^{-3}d^{-1}$ of sCOD removal. Higher removal performance in R2 and R5 could be due to the higher biomass concentration in these two reactors compare to the other ones (See section 3.2.7). Yet, p-values of 0.45 and 0.0001 were achieved for the efficiency difference between reactors working at SALRs of 9 and 4 g BOD $m^{-2}d^{-1}$ and 9 and 18 g BOD $m^{-2}d^{-1}$, respectively. This showed that the sCOD removal variation was not significant for the reactors working at the SALR of 4 and 9 g BOD $m^{-2}d^{-1}$, while the difference was remarkable for the ones operating at 9 and 18 g BOD $m^{-2}d^{-1}$.

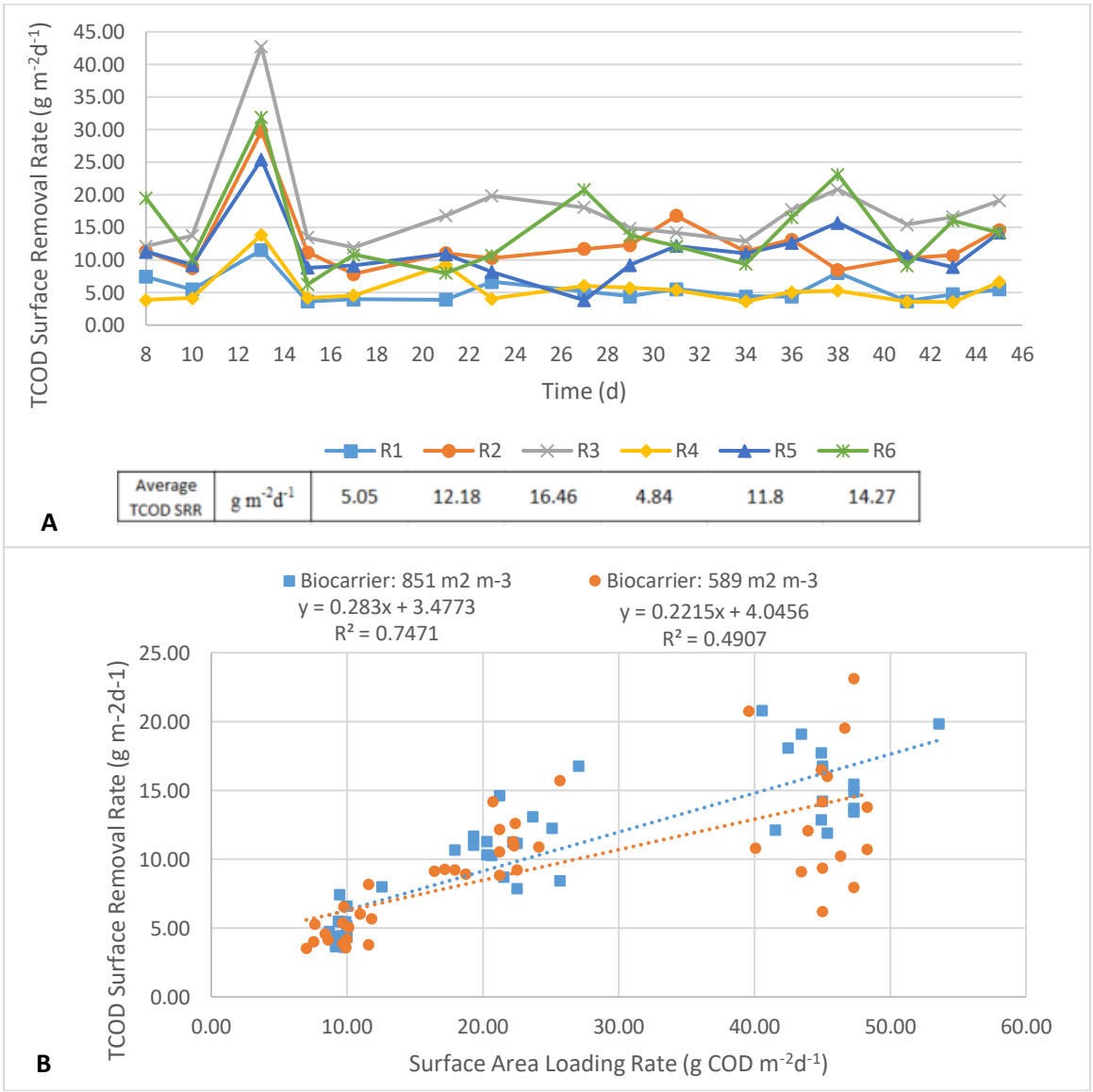
Different types of equations such as linear, exponential, logarithmic, and power were examined to find the best correlated one. However, relatively weak correlation coefficients of 0.1824 and 0.383 were found between sCOD removal and SALR for biocarriers with surface areas of 851 and 589 m^2m^{-3}, respectively, for linear equation. Yet, based on the results, SALR should be less than 17 and 14 g BOD $m^{-2}d^{-1}$, corresponding to 42.5 and 35 g COD $m^{-2}d^{-1}$, for biocarriers with surface areas of 851 and 589 m^2m^{-3}, respectively, to assure sCOD removal greater than 70%.

3.3.4.2. Carrier surface area influence

sCOD removal was almost independent of carrier surface area at all three SALRs. Yet, the media with surface area of 851 m^2m^{-3} showed better performance than the media with surface area of 589 m^2m^{-3} reacting to the SALR increase. Reactors containing media with the surface area of 851 m^2m^{-3} were able to remove 70 to 79% while the other ones removed 68-77.5% of sCOD. This

was most probably because of the higher amount of biofilm formed on the media with higher available surface area (see section 3.2.7). Still, a difference in average removal of sCOD by two types of media of 0.1 kg $m^{-3}d^{-1}$, with a p-value of 0.81, inconsistent with the hypothesis that the sCOD removal by media with the surface area of 851 m^2m^{-3} was significantly higher than the other media type. Therefore, the performance of the two media types could be considered almost equal reacting to the SALR increase.

3.3.5. Surface Removal Rate (SRR)



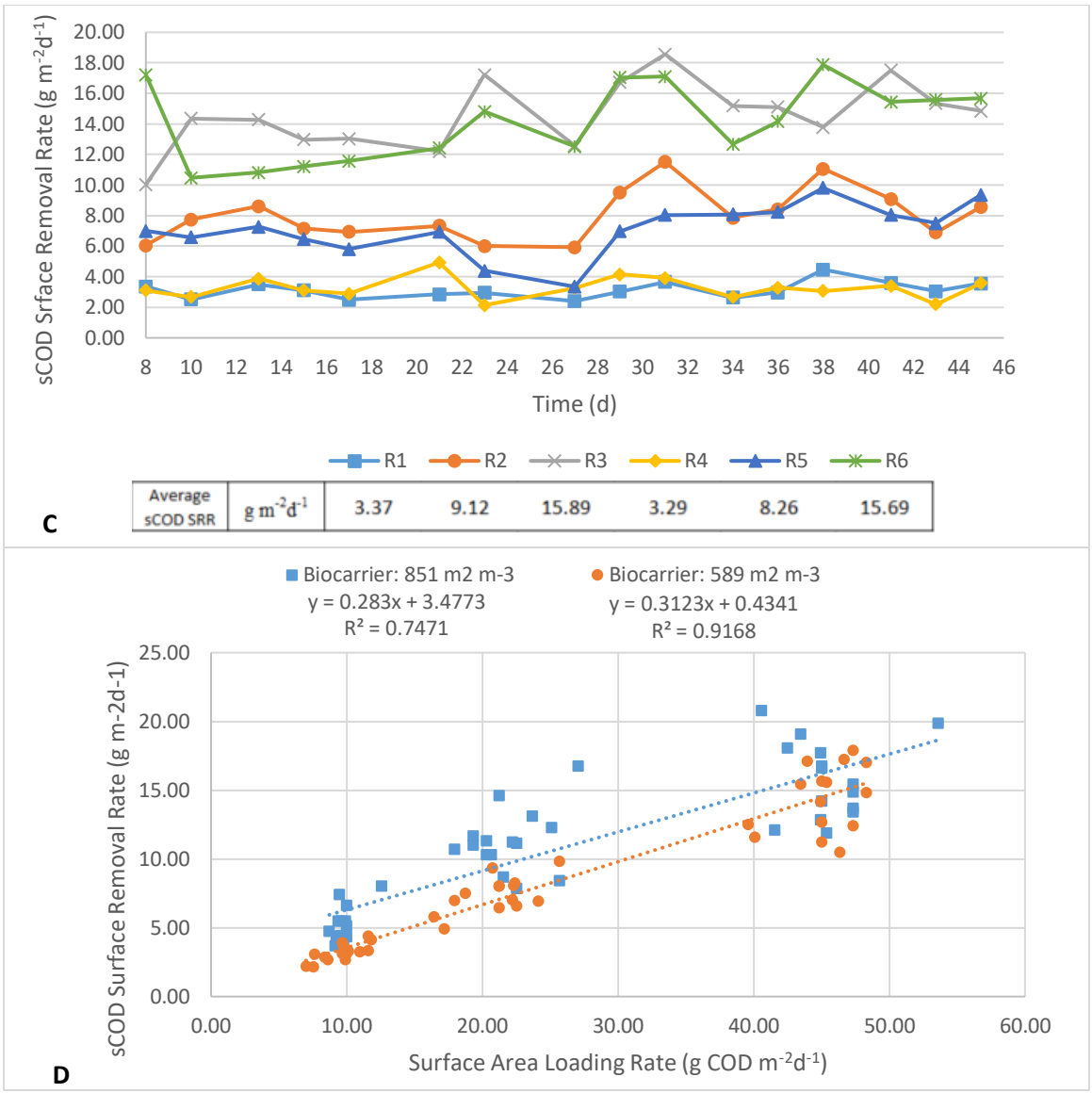


Figure 13- A) MBBRs performance in TCOD Surface Removal Rate (SRR); B) TCOD SRR correlation to SALR; C) MBBRs performance in sCOD Surface Removal Rate (SRR); D) sCOD SRR correlation to SALR

The surface removal rate is a good representative of biomass activity. Daily SRRs, calculated based on Equation 9, are plotted for TCOD and sCOD in Figure 13-A and Figure 13-C, respectively.

According to Metcalf and Eddy [4] (adapted from McQuarrie and Boltz [68] and WEF [57]), the typical BOD surface removal rate for secondary treatment by MBBR ranges from 5 to 15 g BOD $m^{-2}d^{-1}$. Again, by considering the average BOD/COD ratio of 0.4 in influent (See Table

6) and 0.2 in effluent [4], the average BOD removal in R1 to R6 could be calculated as 2.85, 7.22, 12.57, 2.73, 6.81, and 12.30 g BOD $m^{-2}d^{-1}$, respectively. The results showed that all reactors worked at the typical BOD surface removal rate for secondary treatment, but R1 and R4, of which the removal rates were lower than 5 g BOD $m^{-2}d^{-1}$ due to their influent SALR of 4 g BOD $m^{-2}d^{-1}$.

3.3.5.1. SALR influence

As it is noticeable by the results plotted in Figure 13, the SRR (i.e., biomass activity) increased when the SALR increased. The reason is that based on Equation 9, when the media surface area, FF, and the reactor's volume are constant, the SRR would change according to the influent flow rate (i.e., Q) and the removed substrate (i.e., S_0-S_e) shifts. In this study, increasing the SALR took place by raising the Q from 93 to 466 ml min^{-1}, which was that great that caused SRR to increase. The highest average SRRs for TCOD and sCOD were in the range of 14-17 and 15-16 g $m^{-2}d^{-1}$, respectively, and achieved at the HRT of 0.72 h.

Correlation coefficients of 0.7471 and 0.4907 were found between TCOD SRR and SALR for biocarrier with the surface area of 851 and 589 m^2m^{-3}, respectively, using a linear equation. Using the same equation, correlations of 0.7471 and 0.9168 were achieved between sCOD SRR and SALR for biocarrier with the surface area of 851 and 589 m^2m^{-3}, respectively. The results suggested that biofilms formed on both types of media could handle a SALR as high as 20 g BOD $m^{-2}d^{-1}$.

3.3.5.2. Carrier surface area influence

The t-test proved that sCOD SRR was almost independent of carrier surface area by comparing SRRs in R1 to R4 (p-value: 0.78), R2 to R5 (p-value: 0.20), and R3 to R6 (p-value: 0.81). However, the media with the surface area of 851 m^2m^{-3} showed slightly better performance than the 589 m^2m^{-3} media at all SALRs, especially at SALR of 9 g BOD $m^{-2}d^{-1}$.

In the case of TCOD SRR, it seemed that carrier surface area played a more critical role as the results were more diverse, especially at SALR of 18 g BOD $m^{-2}d^{-1}$ that the SRR in R3 was almost 2 g TCOD $m^{-2}d^{-1}$ higher than in R6. However, the p-value achieved by the t-test for SRR difference between R3 and R6 was 0.26, which proved that there was not enough evidence to conclude that one SRR was significantly greater than the other one. Therefore, TCOD SRR could also be considered independent of carrier surface area.

Yet, the overall results showed that the biofilms grown on each square meter of media in R1, R2, and R3 were capable of removing more substrates per day than the biofilms in the other three reactors. This can be justified by the higher quantity of biomass on each square meter of media in the first three reactors (See section 3.3.7).

3.3.6. Total and Volatile Suspended Solids (TSS and VSS)

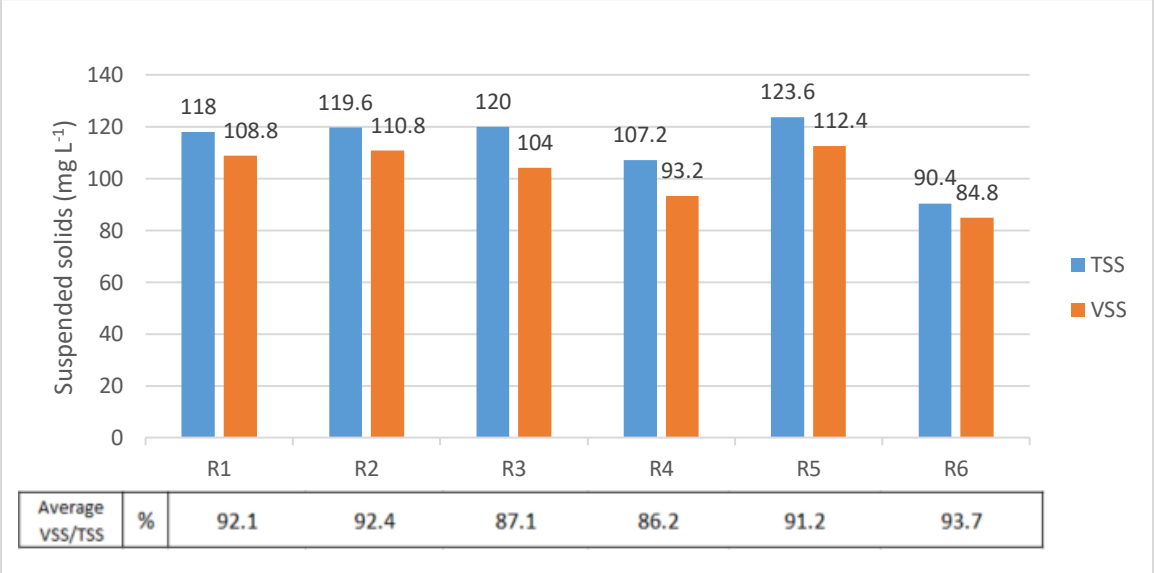


Figure 14 - Total and Volatile Suspended Solids in each reactor

The average effluent TSS, VSS, and their ratio during the steady-state period are reported in Figure 14. The data collected during the first month of the experiment (i.e., unsteady-state period) was not used in the calculation due to unsteady-state fluctuations.

Based on the Literature, the mixed liquor suspended solids (MLSS) concentration in the effluent of a MBBR may be in the range of 100 to 250 mg L^{-1} [4], [68]. The same condition was observed in all reactors but R6, of which the TSS average was even less than 100 mg L^{-1}. Overall, the TSS and VSS produced by reactors were all about ten times less compared to CAS solids production [4], [68], which proved that MBBR is a good option in case of its slight solids production and not requiring solids recycling.

The SS part of effluent is a composition of bacteria cells detached from the carriers, soluble microbial products, non-biodegradable organic matter (colloidal and soluble), and a portion of influent particulate matter that passes through the system [43]. Considering the high VSS/TSS ratios obtained in this experiment, it could be concluded that most of the SS part was formed by bacteria cells detached from the carriers, which could be used for bioenergy production.

3.3.7. Attached Biofilm Quantification

The biocarriers agglomerated with attached biomass and their biofilm quantities are shown in Figure 15. The biofilm quantification and captured microscopic images all have been obtained during the steady-state condition. Biofilm thickness was measured as explained in section 3.1.3, and the achieved results along with the biofilm mass data were then used to calculate biomass density as summarized in Table 9. A sample of calculations is provided in Appendix A.

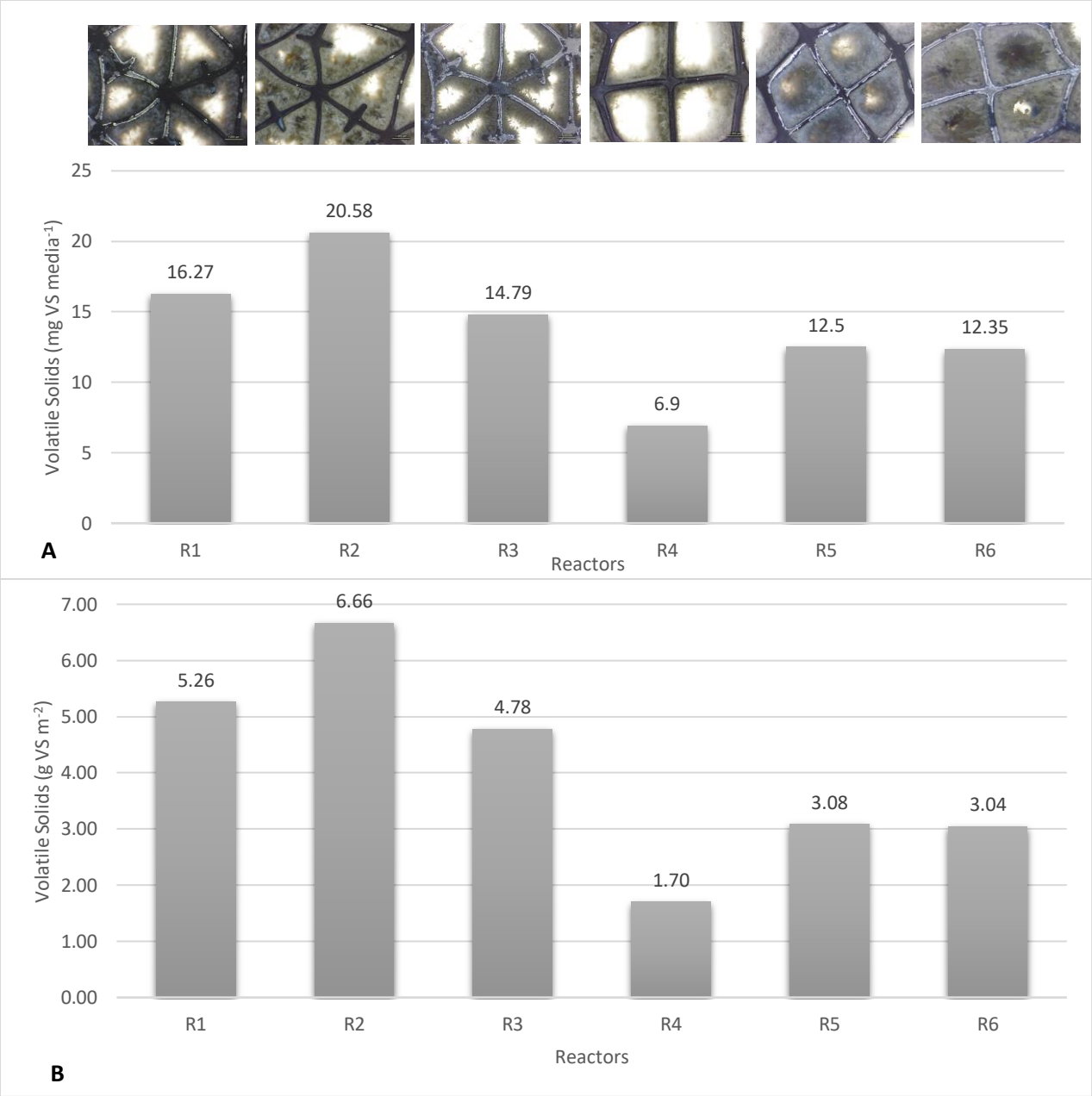


Figure 15 - Attached volatile solids concentrations: A) VS per one unit media and spectroscopic image of the middle opening of the media in each reactor; B) VS quantity per each square meter of media

Table 9 - Biofilm mass, thickness, and density at steady-state condition

Parameter	R1	R2	R3	R4	R5	R6	Unit
Biofilm mass per media	0.018	0.023	0.016	0.007	0.015	0.014	g TS media^{-1}
Biofilm thickness	347.69	460.83	403.33	439.39	529.26	463.89	µm
Biofilm density	0.016	0.015	0.013	0.004	0.007	0.007	g TS cm^{-3}

3.3.7.1. SALR influence

Although increasing biofilm mass and thickness by increasing SALR was expected, reactors working under the SALR of 9 g BOD $m^{-2}d^{-1}$ (i.e., R2 and R5) contained the most amounts of biofilm with the highest thickness compared to the other reactors including the same media. This might be because the HRT in R3 and R6 was that low that microorganisms did not have much time to uptake food and replicate. This has also been proven by relatively low TCOD and sCOD removal percentages in these reactors.

Another unexpected result was that the amount of biofilm attached to the media in R1 was more than the one in R3 despite its lower SALR. Still, biomass activity analysis showed that the attached biofilm in R1 is less active compare to R3 in COD removal (See section 3.2.8). Analyzing the ammonia removal results, on the other hand, showed that a part of biomass in R1 is composed of nitrifying bacteria as the nitrite and nitrate concentrations in this reactor are relatively high compare to the other ones (See section 3.2.9). Therefore, it could be concluded that the low SALR, corresponding to the low amount of available COD, provided a suitable condition for AOBs and NOBs to grow.

3.3.7.2. Carrier surface area influence

As it is clear by biofilm mass measurements, the amount of biofilm formed on media with the surface area of 851 m^2m^{-3} was higher than the other media type while it was thinner and denser. This could simply be justified by the greater available surface area of the mentioned media, which lets more biomass attach and disperse along the available surface.

3.3.8. Kinetics Studies

A kinetic test was performed on media, in the absence of suspended solids, out of the main reactors to estimate the contribution of attached biofilm, and consequently the suspended biomass,

to the overall performance. Figure 16 shows TCOD and sCOD utilization per time achieved by the test, which was used to calculate substrate utilization rate in each reactor.

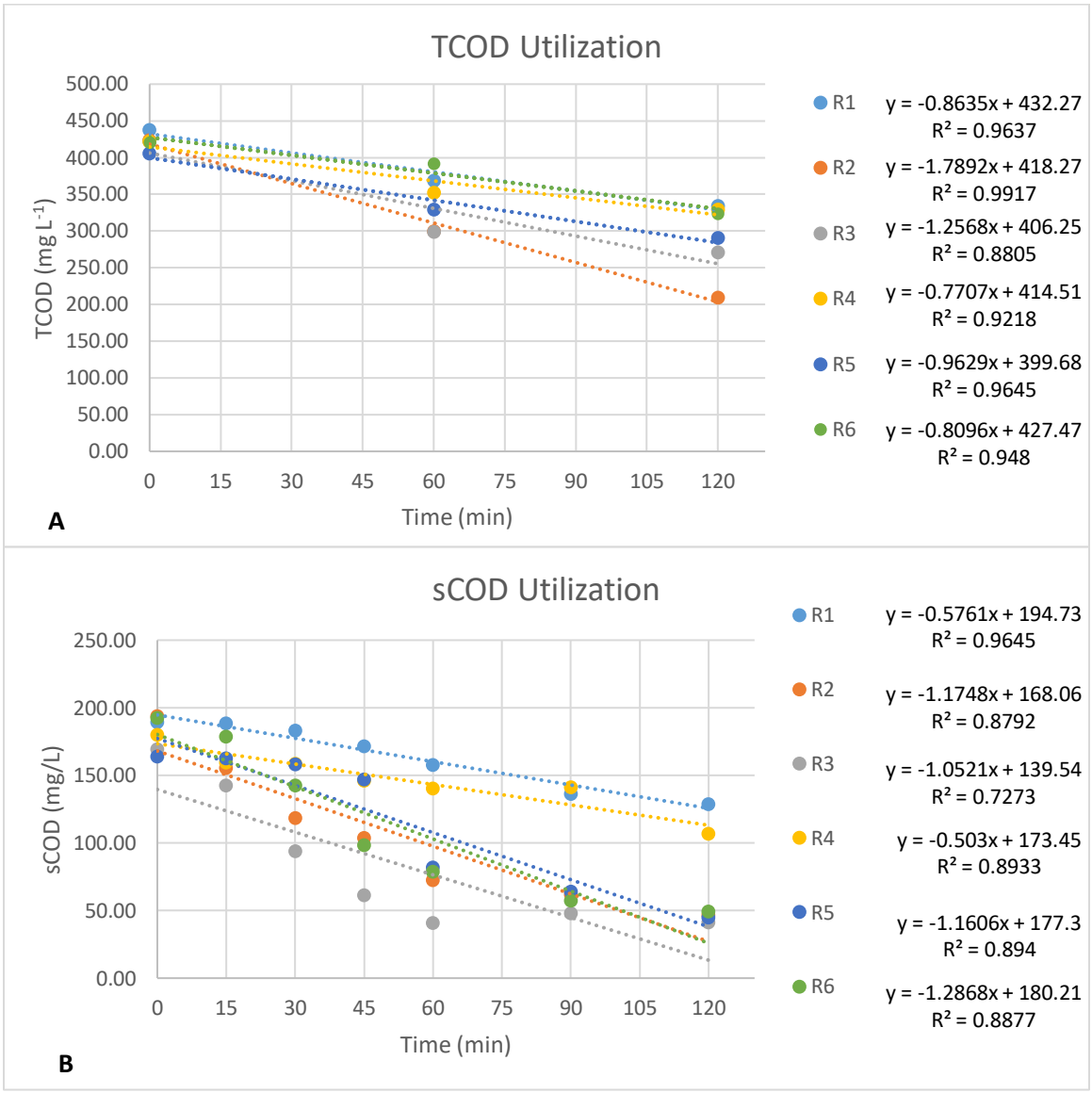


Figure 16 - Kinetic test results: A) TCOD utilization; B) sCOD utilization

Calculated removal rates (i.e., substrate utilization rates) are summarized in Table 10, and a sample of calculations is provided in Appendix A.

Table 10 - Substrate utilization rates

Parameter	R1	R2	R3	R4	R5	R6	Unit
TCOD Utilization rate	1.24	2.58	1.81	1.11	1.39	1.17	kg $m^{-3}d^{-1}$
	0.93	1.52	1.48	2.56	1.77	1.50	mg $mgVS^{-1}d^{-1}$
sCOD Utilization rate	0.85	1.69	1.52	0.72	1.67	1.85	kg $m^{-3}d^{-1}$
	0.63	1.00	1.24	1.67	2.13	2.39	mg $mgVS^{-1}d^{-1}$

The achieved data corresponded to the attached biofilm activity as the VSS present in the main reactors was eliminated for the kinetic test. Therefore, by comparing the total sCOD removal from section 3.3.4 and the achieved data by this test, the contribution of attached biofilm, and consequently the suspended biomass, to the overall performance could be estimated as shown in Figure 17.

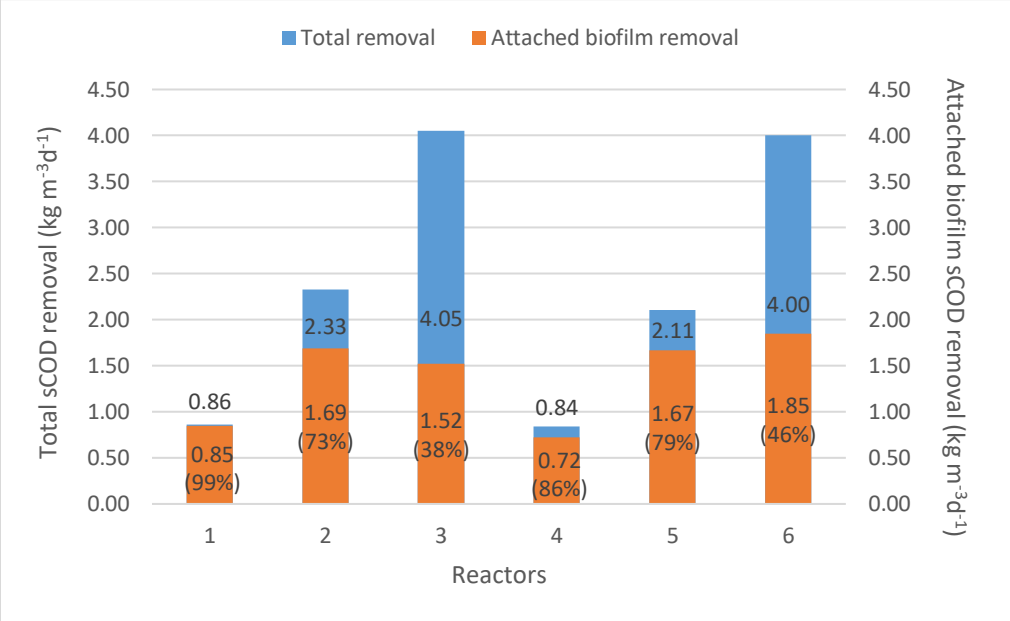


Figure 17 - Total sCOD removal compared to sCOD removal by attached biofilm in each reactor

3.3.8.1. SALR influence

Overall, increasing the SALR caused more sCOD to be removed, while the role of suspended biomass in removal got more significant at lower HRTs. This suggested that low HRT,

especially HRT of 0.72 h in this experiment, provided mass transfer limitations through the attached biofilm. Instead, suspended biomass had the opportunity to consume sCOD as a higher level of sCOD is available.

3.3.8.2. Carrier surface area influence

Comparing R1 to R4, R2 to R5, and R3 to R6, which were working at the same SALR but included different biocarrier types, showed that not only the overall sCOD removal but also the contribution of the attached biofilm in sCOD removal were almost independent of carrier surface area. However, at the SALR of 4 g BOD $m^{-2}d^{-1}$, attached biofilm to the media with the surface area of 851 m^2 m^{-3} showed more activity than the other type (99% contribution in R1 compared to 86% contribution in R4). On the other hand, at two other SALRs, the attached biofilms to the media with the surface area of 589 m^2 m^{-3} were more involved in sCOD removal than the other media type. This showed that the mass transfer limitation was slightly stronger in the biofilm form on the media with the surface area of 851 m^2 m^{-3} at high SALRs than the other media type, which was expected due to its higher biofilm mass and density.

3.3.9. Ammonium Removal

The Flow Injection Analysis (FIA) was done for ammonium, nitrite, and nitrate concentrations assessment to assure that the population of the AOBs and NOBs was negligible and most of the active microorganisms were responsible for COD removal. The average of obtained results are as follows:

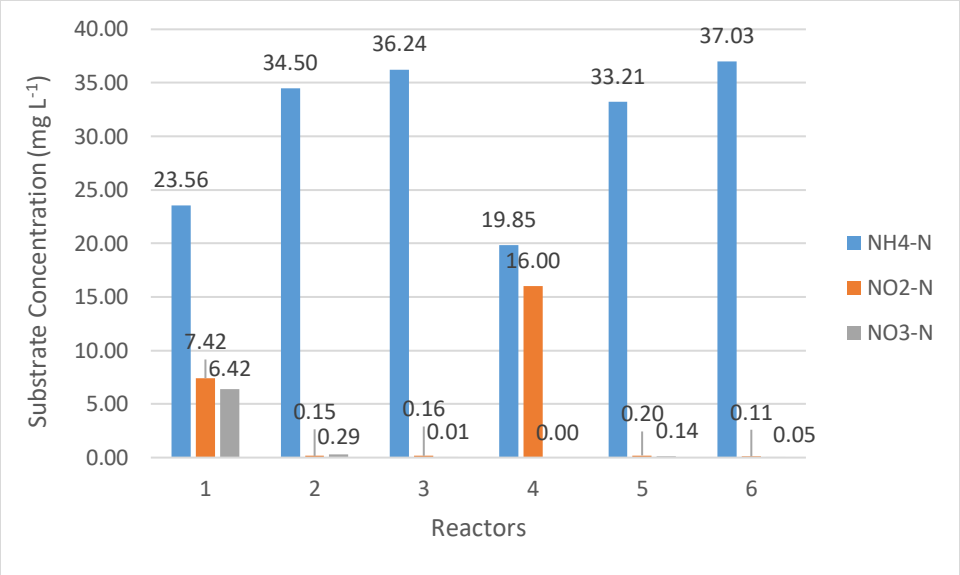


Figure 18 - Average Ammonium, Nitrite, and Nitrate concentrations in each reactor

As shown in Figure 18, the operational condition in R1 and R4 caused nitrifying bacteria growth contrary to other reactors. A part of biomass in R1 was composed of both AOBs and NOBs, while the biomass in R4 included AOBs. These results could justify the relatively low biomass activity in sCOD removal within the two mentioned reactors, especially in R1.

3.4. Conclusions and Future Work

3.4.1. Conclusion

Six pilot-scale MBBRs were set up and operated under various operational conditions treating real municipal wastewater. Three of them were filled up to 30% of their volume by media with the surface area of 851 m^2m^{-3}. The other three were filled up to 43.3% by media with the surface area of 589 m^2m^{-3}, providing 5 m^2 of available surface area in each reactor. The operational SALRs for every three rectors were 4, 9, and 18 g BOD $m^{-2}d^{-1}$, corresponding to the HRTs of 3.58, 1.43, 0.72 h, respectively. The influences of media surface area and SALR on MBBR performance in COD removal were studied.

By assessing the impact of SALR, it was found that MBBRs were able to remove sCOD with efficiency above 70% at the SALRs of 4 and 9 g BOD $m^{-2}d^{-1}$. However, the highest performance in sCOD removal (79.10%) was achieved by media with the surface area of 851 m^2m^{-3} at the SALR of 9 g BOD $m^{-2}d^{-1}$ (i.e., by R2), which corresponded to removing 2.33 kg $m^{-3}d^{-1}$ of sCOD and 1.84 kg $m^{-3}d^{-1}$ of BOD. The biomass present in this reactor removed 12.18 g TCOD and 9.12 g sCOD per square meter of media per day that represented the biomass activity. The performance decreased to 70% and slightly less than that (i.e., 68.69%) by increasing the SALR to 18 g BOD $m^{-2}d^{-1}$ (i.e., in R3 and R6). Still, about 4 and 3 kg $m^{-3}d^{-1}$ of sCOD and BOD were removed, respectively, by the mentioned rectors while their biomasses were able to remove 15.5-16 g sCOD $m^{-2}d^{-1}$.

Correlation curves describing the percentage of substrate removal vs. surface area loading rate were generated for both TCOD and sCOD. By Employing linear equations, correlation coefficients of 0.5942 and 0.6751 were found between TCOD removal and SALR for biocarrier with the surface area of 851 and 589 m^2m^{-3}, respectively. Relatively weak correlation coefficients of 0.1824 and 0.383 were also found between sCOD removal and SALR for biocarriers with surface areas of 851 and 589 m^2m^{-3}, respectively. Therefore, more SALR variations and their impact on COD removal need to be investigated to provide strong correlated equations relating the two parameters.

It was found that the carrier shape and surface area did not significantly influence MBBRs performance under the circumstances of this experiment. Even though R1, R2, and R3 were able to remove slightly more percentage of sCOD, the efficiency differences to the reactors containing the other type of media (i.e., R4, R5, and R6) were that small that could be neglected (i.e., 70-79% compared to 68-77% of sCOD removal). Moreover, the average sCOD removal (kg $m^{-3}d^{-1}$), total

biomass activity (SRR), and the attached biofilm activity (achieved by the kinetic test) were also very close for the reactors with different media working at the same SALR. This confirmed the almost equal efficiencies of different media types and the independency of MBBRs performance of carrier surface area. Still, the media with the surface area of 851 m^2 m^{-3} developed a thiner biofilm without providing mass transfer limitation compared to the other carrier type.

TSS and VSS produced by reactors were all in the range of 90 to 125 mg L^{-1}. This range was in agreement with the MLSS concentration of MBBRs reported in the litretures and was about ten times less compared to CAS solids production.

Kinetic studies showed that the attached biofilms formed on both media types at the SALR of 4 g BOD $m^{-2}d^{-1}$ were the most active ones (i.e., with the lowest mass transfer limitations) with 86-99% involvement in sCOD removal. Although increasing the SALR caused enhancement in sCOD removal (from about 0.8 to 4 kg $m^{-3}d^{-1}$ of sCOD), the role of suspended biomass got more significant at lower HRTs. This suggested that low HRT (especially HRT of 0.72 h, corresponding to the SALR of 18 g BOD $m^{-2}d^{-1}$) provided mass transfer limitations through the attached biofilm. Consequently, suspended biomass had the opportunity to consume sCOD.

3.4.2. Future Work

As it was found that the reactor's performance improved by increasing the SALR from 4 to 9 g BOD $m^{-2}d^{-1}$ and then deteriorated at the SALR of 18 g BOD $m^{-2}d^{-1}$, it would be beneficial to find the exact SALR at which the maximum efficiency may occur. Therefore, assessing the impact of SALR greater than 9 and smaller than 18 g BOD $m^{-2}d^{-1}$ on sCOD removal can be a potential experiment to be done in future. This may help find the optimum SALR with greater efficiency than 79% while working at a relatively low HRT.

Chapter 4: Sugar Industry Wastewater Treatment

4.1. Introduction

Sugar industry wastewater includes high levels of biological oxygen demand, 1.7 - 7 g BOD L^{-1}, and chemical oxygen demand, from 2.3 up to 10 g COD L^{-1}, due to the presence of sugar and organic matters from cane or beet [48], [80]. Based on the Environmental, Health, and Safety (EHS) guidelines for sugar manufacturing, this sector's effluent must include less than 50 mg BOD L^{-1}, 250 mg COD L^{-1}, and 50 mg TSS L^{-1} to be discharged directly to surface waters for general use. However, site-specific discharge levels may vary based on associated sewage collection and treatment systems [48]. In Canada, for instance, it is expressed in the general regulation that the average carbonaceous BOD and suspended solids should not exceed 25 mg BOD L^{-1} and 25 mg TSS L^{-1}, respectively [54].

Various types of anaerobic treatment processes such as anaerobic batch reactor, anaerobic fixed-bed reactor (AFR), up-flow anaerobic fixed-bed (UAFB), and up-flow anaerobic sludge blanket (UASB) have been studied for sugar industry wastewater treatment as reviewed by Kushwaha [81]. However, lack of capability of anaerobic processes in the degradation of oil and grease [82] and not meeting the effluent discharge restrictions are the main disadvantages of utilizing these systems alone. Therefore, combinations of anaerobic and aerobic systems have become a typical practice for organic matter removal from sugar industry wastewater. Still, shortage of space and extreme odor emission can be limiting factors for some plants to use combined systems, making aerobic systems a potential option to be employed alone. Aerobic systems such as aerated lagoons and pounds, batch reactors, and aerated submerged fixed-film (ASFF) processes have been examined for sugar industry wastewater treatment [83]–[85]. Yet, further studies on relatively new technologies are still sought to provide an efficient WWTP.

In this study, aerobic moving bed biofilm reactor (MBBR) efficiency in organic matter removal from synthetic sugar industry wastewater is evaluated. The functionality of BioPorts media with different surface areas under various Surface Area Loading Rates (SALRs) is analyzed to find both optimum media surface area and SALR for MBBR treating very strong and concentrated wastewater.

4.2. Material and Methods

4.2.1. MBBR Setup

The second phase of the experiment was conducted to evaluate the MBRR's performance in COD removal from very concentrated wastewater, synthetic sugar industry wastewater, with initial BOD and COD of 5000 mg BOD L^{-1} and 8650 mg COD L^{-1}, respectively. Just as in the previous phase, the influences of carrier surface area and SALR on MBBRs' efficiency were investigated.

In this stage, three 20 L aerobic moving bed biofilm reactors were set up and filled to 43.3, 30, and 27.1% by volume with BioPorts media with a surface area of 589, 851, and 942 m^2 m^{-3}, respectively. The filling fractions were selected, providing 5.1 m^2 of protected surface area available for biofilm development in each reactor. Three types of biocarriers used in this study are shown in Figure 5.

Reactors were receiving influent at the SALR starting from 1 g BOD $m^{-2}d^{-1}$ and increasing to 28 g BOD $m^{-2}d^{-}1$ gradually for the first month of operation to achieve bacteria growth and stabilization. The SALR was kept at 28 g BOD $m^{-2}d^{-1}$ for one month to investigate the MBBRs' performance during the stabilized and steady-state condition. It was then increased to 55 g BOD $m^{-2}d^{-1}$ and kept as is for the next two months as the steady-state condition was not reached by one-

month operation. Finally, reactors were again receiving influent with the SALR of 28 g BOD $m^{-2}d^{-1}$ for the last five weeks of the experiment to investigate the required time for excess bacteria detachment, biofilm thickness adjustment, and efficiency recovery.

Air was diffused, at a rate of 45.3 L min^{-1}, to supply oxygen to the microorganisms for biological activity and provide mixing. The reactor design parameters for this phase are summarized in Table 11.

Table 11 - Reactor configurations for BOD removal from sugar industry wastewater

	Parameters	Reactor 1	Reactor 2	Reactor 3	Unit
	Biocarrier SA	589	851	942	$m^2 m^{-3}$
	FF	43.3	30	27.1	%
	Available SA	5	5	5	m^2
	$V_{reactor}$	20	20	20	L
	Airflow	45.3	45.3	45.3	L min^{-1}
First stage	**SALR**	28	28	28	g BOD $m^{-2}d^{-1}$
	Q	20	20	20	ml min^{-1}
	HRT	17.05	17.05	17.05	h
Second stage	**SALR**	55	55	55	g BOD $m^{-2}d^{-1}$
	Q	39	39	39	ml min^{-1}
	HRT	8.53	8.53	8.53	h
Third stage	**SALR**	28	28	28	g BOD $m^{-2}d^{-1}$
	Q	20	20	20	ml min^{-1}
	HRT	17.05	17.05	17.05	h

Just as in the previous phase, no seeding was required for the heterotrophic bacteria attachment and growth. The biofilm started to form on the carriers' protected surface area by introducing the wastewater and air to the reactors. However, despite the municipal wastewater treatment phase, the accumulation and reaching to the steady-state condition were much more challenging for the industrial wastewater treatment. Huge foaming and pH drop were continuously happening before the steady-state condition. Yet, after almost one month, reactors got stabled, and foaming and pH drops were stopped.

This phase lasted five months in total; one month was spent for the accumulation and getting stabled while the rest four months were spent on testing the reactors' efficiency under different SALRs.

4.2.2. Wastewater Characteristics

During the experiment period, reactors were continuously receiving the synthetic sugar industry wastewater. Conducted tests during the accumulation period showed a significant drop in pH (from ~7 to ~4.5) and COD level (from ~90000 to ~7000 mg COD L^{-1}) of the feed after almost four and half days. Therefore, the fresh feed was being prepared two to three times a week to avoid anaerobic microorganism growth in the feed tank, which was the reason for pH and COD drop due to a very high level of available food (i.e., COD).

Synthetic wastewater was being prepared so that its COD concentration meets the average amount of 8650 mg COD L^{-1}. This COD corresponds to 5000 mg BOD L^{-1} as the average BOD/COD ratio was measured to be 0.58, which agrees with the literature where BOD/COD ratios in the range of 0.31 to 0.69 have been reported for sugar industry wastewater [86]–[88]. Measured TCOD and sCOD, however, were in the range of 7500-10500 mg COD L^{-1} during the experiment period. The feed's ammonia and phosphorus concentrations were kept as 500 mgNH4-N L^{-1} and 50 mgPO4-P L^{-1}, respectively, to assure bacteria growth under BOD:N:P ratio of 100:10:1. 1 g L^{-1} of Sodium bicarbonate was also added to the feed to avoid pH drop in the feed tank as a result of possible anaerobic bacteria growth and activity due to very high COD content. Both TSS and VSS were almost 100 mg L^{-1}, based on the measurements, and the PH value was 6 ± 0.5. The synthetic sugar industry wastewater recipe and characteristics are outlined in Table 12.

Table 12 - Synthetic sugar industry recipe and characteristics

Recipe		Characteristics (g L^{-1})
Chemical	**Amount (g L^{-1})**	
Sugar	8	BOD_5 = 5 ± 1 TCOD = 9 ± 1.5 sCOD = 9 ± 1.5 NH4-N = 0.5 PO4-P = 0.05 TSS = 0.102 ± 0.06 VSS = 0.102 ± 0.06 Alkalinity = 0.815 $CaCO_3$
Ammonium Chloride	2.5	
Potassium Phosphate	0.25	
Sodium bicarbonate	1	

4.2.3. Analytical Methods

Operational parameters including DO, temperature, and pH were recorded using a Thermo Scientific STARA2230 DO Meter and Alpha pH200 meter (Eutech Instruments, USA), respectively, three times a week to make sure that reactors are working under the suitable condition. The MBBRs' influent and effluents were tested three times a week for TCOD and sCOD through spectrophotometric methods according to the Standard Methods [78]. The obtained data was then used for calculating the percentages of the removed TCOD and sCOD and surface removal rate using Equation 9.

Effluent TSS and VSS were also measured using 20 ml of effluent sample from each reactor, which was filtered into a single crucible equipped with glass microfiber filters for TSS and VSS determination based on the Standard Methods [78]. After filtration, the samples were dewatered at 105˚C for 24h and weighted for the TSS calculations. Then, they were placed in a 550˚C oven for 2h and weighted again for the VSS calculations.

To monitor biofilm development, three biocarriers were removed from each reactor as triplicates once a week. Zeiss SteREO Discovery.V8 Microscope was used for observing the biofilm shape, distribution, and thickness formed on the carriers. Despite the previous phase, municipal wastewater treatment, measuring the biofilm thickness using CMEIAS-IT software was not doable while treating industrial wastewater since all media types under the experimental conditions got almost clogged (but the media with the surface area of 589 m^2 m^{-3} at SALR of 28 g BOD $m^{-2}d^{-1}$). This phenomenon made the boundary of the attached biofilm unclear and hard to be detected for thickness measurements.

Biocarriers total solids (TS) and volatile solids (VS) were quantified by mechanical (i.e., manual) biofilm removal using two biocarriers in a 100 mL beaker in triplicates. After manually removing biofilm from biocarriers using a brush and deionized water, the same steps as TSS and VSS measurements were done to achieve biocarriers TS and VS. The density of the biofilm, however, remained unknown due to difficulties in thickness measurements. Biofilm quantification was performed once at the steady-state condition for the first and second stages (i.e., SALRs of 28 and 55 g BOD $m^{-2}d^{-1}$).

To achieve the organic matter removal rate representing attached biofilm performance, the Kinetic test was done once at steady-state in the last week of each first and second stage. Two 1 L beakers were filled with 0.433 L of media with surface area of 589 m^2m^{-3} (FF = 43.3%), two others were filled with 0.3 L of media with surface area of 851 m^2m^{-3} (FF = 30%), and the last two were filled with 0.271 L of media with surface area of 942 m^2m^{-3} (FF = 27.1%) as duplicates. Reactors were filled up to 1 L with the synthetic wastewater and started receiving air at a 2.27 L min^{-1} flowrate. Samples were collected every 15 minutes, starting from time zero to 1 hour and every 30

minutes for the next 1 hour. Finally, TCOD and sCOD tests were accomplished to achieve the rate of organic matter removal.

The MBBRs' effluents were tested three times during the steady-state conditions for ammonium, nitrite, and nitrate concentrations assessment by Flow Injection Analysis (FIA) according to the Standard Methods [78]. This test was done to assure that the population of the AOBs and NOBs was negligible and most of the active microorganisms were responsible for BOD removal.

4.3. Results and Discussion

4.3.1. Operational Condition

The first month of the experiment was considered an accumulation and stabilization period. Therefore, data obtained during that time was not considered in any calculation.

The averages of monitored pH, temperature, and DO of the mixed liquid in the reactors after the stabilization period are presented in Table 13. As a result of anaerobic activities in the feed tank, a pH drop in influent was occasionally happening, causing a pH drop in reactors. Even though the variation range was 5 to 7.5, no alternation in microbial activity in COD removal was observed. Still, pH below 6 was immediately raised to above 7 by adding sodium bicarbonate to the reactors.

Table 13 - Operational parameters

Parameter	R1	R2	R3	Unit
pH	5.8 ± 0.8	6 ± 1	6.2 ± 1.1	-
T	19.7 ± 0.3	19.1 ± 0.4	18.7 ± 0.4	˚C
DO	5.9 ± 0.9	6.4 ± 1.1	6.7 ± 1.3	mg L^{-1}

4.3.2. Total Chemical Oxygen Demand (TCOD) Removal

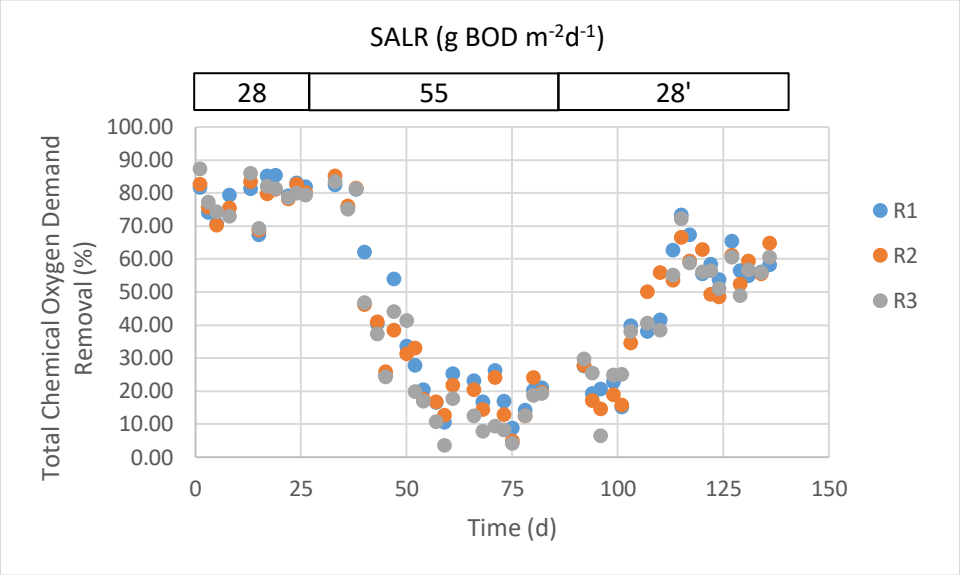


Figure 19 - MBBRs performance in Total Chemical Oxygen Demand (TCOD) removal at different SALRs

After the first month of stabilization, reactors were almost at the steady-state condition with an average TCOD removal of 78-79%. However, after increasing the SALR to 55 g BOD $m^{-2}d^{-1}$, reactors' efficiencies gradually declined. It took almost 26 days for the reactors to reach steady-state, with the average TCOD removal of 11-19%, under the new operational condition. After the second operational adjustment (i.e., decreasing the SALR from 55 to 28 g BOD $m^{-2}d^{-1}$), systems' efficiencies gradually improved again, which caused an unsteady-state condition for 22 days. After that, relatively stabled performances with the average TCOD removal of 57-61% were achieved.

The averages of TCOD removal by each reactor during the steady-state condition at different operational SALRs are reported in Table 14.

Table 14 - Average TCOD removal by each reactor at different SALRs

Reactors	**R1**			**R2**			**R3**			**Unit**
SALR	28	55	28'	28	55	28'	28	55	28'	g BOD $m^{-2}d^{-1}$
Average TCOD removal	79.03	18.34	60.19	78.01	16.81	57.62	78.97	11.81	57.53	%
Average TCOD removal	10.73	5.54	7.53	10.58	5.07	7.21	10.72	3.58	7.20	kg $m^{-3}d^{-1}$

4.3.2.1. SALR influence

At the HRT of 17 h, corresponding to SALR of 28 g BOD $m^{-2}d^{-1}$, MBBRs removed about 78-79% of TCOD, while the removal efficiency started to decrease significantly once the SALR was increased to 55 g BOD $m^{-2}d^{-1}$ as shown by Figure 19 and reported in Table 14. After one month of stabilization under the new operational condition, averages of TCOD removal in all reactors declined to the range of 11 to 19% at the SALR of 55 g BOD $m^{-2}d^{-1}$, corresponding to the HRT of 8.5 h. Moreover, the average amount of removed TCOD (kg $m^{-3}d^{-1}$) also reduced significantly from >10.5 to 4.67±1 kg $m^{-3}d^{-1}$ by increasing the SALR in all three reactors. This apparent efficiency reduction could be justified by the excess biofilm growth on the internal surface of all types of media, limiting mass transfer and causing performance diminution (See section 4.2.6).

Once SALR was set to 28 g BOD $m^{-2}d^{-1}$ again, reactors' efficiencies increased gradually by 41.8%, 40.8%, and 45.7% in R1, R2 and R3, respectively. This recovery could be justified by biofilm thickness adjustments (See section 4.2.6), which took place due to increasing the HRT from 8.5 to 17 h, reducing the mass transfer limitations. Even though both TCOD removal and the average amount of removed TCOD (kg $m^{-3}d^{-1}$) were enhanced remarkably in all three reactors, a 100% recovery (i.e., obtaining the same results as the first stage) could not be reached in any of them. This was possibly due to the uneven biofilm thickness adjustment, which is clear by microscopic pictures in Table 19, preventing the mass transfer limitation from being totally eliminated.

4.3.2.2. Carrier surface area influence

At the SALR of 28 g BOD $m^{-2}d^{-1}$, TCOD removal was almost independent of media surface area. Yet, media with the surface area of 589 m^2m^{-3} showed the highest TCOD removal, which

was 79.03%, corresponding to the removal of 10.73 kg $m^{-3}d^{-1}$ of TCOD. The best performance of this type of media got more considerable at the SALR of 55 g BOD $m^{-2}d^{-1}$, where R1 removed 18.34% of TCOD, corresponding to the removal of 5.54 kg COD $m^{-3}d^{-1}$, compare to the 16.81 and 11.81% of TCOD removal in R2 and R3, respectively. This showed that the lower amount of biofilm formed on the media with the surface area of 589 m^2m^{-3} (See section 4.2.6), along with its wider openings, caused less TCOD mass transfer limitations at the first two stages. The p-values achieved by t-test for removal difference between R1 and R2 (p-value: 0.53) and R1 and R3 (p-value: 0.01) proved that the performance of media with the surface area of 589 m^2m^{-3} in TCOD removal (kg $m^{-3}d^{-1}$) was more remarkable than the media with the surface area of 942 m^2m^{-3}. Still, its efficiency was not notable compared to media with the surface area of 851 m^2m^{-3}.

At the second set of SALR of 28 g BOD $m^{-2}d^{-1}$, R1 showed the best performance again with the steady-state average of 60.19% of TCOD removal, while R2 and R3 were able to remove 57.62 and 57.53% of TCOD, respectively. This carrier type's relatively good recovery performance was again due to its even and wide openings, which caused the shear force to be distributed almost evenly, leaving a biofilm with a sufficient thickness and quantity (See section 4.2.6). However, based on the calculated p-values, 0.55 and 0.53 for removal difference between R1 - R2, and R1 - R3, respectively, there was not enough evidence to conclude that the mentioned differences are notable. Therefore, the recovery could also be considered independent of carrier surface area.

4.3.3. Soluble Chemical Oxygen Demand (sCOD) Removal

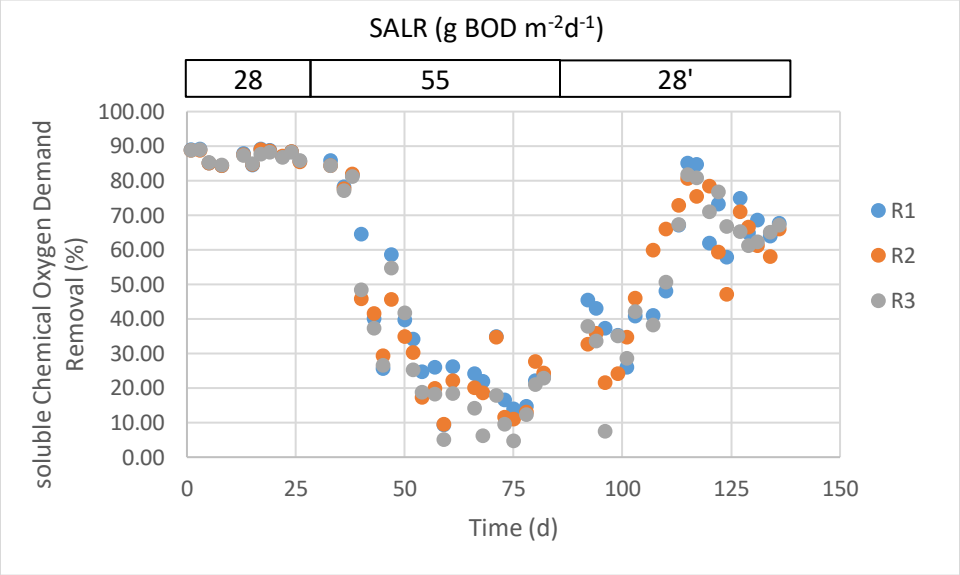


Figure 20 - MBBRs performance in Soluble Chemical Oxygen Demand (sCOD) removal at different SALRs

Reaching steady-state condition for sCOD removal was the same as TCOD removal in each stage, as shown in Figure 20. Tests were started to be done when the reactors were at the steady-state condition at the first stage, while it took 26 and 22 days for the reactors to stabilize after the first (28 to 55 g BOD $m^{-2}d^{-1}$) and second (55 to 28 g BOD $m^{-2}d^{-1}$) SALR shifts, respectively.

The averages of sCOD removal by each reactor during the steady-state condition at operational SALRs are reported in Table 15.

Table 15 - Average sCOD removal by each reactor at different SALRs

Reactors	**R1**			**R2**			**R3**			**Unit**
SALR	28	55	28’	28	55	28’	28	55	28’	g BOD $m^{-2}d^{-1}$
Average sCOD removal	87.17	21.49	70.02	87.06	19.18	66.95	86.97	14.10	69.58	%
Average sCOD removal	11.28	6.25	8.35	11.25	5.58	8	11.23	4.14	8.28	kg $m^{-3}d^{-1}$

Some biological systems used for sugar industry wastewater treatment, reported in the literature, are summarized in Table 16. By comparing the performance of the MBBRs in this research to other reactor types, it could be stated that MBBRs with all three types of media showed sufficient efficiency, removing ~87% and >11 kg $m^{-3}d^{-1}$ of sCOD while working at the SALR of 28 g BOD $m^{-2}d^{-1}$ and HRT of 17 h. HRT of 8.5 h, on the other hand, did not provide an acceptable proficiency for such a high SALR (i.e., 55 g BOD $m^{-2}d^{-1}$), corresponding to a loading rate of 24.27 kg COD $m^{-3}d^{-1}$ and 14.02 kg BOD $m^{-3}d^{-1}$, for none of the media types. Therefore, according to the literature, an aerated submerged fixed-film reactor could be a better option than MBBR for such high SALRs, 5-120 g BOD $m^{-2}d^{-1}$, providing COD removal in the range of 67.8 to 73.6% [85].

Table 16 - Case studies on sugar industry wastewater treatment using biological methods

Reactor type	Waste type	OLR	SALR	HRT	%COD removal	Reference
Aerated submerged fixed-film reactor	Cane Sugar	-	5-120 g BOD $m^{-2}d^{-1}$	8 - 2 h	67.8 - 73.6%	[85]
Up-flow anaerobic fixed bed	Beet Sugar	7.8-9.6 kg COD $m^{-3}d^{-1}$	-	20 h	75 - 93%	[86]
Up-flow anaerobic sludge blanket	Cane Sugar	8-16 kg COD $m^{-3}d^{-1}$	-	6 h	80.6 - 89.4%	[89]
Anaerobic down-flow stationary fixed-film	Synthetic	-	-	48 - 6 h	41 - 81.8%	[90]

4.3.3.1. SALR influence

The impact of SALR on sCOD removal was observed to be the same as its influence on TCOD removal efficiency. As shown by Figure 20 and reported in Table 15, the sCOD removal was about 87%, removing 11.25±0.02 kg COD $m^{-3}d^{-1}$ at HRT of 17 h, corresponding to the SALR of 28 g BOD $m^{-2}d^{-1}$, independent of the media surface area. At the SALR of 55 g BOD $m^{-2}d^{-1}$, on the other hand, the sCOD removal decreased to 14 to 22% and 4.14 to 6.25 kg $m^{-3}d^{-1}$. The excess

biofilm growth on the internal surface of all types of media, limiting mass transfer and causing performance diminution, was again the reason for poor performance at such a low HRT.

By lessening the SALR from 55 to 28 g BOD $m^{-2}d^{-1}$, reactors' performances in sCOD removal were recovered gradually by 48.5, 47.8, and 55.5% in R1, R2, and R3, respectively. Biofilm thickness adjustment and mass transfer limitation reduction were the main reasons for what was mentioned. Even though a 100% recovery (i.e., obtaining the same results as the first stage) could not be reached in any of the reactors, sCOD removals in the range of 67 to 70%, corresponding to removal of 8-8.35 kg $m^{-3}d^{-1}$ of sCOD, were achieved after the last SALR adjustment.

4.3.3.2. Carrier surface area influence

As mentioned above, the reactors' performances were almost independent of the carrier surface area while working at the HRT of 17 h. Yet, media with the surface area of 589 m^2m^{-3} showed the highest sCOD removal, 87.17%, corresponding to the removal of 11.28 kg COD $m^{-3}d^{-1}$. The best performance of this type of media got more considerable at the SALR of 55 g BOD $m^{-2}d^{-1}$, where R1 removed 21.49% of sCOD, corresponding to 6.25 kg $m^{-3}d^{-1}$, compared to 19.18 and 14.10% of sCOD removal in R2 and R3, respectively. This again showed that the lower amount of biofilm formed on the media with the surface area of 589 m^2m^{-3}, along with its wider openings, caused less mass transfer limitations at low HRTs. The p-values achieved by t-test for removal differences between R1 and R2 (p-value: 0.47) and R1 and R3 (p-value: 0.02) also proved that the performance of media with the surface area of 589 m^2m^{-3} in sCOD removal (kg $m^{-3}d^{-1}$) was more remarkable than the media with the surface area of 942 m^2m^{-3}. Still, its efficiency was not notable compared to media with the surface area of 851 m^2m^{-3}.

At the second set of SALR of 28 g BOD $m^{-2}d^{-1}$, reactors' performances showed to be almost independent of the carrier surface area again. R1 and R3 showed very close efficiency with the steady-state average of 70.02 and 69.58% of TCOD removal, respectively, while R2 removed 66.95% of TCOD. The least biofilm thickness adjustment that media with the surface area of 851 m^2 m^{-3} experienced (See Table 19) could be the reason for its relatively lower recovery.

4.3.4. Surface Removal Rate (SRR)

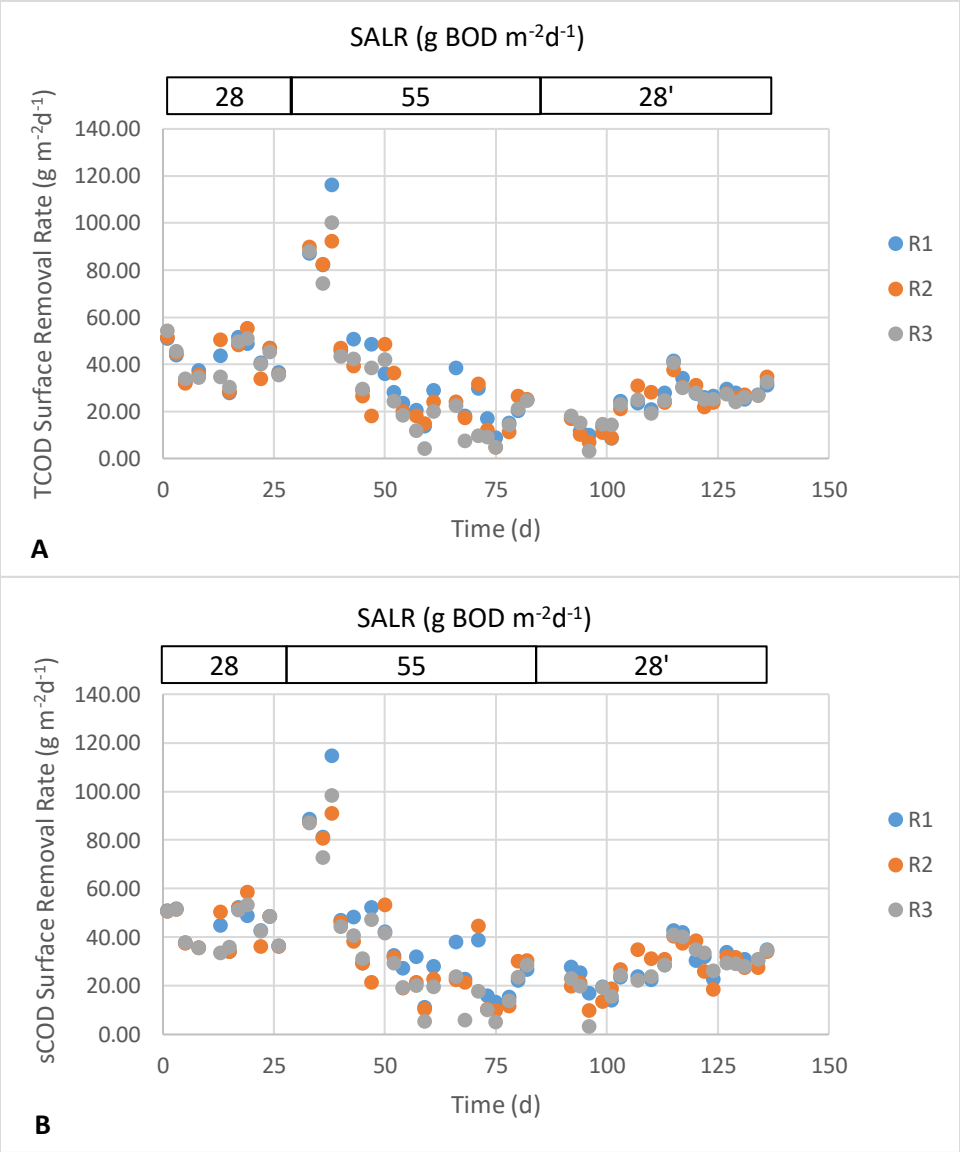


Figure 21 - MBBRs performance in: A) TCOD surface removal rate; B) sCOD surface removal rate

Daily SRRs, calculated based on Equation 9, are plotted for TCOD and sCOD in Figure 21 A and B, respectively. Also, the average SRRs for each reactor during the steady-state condition at different SALRs are reported in Table 17.

Table 17 - Average TCOD and sCOD surface removal rates for each reactor at different SALRs

Reactors	**R1**			**R2**			**R3**			**Unit**
SALR	28	55	28'	28	55	28'	28	55	28'	g BOD $m^{-2}d^{-1}$
Average TCOD SRR	42.08	21.71	29.53	41.49	19.90	28.29	42.04	14.04	28.24	g TCOD $m^{-2}d^{-1}$
Average sCOD SRR	44.22	24.50	32.76	44.11	21.88	31.38	44.05	16.22	32.47	g sCOD $m^{-2}d^{-1}$

4.3.4.1. SALR influence

Based on Equation 9, when the media surface area, FF, and the reactor's volume are constant, the SRR would change according to the influent flow rate (i.e., Q) and the removed substrate (i.e., S_0-S_e) shifts. In this study, increasing the SALR took place by raising the Q from 20 to 39 ml min^{-1}, which caused expecting the SRR to increase. However, the drop in the other factor (i.e., S_0-S_e) was so significant, from ~7420 to ~1626 mg TCOD L^{-1} and from ~7820 to ~1825 mg sCOD L^{-1}, that caused SRR to decline by almost more than half in all three reactors. Returning the SALR to the first state (i.e., 28 g BOD $m^{-2}d^{-1}$), on the other hand, increased the substrate removal notably, from ~ 1626 to ~ 5741 mg TCOD L^{-1} and from 1825 to 5707 mg sCOD L^{-1}, that enhanced the SRRs despite the Q reduction (from 39 to 20 ml min^{-1}).

This again showed an insufficient biomass activity in all reactors at the HRT of 8.5 h comparing to their performance at the HRT of 17 h, which was due to media clogging leading to mass transfer limitations.

4.3.4.2. Carrier surface area influence

At both sets of SALR of 28 g BOD $m^{-2}d^{-1}$, all media types showed very close proficiency in both TCOD and sCOD SRRs. Still, media with the surface area of 589 m^2 m^{-3} presented the highest TCOD and sCOD SRRs, 42.08 and 44.22 g $m^{-2}d^{-1}$, respectively. At the HRT of 8.5 h, however, the larger the media surface area, the less TCOD and sCOD SRRs. TCOD and sCOD SRRs decreased from 21.71 to 14.04 and 24.50 to 16.22 g $m^{-2}d^{-1}$, respectively, by increasing the media surface area from 589 to 942 m^2 m^{-3}. The same reasons of sufficient biomass quantity in R1 and wider media opening could justify the results. Yet, based on the achieved p-values from the t-test, 0.55 and 0.53, while comparing media with the surface areas of 589 to 859, and 589 to 942 m^2 m^{-3}, there was not enough evidence that the TCOD SRRs differences are significant.

4.3.5. Total and Volatile Suspended Solids

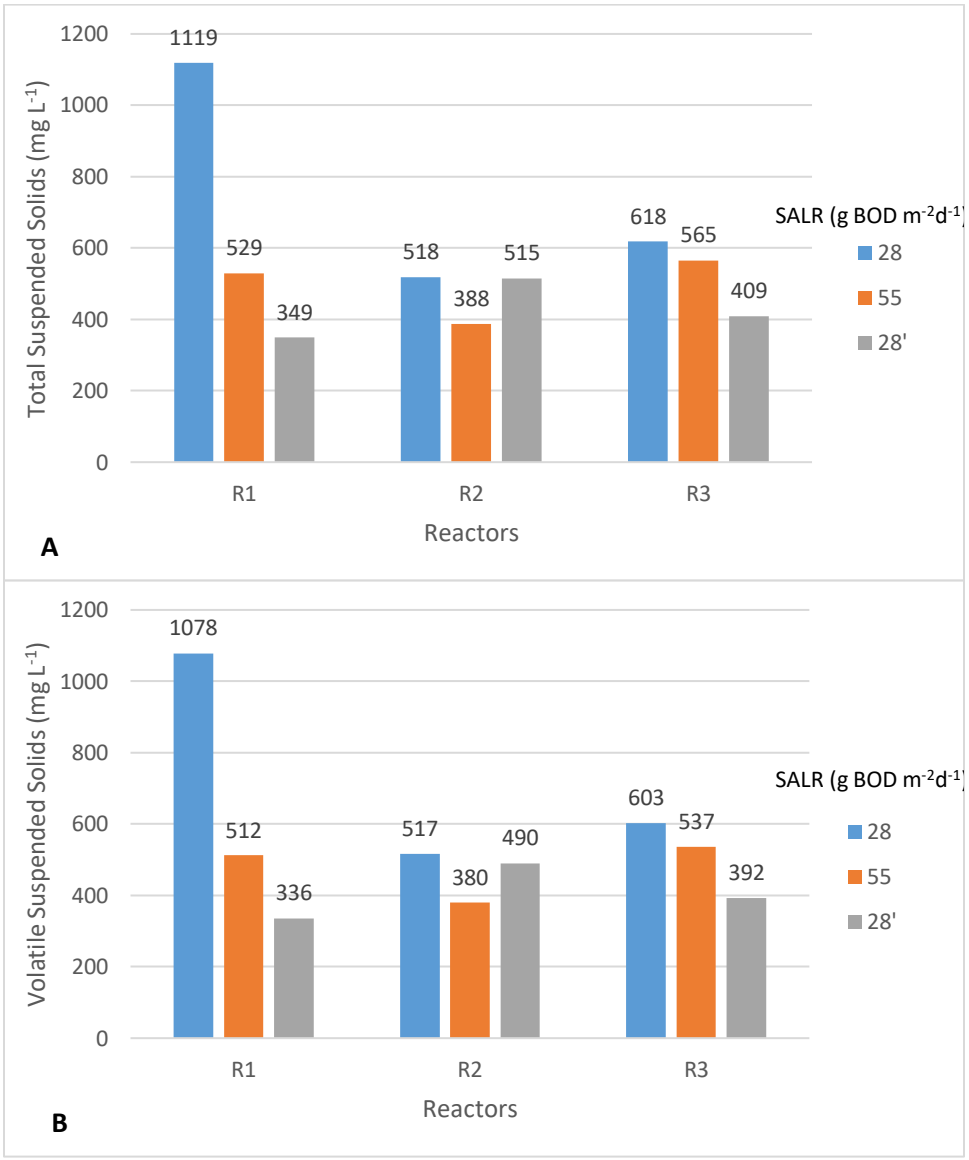


Figure 22 – A) Total and B) Volatile Suspended Solids in each reactor at different SALRs

The average effluent TSS, VSS, and their ratio during the steady-state period are reported in Figure 22 A and B, and Table 18, respectively.

Table 18 - VSS to TSS ratio in each reactor at different SALRs

Reactors		**R1**			**R2**			**R3**			**Unit**
SALR		28	55	28'	28	55	28'	28	55	28'	g BOD $m^{-2}d^{-1}$
VSS/TSS	Average	95.94	96.63	96.87	99.8	98.06	95.78	99.1	95.51	96.77	%
	Standard deviation	4.45	2.77	2.22	3.59	3.59	2.73	5.01	2.50	2.23	

As reviewed and reported by Kushwaha [81], untreated sugar industry wastewater includes a relatively high level of total suspended solids, even up to 9212 mg TSS L^{-1}. Therefore, synthetic wastewater in this study, which contained ~111 mg TSS L^{-1} with a VSS to TSS ratio of almost 100%, could not be a sufficient representative of a real sugar industry wastewater in terms of TSS and VSS removal. On the other hand, in terms of solids production, the condition of this research could be considered an ideal one since most of the solids present in the effluents were produced within the reactors.

Based on the results shown in Figure 22, relatively high levels of suspended solids were produced in all three reactors compared to MBBRs treating municipal wastewater, in which the MLSS concentration was in the range of 90 to 125 mg L^{-1}. This was simply due to a very high concentration of COD in synthetic sugar industry wastewater, leading to an increase in bacteria growth, attachment and detachment. Therefore, higher efforts for solids treatment need to be considered under the same circumstances.

4.3.5.1. SALR influence

Some previous studies have suggested increasing the SALR causes TSS and VSS concentration rise in MBBR effluent [91], [92]. However, in this study, increasing the SALR from 28 to 55 g BOD $m^{-2}d^{-1}$ caused a decrease in MBBRs' solids production, especially in R1, where the VSS concentration decreased from 1078 to 512 g VSS L^{-1}. This was because excess biofilm growth on all media types at the SALR of 55 g BOD $m^{-2}d^{-1}$ led to carrier clogging and, consequently, reduction in shear forces that cause detachment.

Different solid production performances (i.e., both increase and decrease) were observed in various reactors when setting the SALR to 28 g BOD $m^{-2}d^{-1}$ for the second time. Therefore,

more studies are needed to understand the overall effect of SALR reduction on TSS and VSS production.

4.3.5.2. Carrier surface area influence

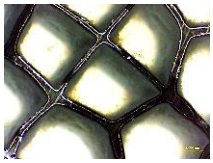
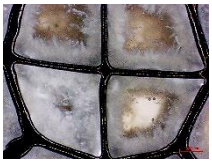
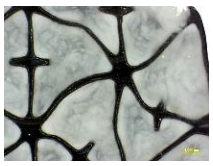
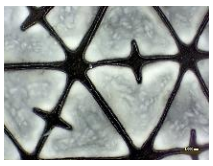
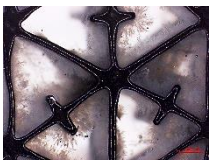
Media with the surface area of 851 m^2 m^{-3} (in R2) showed the best performance in producing relatively lower quantities of TSS and VSS at two first operational SALRs (518 and 388 mg TSS L^{-1} at SALRs of 28 and 55 g BOD $m^{-2}d^{-1}$, respectively). The lower amount of detached bacteria from this media type could also be proved by its highest quantity of attached biofilm (7.89 and 12.5 g VS m^{-2} at SALRs of 28 and 55 g BOD $m^{-2}d^{-1}$, respectively) compared to the other two media types (See section 4.3.6). The high amount of TSS and VSS produced in R1 showed that the wide openings of media with the surface area of 589 m^2 m^{-3} let the shear force cause bacteria detachment, especially at SALR of 28 g BOD $m^{-2}d^{-1}$ where the biofilm was thin enough to let the water pass freely. In R3, on the other hand, the substantial mass transfer limitation in media with the surface area of 942 m^2 m^{-3} might have caused more bacteria decay and detachment compared to the one with the surface area of 851 m^2 m^{-3}.

After the recovery time, however, various carriers showed different biofilm recovery performances. At the second set of SALR of 28 g BOD $m^{-2}d^{-1}$, the highest quantity of TSS and VSS were produced in R2 (i.e., 515 mg TSS L^{-1} and 490 mg VSS L^{-1}) while R1 provided the lowest amounts (i.e., 349 mg TSS L^{-1} and 336 mg VSS L^{-1}), which was entirely unlike the first set of SALR of 28 g BOD $m^{-2}d^{-1}$. Wider openings of R1 and more bacteria decay in R3 could probably be the reasons for faster responses to biofilm thickness adjustments (See section 4.3.6), which caused lower TSS and VSS during the steady-state period in these two reactors compare to R2.

4.3.6. Attached Biofilm Quantification

The biocarriers agglomerated with attached biomass and their biofilm quantities are shown in Table 19 and Figure 23, respectively. The biofilm quantification and captured microscopic images all have been obtained during the steady-state condition.

Table 19 - Microscopic images of the middle opining of different carries agglomerated with attached biofilm at operational SALRs

SALR / **Media**	**28 (g BOD $m^{-2}d^{-1}$)**	**55 (g BOD $m^{-2}d^{-1}$)**	**28' (g BOD $m^{-2}d^{-1}$)**
589 (m^2 m^{-3})			
851 (m^2 m^{-3})			
942 (m^2 m^{-3})			

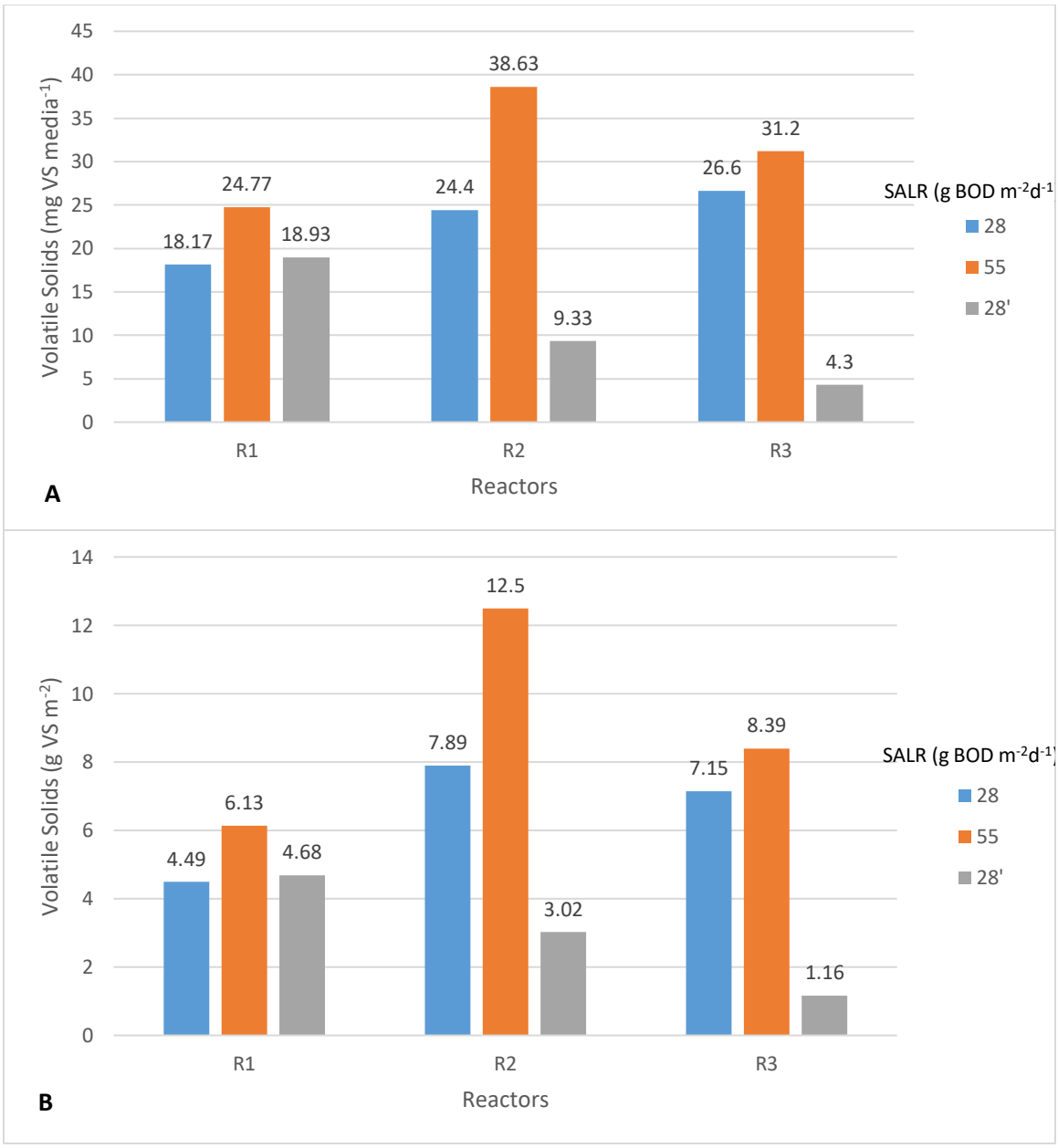


Figure 23 - Attached volatile solids concentrations: A) VS per one unit media; B) VS quantity per each square meter of media

4.3.6.1. SALR influence

As it is clear by both microscopic images and biofilm quantifications, increasing the SALR from 28 to 55 g BOD $m^{-2}d^{-1}$ caused more biomass growth on the internal surface of all types of media. This was expected due to more available food (i.e., organic matters) to microorganisms at

higher SALR. Yet, these excess developments, which were in the range of 1.24 to 4.61 g VS m^{-2}, were beyond the capacity of all three types of carriers, leading to blocking the openings and limiting the food and oxygen mass transfer through the biofilm. Therefore, it was proved that the SALR as high as 55 g BOD $m^{-2}d^{-1}$, corresponding to the HRT of 8.5 h, cannot be a suitable condition for MBBRs containing three media types used in this research.

Restoring the SALR to 28 g BOD $m^{-2}d^{-1}$ brought biofilm thickness and quantity reduction in all three reactors. Water pathways were developed through clogged biofilms, improving shear force impacts, biofilm detachment, and providing relatively thin biofilms. This proved that even after an almost complete media clog, there is a chance to recover thin and efficient attached biofilms just by decreasing the load or increasing the HRT.

4.3.6.2. Carrier surface area influence

As was anticipated, the media with the surface area of 589 m^2 m^{-3} contained the minor biomass quantities, 18.17 and 24.77 g VS $media^{-1}$ at HRTs of 17 and 8.5 h, respectively. Its small surface area compared to the other two types and its wider openings caused less biofilm attachment and more detachment due to friction forces, providing a sufficient biofilm thickness and the least mass transfer limitations. This also was proved by the R1's efficiency in sCOD removal (See section 4.3.3).

Two other types of carriers, on the other hand, showed different performances in terms of biofilm growth at different SALRs. At the SALR of 28 g BOD $m^{-2}d^{-1}$, media with larger surface area (i.e., 942 m^2 m^{-3}) contained more biomass, 26.6 g VS $media^{-1}$, compared to the other one, 24.4 g VS $media^{-1}$, as was expected. This occurrence, followed by creating a thicker biofilm, as shown in Table 19, caused more mass transfer limitation in R3 than R2, which was also proved by the reactors efficiencies in sCOD removal (See section 4.3.3). At the SALR of 55 g BOD $m^{-2}d^{-1}$, on

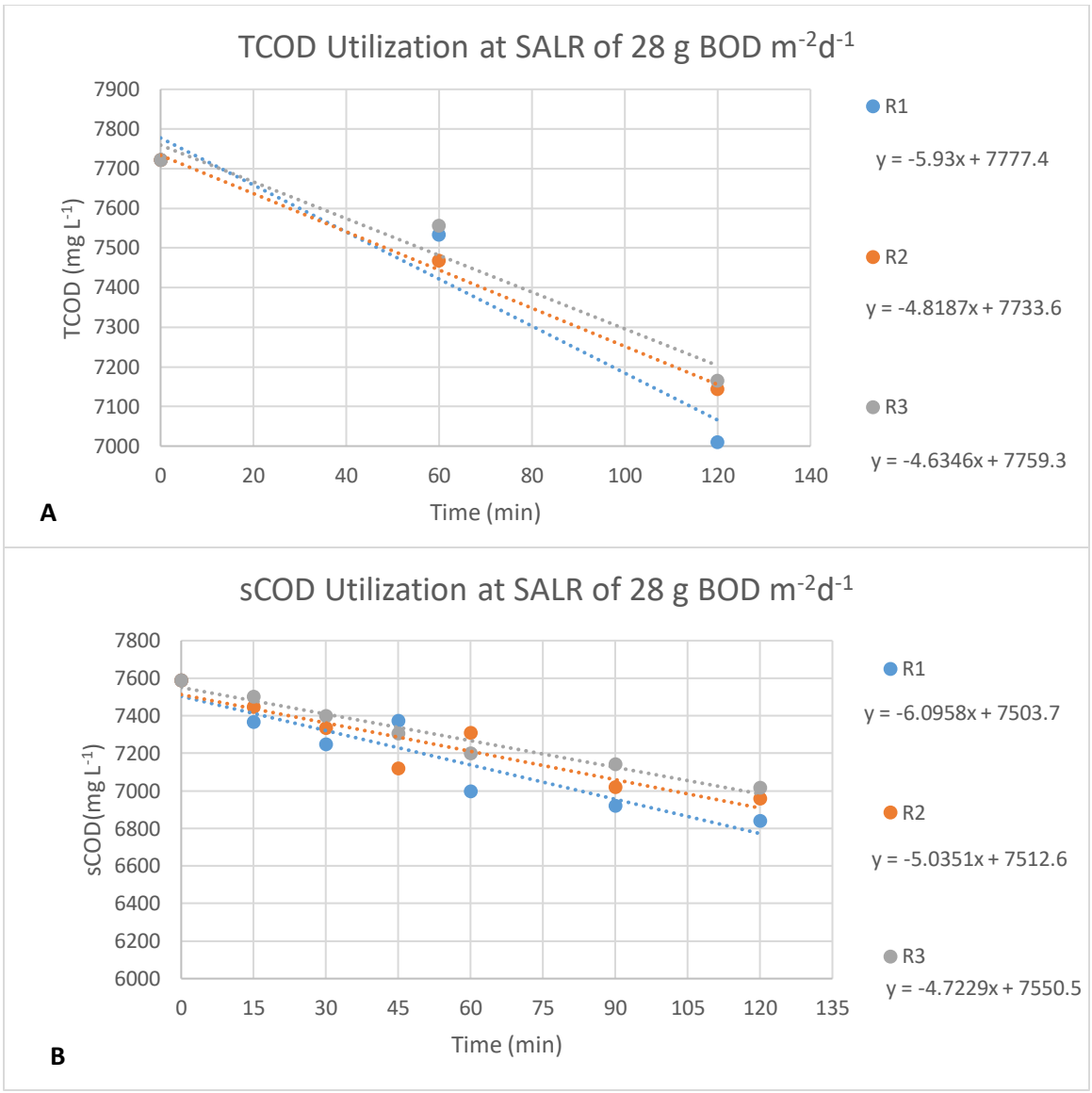
the other hand, the biofilm quantity was obtained to be less in R3 (31.2 mg VS media^{-1}) than R2 (38.63 mg VS media^{-1}). This could be justified by the reason that since the mass transfer limitation had already existed in R3, increasing the SALR to 55 g BOD m^{-2}d^{-1} did not help much biofilm grow on media. Hence, the quantity of biomass got greater on media with the surface area of 851 m^2 m^{-3} compared to 942 m^2 m^{-3}.

At the recovery stage, however, carriers showed totally different performances in the case of attached biofilm thickness and quantities. As shown in Figure 23, the larger the media surface area, the less the bound biomass quantity; 4.68, 3.02, and 1.16 g VS m^{-2} in R1, R2, and R3, respectively. The following reason could justify this interesting happening. Despite the higher biomass quantity in R2 and R3 at the SALR of 55 g BOD m^{-2}d^{-1}, their low performance in sCOD removal showed that their biofilm might include more significant amounts of dead microorganisms compare to R1, which were detached thoroughly once decreasing the SALR, leading to less remained biofilm on those types of media.

Overall, media with the surface area of 589 m^2 m^{-3} showed the best performance in providing a sufficient biofilm quantity at all tested SALRs.

4.3.7. Kinetic Studies

Kinetic tests were performed on media, in the absence of suspended solids, out of the main reactors to estimate the contribution of attached biofilm, and consequently the suspended biomass, to the overall performance. The tests were done twice in the last week of operating at the SALRs of 28 and 55 g BOD m^{-2}d^{-1} while meeting the steady-state condition. Figure 24 shows TCOD and sCOD utilization per time at different SALRs achieved by the test.


TCOD Utilization at SALR of 28 g BOD m-2d-1
TCOD (mg L-1)
7900
7800
7700
7600
7500
7400
7300
7200
7100
7000
0
20
40
60
80
100
120
140
Time (min)
R1
y = -5.93x + 7777.4
R2
y = -4.8187x + 7733.6
R3
y = -4.6346x + 7759.3
A
sCOD Utilization at SALR of 28 g BOD m-2d-1
sCOD(mg L-1)
7800
7600
7400
7200
7000
6800
6600
6400
6200
6000
0
15
30
45
60
75
90
105
120
135
Time (min)
R1
y = -6.0958x + 7503.7
R2
y = -5.0351x + 7512.6
R3
y = -4.7229x + 7550.5
B

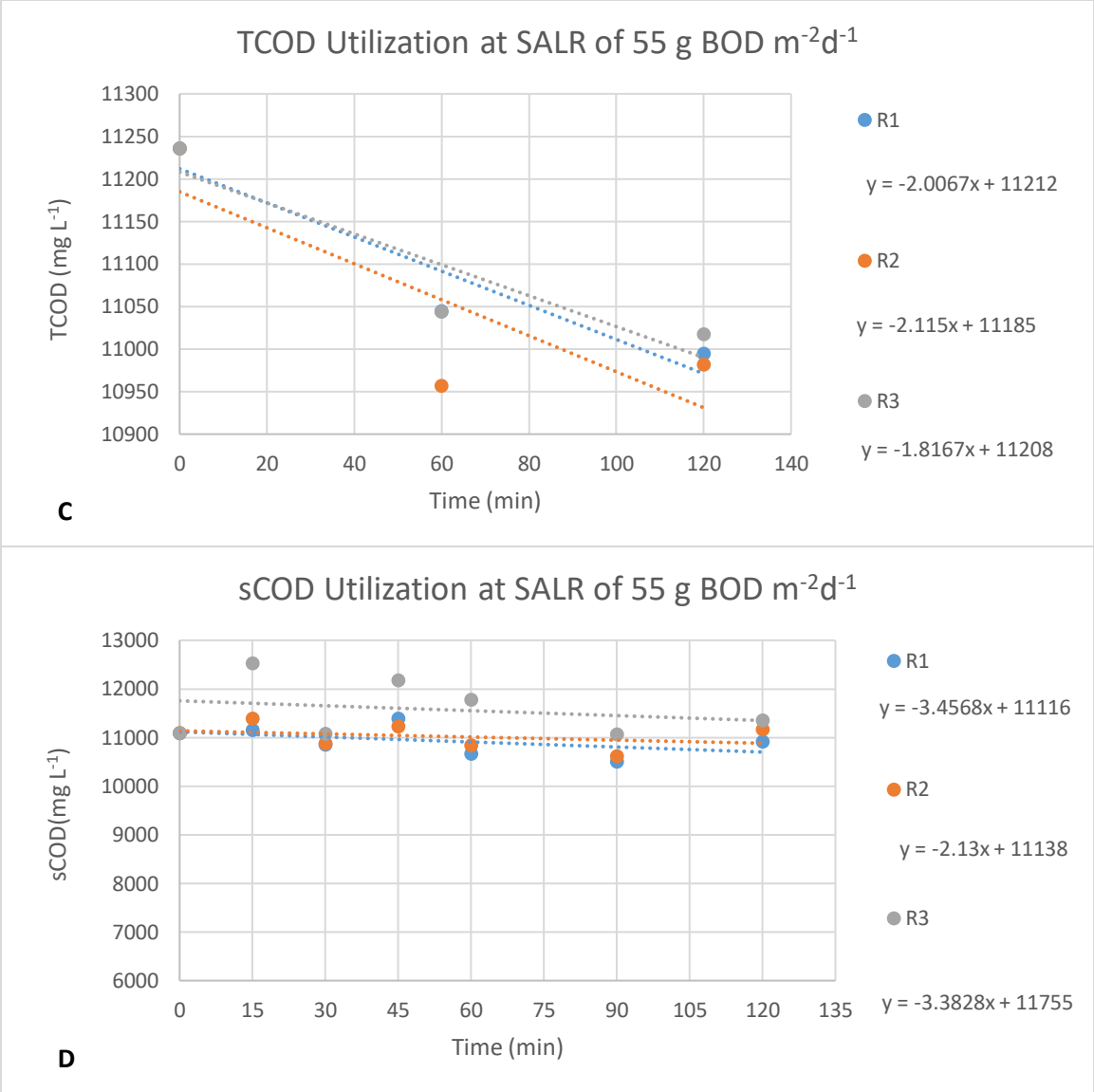


Figure 24 - Kinetic test results at SALR of 28 g BOD $m^{-2}d^{-1}$: A) TCOD utilization; B) sCOD utilization; and kinetic test results at SALR of 55 g BOD $m^{-2}d^{-1}$: C) TCOD utilization; D) sCOD utilization

Calculated removal rates (i.e., substrate utilization rates) at both operational SALRs are summarized in Table 20.

Table 20 - Substrate utilization rates in each reactor at different SALRs

Parameter	R1		R2		R3		Unit
SALR	28	55	28	55	28	55	g BOD $m^{-2}d^{-1}$
TCOD Utilization rate	8.54	2.89	6.94	3.05	6.67	2.62	kg $m^{-3}d^{-1}$
	7.45	1.85	3.45	0.96	3.66	1.22	mg $mgVS^{-1}d^{-1}$
sCOD Utilization rate	8.78	4.98	7.25	4.79	6.80	3.55	kg $m^{-3}d^{-1}$
	7.66	3.19	3.60	1.50	3.73	1.66	mg $mgVS^{-1}d^{-1}$

The achieved data corresponded to the attached biofilm activities as the VSS present in the main reactors was eliminated for the kinetic test. Therefore, by comparing the total removal and the achieved data by this test, the contribution of attached biofilm, and consequently the suspended biomass, to the overall performance could be estimated as shown in Figure 25.

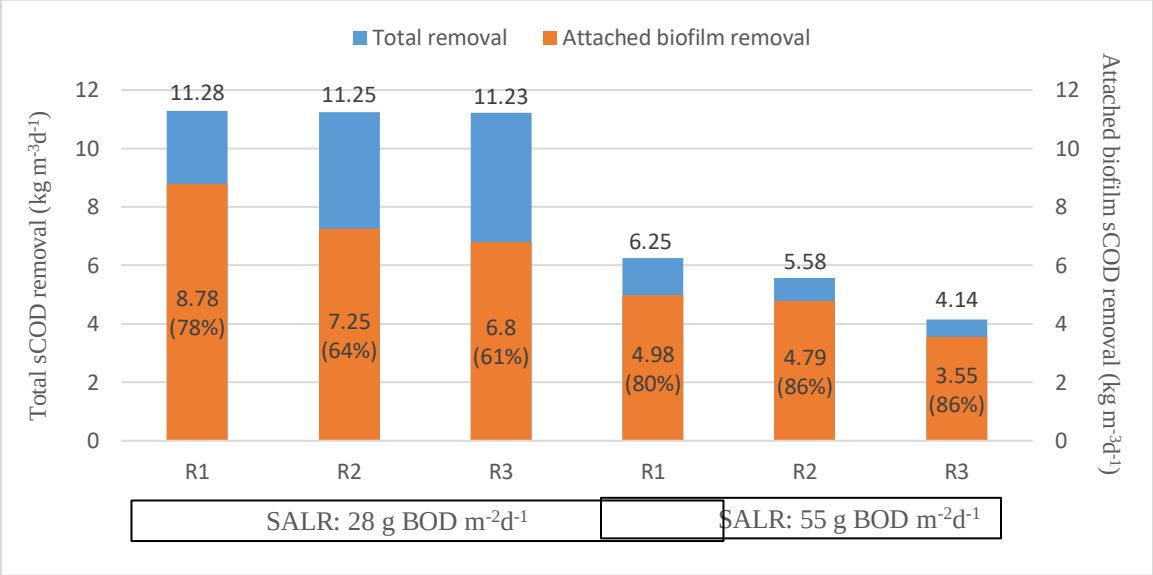


Figure 25 - Total sCOD removal compared to sCOD removal by attached biofilm in each reactor at different SALRs

4.3.7.1. SALR influence

Overall, increasing the SALR caused a considerable decline in sCOD removal both in total and by the attached biofilm. The sCOD removals were reduced by 44-63% in MBBRs, while the decrements in attached biofilm removal were in the range of 34 to 48%. Still, the contribution of the attached biofilm to the total removal increased by decreasing the HRT in all three reactors. The higher quantity of the attached biofilm and lower amount of volatile suspended solids at the HRT of 8.5 h than the HRT of 17 h (See sections 4.3.5 and 4.3.6) could be the reason for the mentioned observation.

4.3.7.2. Carrier surface area influence

At the SALR of 28 g BOD $m^{-2}d^{-1}$, the total sCOD removal rate was almost independent of the media type as all three reactors were working at 11.25±0.03 kg $m^{-3}d^{-1}$ sCOD removal rate. However, the attached biofilm to the carrier with the surface area of 589 m^2 m^{-3} showed the highest activity (i.e., sCOD removal rate), which was equal to 8.78 kg $m^{-3}d^{-1}$, while the attached biofilm in R2 and R3 could consume 7.25 and 6.8 kg of sCOD per cubic meter per day, respectively. Thin biofilm, which provided a sufficient pathway for the water stream, could be the main reason for the relatively high biofilm activity in R1.

On the other hand, at the HRT of 8.5 h, both total and attached biofilm removal rates depended on carrier surface area. The more the carrier surface area, the less the sCOD removal rates. Hence, the carrier with the surface area of 589 m^2 m^{-3} showed the best performance, 6.25 and 4.98 kg $m^{-3}d^{-1}$ of sCOD removal in total and by the attached biofilm, respectively. The relatively poor carriers' performances in R2 and R3 were again due to extreme mass transfer limitations through the biofilm due to the excess biomass growth.

4.3.8. Ammonium Removal

The Flow Injection Analysis (FIA) was done for ammonium, nitrite, and nitrate concentrations assessment to assure that the population of the AOBs and NOBs was negligible and most of the active microorganisms were responsible for BOD removal. The average of obtained results are as follows:

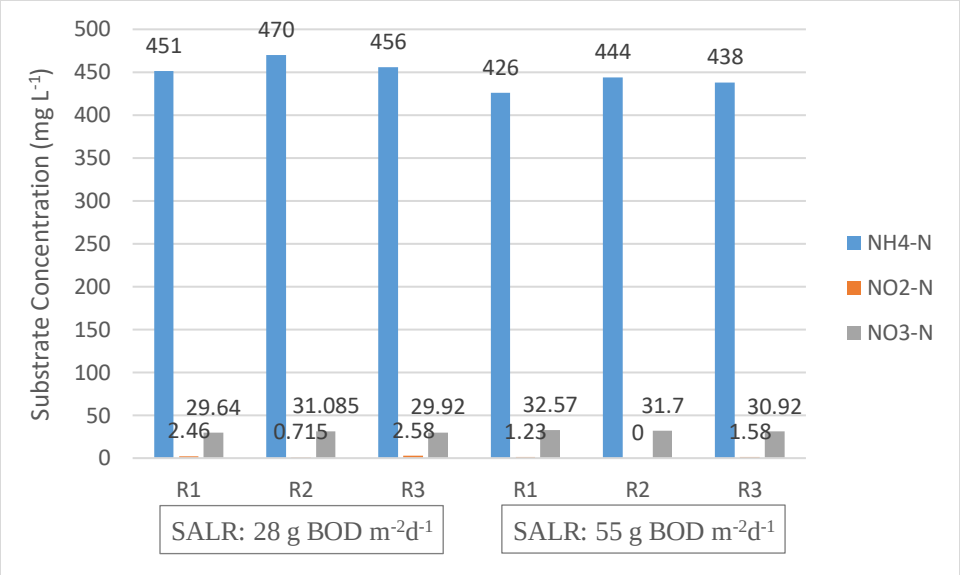


Figure 26 - Average Ammonium, Nitrite, and Nitrate concentrations in each reactor at different SALRs

Based on the achieved results, from the average amount of 500 mgNH_4-N L^{-1} in the influent, a negligible amount was turned to nitrite and nitrate. This confirmed a small population of AOBs and NOBs in all three reactors, showing an unfavorable condition for nitrifiers' growth at both tested SALRs.

Still, increasing the SALR increased the activity of nitrifiers and probably denitrifiers slightly, as the levels of ammonia were lower in all three reactors, while the nitrate levels were a bit higher.

On the other hand, comparing the media performance proved that the carrier with a surface area of 589 m^2 m^{-3} provided more favorable conditions for nitrifiers' growth and activity since more ammonia was converted in R1 than R2 and R3 under both SALRs. This again showed that the lower amount of biofilm formed on the media with the surface area of 589 m^2m^{-3} (See section 4.2.6) and its wider openings caused less mass transfer limitations, causing more ammonia removal.

4.4. Conclusions and Future Work

4.4.1. Conclusion

Three lab-scale MBBRs were set up and operated under various operational conditions treating synthetic sugar industry wastewater. R1 was filled up to 43.3% of its volume with media with the surface area of 589 m^2m^{-3} while R2 and R3 were filled up to 30% and 27.1% of their volumes with media with the surface area of 851 and 942 m^2m^{-3}, respectively, providing 5 m^2 of available surface area in each reactor.

The operational SALRs for each rector were 28, 55, and the second set of 28 g BOD $m^{-2}d^{-1}$, corresponding to the HRTs of 17, 8.5, and 17 h, respectively. The influence of media surface area and SALR on MBBR performance in COD removal was studied for the first two stages. After carrier clog happening and significant efficiency drop, the SALR was set to 28 g BOD $m^{-2}d^{-1}$ again, and the recovery time and removal performance of each reactor were investigated at the third stage.

By assessing the impacts of SALR on sCOD removal, it was found that all MBBRs were able to remove sCOD with ~87% efficiency. The reactors removed 11.25±0.02 kg COD $m^{-3}d^{-1}$, at the HRT of 17 h, corresponding to the SALR of 28 g BOD $m^{-2}d^{-1}$, independent of the media surface area. At the SALR of 55 g BOD $m^{-2}d^{-1}$, on the other hand, an excess biofilm growth on the internal surface of all types of media happened, limited the mass transfer and caused performance to degrade to 14-22% of sCOD removal.

It was found that the carrier shape and surface area did not significantly influence MBBRs performance when working at the SALR of 28 g BOD $m^{-2}d^{-1}$. Still, carrier with the surface area of 589 m^2 m^{-3} provided the thinnest biofilm with the efficiency equal to the biofilms on other carrier types. At the SALR of 55 g BOD $m^{-2}d^{-1}$, medias with surface areas of 589 and 851 m^2 m^{-3} showed

close efficiencies in sCOD removal, 21.5 and 19.2%, respectively. In comparison, the media with the surface area of 942 m^2 m^{-3} showed the worst performance of removing 14.1% of sCOD. The biomass present in the reactor with the best performance (R1) was able to remove 42.08 g TCOD and 44.22 g sCOD per square meter of media per day at the HRT of 17 h, and 21.71 g TCOD $m^{-2}d^{-1}$ and 24.50 g sCOD $m^{-2}d^{-1}$ at the HRT of 8.5 h.

TSS and VSS produced by reactors were all in the range of 336 to 1119 mg L^{-1}. Increasing the SALR from 28 to 55 g BOD $m^{-2}d^{-1}$ caused a decrease in MBBRs' solids production, especially in R1, where the VSS concentration decreased from 1078 to 512 g VSS L^{-1}. However, different solid production performances (i.e., both increase and decrease) were observed in various reactors when setting the SALR to 28 g BOD $m^{-2}d^{-1}$ for the second time. Therefore, more studies are needed to understand the overall effect of SALR reduction on TSS and VSS production.

Kinetic studies showed that the attached biofilm formed on the media with the surface area of 589 m^2 m^{-3} was the most active one, with 78% involvement in sCOD removal, at the SALR of 28 g BOD $m^{-2}d^{-1}$. This showed that the thinnest biofilm on this media compare to two others provided the least mass transfer limitation. However, the same media type showed the weakest activity, 80% of sCOD removal, at the SALR of 55 g BOD $m^{-2}d^{-1}$, while the other carriers showed 86% activity due to their higher biofilm quantities. The contribution of suspended biomass in COD removal decreased by reducing the HRT from 17 to 8.5 h in all three reactors. This could be due to the higher quantity of the attached biofilm and lower amount of volatile suspended solids at lower HRT.

Although all MBBRs could handle the SALR as high as 55 g BOD $m^{-2}d^{-1}$ for almost 12 days with COD removal above 80%, the efficiency started to decrease significantly ever since the 12^{th} day. Therefore, there could be a chance of managing intermittent high loads before reaching

the maximum handling duration, 12 days in this study. The frequency of applying intermittent high loads could be selected based on the time that microorganisms need to be stabled under the new condition plus at least 2 weeks of operation at the steady-state condition. This total duration was observed to be almost 6 weeks in this study. Based on the observations, the excess biofilm growth on carriers resulted in failure in MBBR operation when the maximum handling duration passed. Still, there was a chance of adjusting the biofilm thickness on the clogged carries and recovering the removal performance in almost one month duration, in the case of this study, just by decreasing the SALR.

By reducing the SALR from 55 to 28 g BOD $m^{-2}d^{-1}$, it took almost 26 days for the reactors to reach the steady-state condition and recover the performance to 67-70% of sCOD removal. Biofilm thickness was adjusted in all three reactors, leading to mass transfer limitation reduction and improved removal efficiency. Even though a 100% recovery (i.e., obtaining the same results as the first stage) could not be reached in any of the reactors, sCOD removal in the range of 67 to 70% corresponding to removal of 8-8.35 kg $m^{-3}d^{-1}$ of sCOD was achieved after the last SALR adjustment.

4.4.2. Future Work

Although using the synthetic wastewater at lab scale could be a good start point, real sugar industry wastewater has notable differences from synthetic one, including the particular concentration of COD, nutrients, metal and solids contents etc. Therefore, setting and operating MBBRs under sufficient operational conditions (most probably at the SALR lower than 28 g BOD $m^{-2}d^{-1}$) treating real sugar industry wastewater could be a potential experimental option to further assessment of MBBR and biocarriers performances.

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Appendix A:

Biomass Density sample calculation:

Measured biofilm thickness on media in R1: 347.69 µm = 0.034769 cm

Biofilm mass per media: 0.018 g TS media^{-1}

Media surface area: 851 m^2 m^{-3} = 8510000 cm^2 m^{-3}

Number of media per each cubic meter: 275000 media m^{-3}

Biomass density (g TS cm^{-3}) = $\frac{0.018\ gTs\ media^{-1} * 275000\ media\ m^{-3}}{8510000\ cm^2 m^{-3} * 0.034769\ cm} = 0.017\ gTS\ cm^{-3}$

Substrate Utilization Rate sample calculation:

TCOD utilization in R1 using Figure 16-A:

Slope of the plotted linear equation: -0.8635 → 0.8635 mg TCOD L^{-1}min^{-1} is removed

$$r_{su} = 0.8635\ \text{mg L}^{-1}\text{min}^{-1} = 1243.44\ mg\ L^{-1}d^{-1} = 1.24\ kg\ m^{-3}d^{-1}$$

Based on the Figure 15-B, attached biofilm concentration in R1 is 1.34 g VS L^{-1} = 1341.97 mg VS L^{-1}, therefore r_{su} (mg TCOD mgVS^{-1}d^{-1}) can be calculated as follows:

$$r_{su} = \frac{1243.44\ mgTCOD\ L^{-1}d^{-1}}{1341.97\ mgVS\ L^{-1}} = 0.927\ mgTCOD\ mgVS^{-1}d^{-1}$$