Design and Fabrication of Open-Cell Foams for Biological Organic Removal from Wastewater

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Abstract

Open-cell foams consist of macroporous structures are designed and fabricated in this study to be used as biofilm carriers for biological wastewater treatment. A manufacturing approach that integrates compression molding and particulate leaching is employed to fabricate the open-cell foams. Polyvinylidene fluoride (PVDF) open-cell foams prepared by sodium chloride (NaCl) as leaching agent possessed large specific surface areas with high degrees of cell-to-cell interconnectivity and demonstrated high potential for biofilm growth and protection. Moreover, PVDF open-cell foams fabricated by 80 wt.% and 85 wt.% of NaCl, showed a soluble chemical oxygen demand (sCOD) removal efficiency of up to 88%. sCOD indicates the soluble organic matters inside wastewater which have a significant role in water pollution.

In the first phase of this study, PVDF foams with different porous structures and total protected surface areas were fabricated by different types (NaCl and sodium acetate (NaOAc)) and different loadings (80 wt.% and 90 wt.%) of leaching agents. The fabricated carriers were used in batch aerobic and continuous aerobic flow wastewater treatment to evaluate their feasibility to promote the growth and protect of biofilms. In the second phase, the focus was on investigating the effects of porosity, cell morphology, and surface properties of open-cell foams on the sustainability of biofilm and their performances in biological organic removal from wastewater. For this purpose, 6 types of open-cell foams with different cell sizes and different porosities were fabricated by PVDF and high-density polyethylene (HDPE) to be used in the continuous aerobic flow wastewater treatment process.

Acknowledgement

I would like to express my deep gratitude to Professor. Leung and Professor. Eldyasti my research supervisors, for their patient, guidance, encouragement and useful critiques through this research work.

I must express my very profound gratitude to my lovely family and friends for providing me with unfailing support and continuous encouragement throughout my years of study.

I dedicate this thesis to my mother and father.

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List of Abbreviations

sCOD	Soluble chemical oxygen demand
BOD	Biological oxygen demand
DO	Dissolved oxygen
RAS	Return activated sludge
VSS	Volatile suspended solid
FSS	Fixed suspended solids
TSS	Total suspended solids
HRT	Hydraulic retention time
FBBR	Fixed bed biofilm reactor
MBBR	Moving bed biofilm reactors
EPS	Extracellular polymeric substance
PE	Polyethylene
PVDF	Polyvinylidene fluoride
PP	Polypropylene
PS	Polystyrene
PU	Polyurethane
PVC	Poly vinyl chloride
HDPE	High Density Polyethylene
PTFE	Polytetraflouroethylene
SEM	Scanning electron microscopy
PSD	Particle size distribution

Chapter 1
Preamble

1.1. Introduction

The scarcity of water, as a crucial pillar of all living organisms, is a substantial challenge which has affected the life quality of humans in different parts of the world. About 70% of the earth surface is covered by water; however, less than 2.5 % of total water on the earth is freshwater and less than 1.3% of total freshwater is accessible surface freshwater [1]. Figure 1.1 shows a graphical distribution of water sources on the earth.

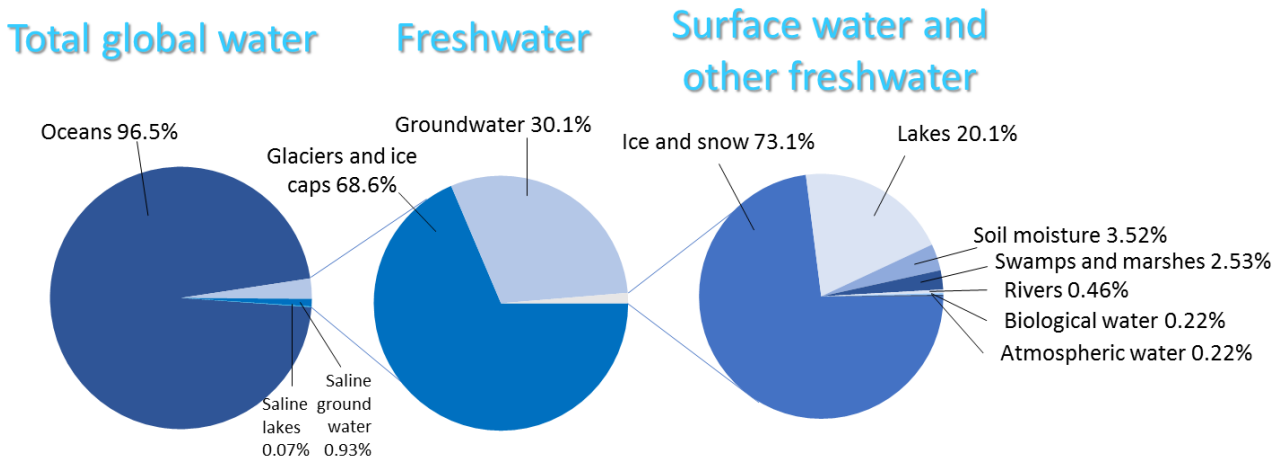

Figure 1.1 Graphical distribution of water sources on the earth [1].

Besides the freshwater shortage, in many parts of the world, climatic variation, industrial development, and urbanization have adversely changed the quality of water including forestry, freshwater, and marine resources. Water pollution has been brought about by the discharge of inadequately treated sewage and industrial wastewaters [2]. Empirical data collected from different sources suggest that globally approximate of 330 km^3 $year^{-1}$ municipal wastewater is produced while only 60% of it, is treated (this percentage differs between high and low income countries)

[3]. It can be concluded that accessibility of freshwater is decreasing at a very fast rate. Meanwhile, surveys show that 30% of all diseases worldwide are caused by polluted water [2]. In conclusion, water crisis is one of the most important challenges of current century and wastewater treatment has been the focal point among scholars to address this challenge [2]. The main wastewater removal methods are based on physical, chemical, and biological means. However, both physical and chemical treatment processes required a high maintenance and operating cost. Additionally, chemical wastewater treatment method include several techniques such as chemical precipitation, adsorption, and chlorine usage for disinfection processes which are being utilized by many wastewater treatment factories [4]. Biological wastewater treatment is one of the solutions which can influence the overall micropollutant removal in wastewater [5]. Dissolved organic materials in wastewater have a significant role in water pollution which are necessary to be removed from wastewater [3]. To tackle this challenge and eliminate their harmful effects on the environment, different organic removal methods have been utilized in the recent century. In this case, biological wastewater treatment can be employed to biodegrade organic pollutions. Comparing to the other treatment methods, biological wastewater treatment has lower operational costs, provide easy handling and also has less harmful effects on the environment [2]. Due to the considerable advantages of biological wastewater treatment method, this process has gained much attention in recent years. Figure 1.2 shows part of wastewater treatment plant diagram.

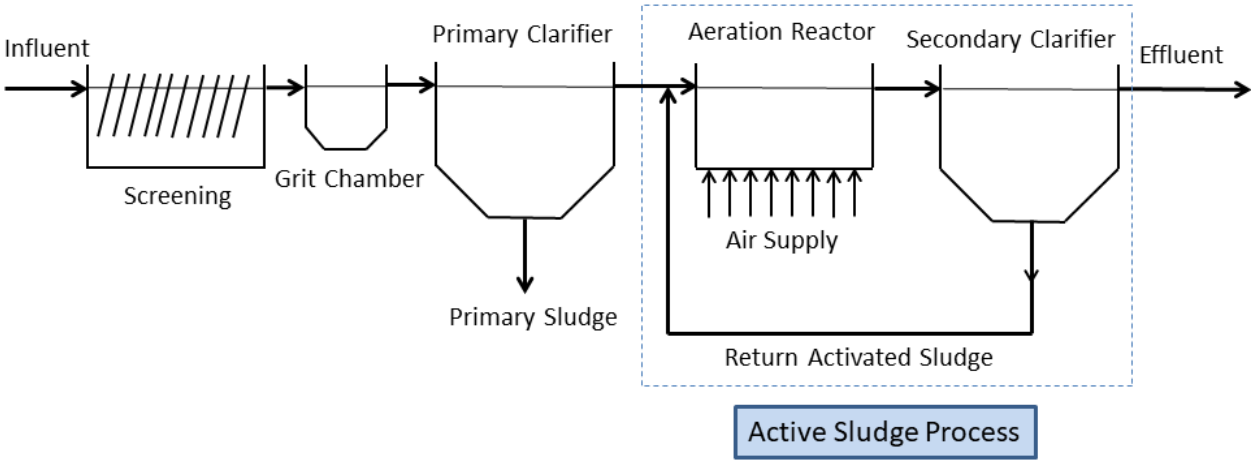

Figure 1.2. Part of wastewater treatment plant diagram [4].

Activated sludge process is one of the important parts of biological wastewater system which relies on suspended microorganism in a bioreactor for organic removal followed by a secondary sedimentation tank (clarifier) to maintain the solids concertation to the organic loading in the bioreactor. However, clarifiers are expensive facilities which occupy a large volume in the wastewater treatment plants. In order to eliminate the need for the second clarifier in treatment plant and moreover, to facilitate organic removal efficiency in the bioreactor, attached growth bioreactor systems have been developed. Attached growth bioreactor systems, as a promising method in the biological wastewater treatment, provide a protected surface area to support microbial attachment and biofilm growth, and thereby promote organic removal efficiency in wastewater treatment. Biofilm attachment in the attached growth bioreactors will enhance microorganisms' population in the system which leads to higher organic removal efficiency in these systems. Moreover, attached growth bioreactors reduce the microorganisms' concentration in the effluent which makes them a proper replacement for second clarifier in the wastewater treatment plant. Supporting carriers, as available surface area for microbial attachment, will play a crucial role in organic removal efficiency of the wastewater treatment systems.

1.2. Research rational

Clarifiers are a major part of a wastewater treatment plant and their design and manufacture is a costly process. Moreover, clarifiers occupy large volume of the entire wastewater treatment plant [6]. Moving bed biofilm reactors (MBBR) carriers can be a potential replacement for clarifiers with lower costs. Presence of biofilm carriers provides high concentration of microorganisms in the aeration tank which leads to higher organic biodegradation and minimal sludge generation [7]. Comparing to the currently MBBR carriers in the market, open-cell foams used in this study would provide larger specific surface areas which enhance the biofilm formation and thereafter wastewater treatment process. Furthermore, the attached biofilm on the existing MBBR carriers would be detached at high shear stresses and high organic loadings [8]. In contrast, open-cell foams have a high porosity and surface roughness which eventually would promote biofilm protection. Microbial cells located on rough surfaces or inside pores would experience suppressed shear forces and would be protected from detachment even at high degree of shear stresses and organic loadings.

1.3. Research goals and objectives

The present study aims to take advantage of the special morphologies of the open-cell foams and tries to utilize them in developing a protected surface for biofilm attachment used for wastewater treatment process. Due to their large overall surface areas and high levels of interconnectivity among their cells, open-cell foams can be a potential habitat for microbial immobilization, which is advantageous for biological wastewater treatment purposes. One of the objectives of this research is to develop and design a biofilm carrier which can provide a larger protected surface area for biofilm formation compared to existing biofilm carriers in the market. Moreover, this design should eliminate biofilm detachment from the carriers at high shear stresses.

The first phase of this research is to study the feasibility of using open-cell PVDF foams as carriers to promote the growth of biofilms and protect them. The second phase is to investigate the effects of porosity, cell morphology, and surface properties of foams on the sustainability of biofilms and their performances in wastewater treatment.

1.4. Thesis structure

In Chapter one, the water crisis in the world and the importance of wastewater treatment are described. This chapter finds biological wastewater treatment as a promising method for organic removal in the wastewater and tries to introduce innovatory design to enhance this treatment method, as well as the goals and objectives of this research. Chapter 2 provides a comprehensive literature survey and review the background of the research. Chapters 3 and 4 introduce the phases of the research. In Chapter 3, the feasibility of using open-cell PVDF foams as carriers promoting biofilm formation in attached growth systems is investigated. Chapter 4 studies the effects of foam morphology on the sustainability of biofilms and their performances in wastewater treatment. Finally, Chapter 5 concludes the contribution of this thesis and provides some potential future directions for this research.

Chapter 2
Background and Literature Review

2.1. Foam

Foam can be described as an enclosed gaseous void in a much denser continuum media, usually a liquid or solid phase. It can be found in nature such as in cellulosic wood or it can be constructed through synthetic processes (i.e. foamed plastics). By changing cell size, quantity, and cell distribution, a wide spectrum of material properties can be obtained.

Cellular structure plays a key role in determining the behavior of polymer foams, therefore these materials can be classified according to their cellular structure. Polymer foams are classified into closed-cell foams, interconnected or partially open cell foams, and open-cell foams. Closed-cell foams consist of discrete cellular structures separated by cell walls. In contrast, open-cell foams contain interconnecting cellular structures due to the presence of pin-holes on the cell walls or the complete rupture of the cell walls. Generally, closed-cell foams contain lower than 10 vol.% open cell structure, while open-cell foams possess 80-90 vol.% of open cell structure [9].

In this context, extensive researches have been conducted to investigate the different open-cell foams fabrication methods with tailored morphologies to meet the needs of different applications. There are several techniques used for open-cell foam fabrication which have been briefly discussed in the following section.

2.1.1. Open-cell foam fabrication methods

2.1.1.1. Thermally induced phase separation

Thermally induced phase separation (TIPS) is an easy way to fabricate polymer foams, in which morphology parameters such as pore size, porosity, and interconnectivity can be controlled. In this method, phase separation is accomplished during the cooling process of a homogenous polymer solution and the homogeneous polymer solvent solution separates into a polymer-rich phase and a polymer-poor phase. In addition to the cooling process, phase separation can also take place using another immiscible solvent. It is worth mentioning that after this step, the porous polymeric structure is obtained by removing the solvent. During solvent removal, shrinkage of polymer foam can happen; however, this phenomenon can be minimized or prevented by using freeze drying, supercritical drying or exchanging the solvent prior to vacuum drying. In fact, TIPS uses thermal energy as a latent solvent to induce phase separation. In this method, by adjusting different thermodynamic and kinetic parameters, different structures can be achieved and they can be used for producing micro-porous foams [10-11].

2.1.1.2. Emulsion freeze drying

In this method, an emulsion is created by homogenizing of a polymer solvent solution and water. Then the emulsion is quickly cooled to lock in the liquid-state structure and the solvent and water are removed by freeze drying.

In order to create a polymer foam from an emulsion of two immiscible phases where the continuous phase is a polymer rich phase and the disperse phase is water, some factors such as volume fraction of dispersed phase and emulsion stability play important roles. Emulsion stability

affects pore structure while creaming, flocculation and coalescence have influences on the thermodynamical stability of the emulsion and consequently cause poorly dispersed pores [12].

2.1.1.3. Interpolymer blending

In this method, foams are fabricated by expanding interpolymers and interpolymer blends. In the first step of this process, polymers and additives are melted and blended in an extruder. Then, the blowing agent is added to the system and is mixed with other components at high temperature and pressure to ensure complete dissolution. Afterwards, the melt is cooled to the foaming temperature while the melt strength should be high enough to prevent cell collapse and stabilize the foam. Finally, the melt is extruded trough a die to room temperature and pressure, consequently, the expansion of molten polymer takes place and the foam is obtained [13-14].

2.1.1.4. Particulate leaching/solvent casting

A relatively new method to fabricate open-cell structures is particulate leaching or solvent casting. In this method, a homogeneous domain is added into the polymer matrix initially. Solid particles, such as sodium chloride and potassium chloride crystals, are introduced into the polymer matrix at the beginning of the process. Afterwards, these particles will be dissolved out of the matrix using a solvent, therefore a porous cellular structure throughout the entire polymer matrix can be obtained. This technology has been commonly used in the production of highly porous bio-scaffolds [15]. Usually, a combination of particulate leaching and compression molding is used to fabricate samples [16].

2.1.2. Foam application

Using foam materials has intensified severely over the last decades. In 1996, more than six billion pounds (three metric tons) of foamed plastics were consumed just in the United States.

Nowadays, there is also a great demand for foamed plastics in almost all aspects of our lives. Table 2.1 presents markets, functions, and polymers for foamed plastics [17]. Polyethylene (PE), polyvinylidene fluoride (PVDF), polypropylene (PP), polystyrene (PS), polyurethane (PU), and polyvinyl chloride (PVC) are common thermoplastics that are mainly used for polymer foam fabrication due to their suitable melt rheology [18].

Table 2.1. Present markets for foamed plastics [17].

Functions	**Markets**	**Attributes**	**Polymers**
Cushioning	Furniture, transportation, construction	Energy absorption	Flexible PU, PE, ABS
Insulations	Construction, automotive	Low thermal conductivity, sound absorption	Rigid PU, PS, PE
Protection	Packaging	Soft and flat surface cushioning	RIM PU, PS bead, PE, PP sheet
Strength/ weight	Construction, medical, household	Strength and softness	RIM PU, x-linked PE, PS, PVC, flexible PU
Thermal	Thermoforming	Thermal strength	PS, x-linked PE
Impact absorption	Automotive, athletics	Sharp energy absorption	Bead PP, x-linked PE

Foams have been widely used in a variety of applications. Closed-cell foams are generally used in the applications where insulation properties are needed since they have lower permeability, while open-cell foams have better absorption capabilities [18]. Open-cell foams have unique characteristics such as lightweight, open porosity, impact absorption, flotation, and acoustic insulation. These characteristics have made them ideal materials in a wide spectrum of applications including filters, separation membranes, sound insulation, and battery electrode supports [19-21]. The present study tries to find another application for open-cellular foams in wastewater treatment technology. Due to the large surface area in the foams, and interconnection between cells in the

open-cellular foams, they can be a potential habitat for microbial immobilization in biological wastewater treatments.

2.2. Biological wastewater treatment process

Biological wastewater treatment is an important and integral part of any wastewater treatment plant that cleans wastewater suspended with soluble organic wastes. During this process, heterotrophic microorganisms decompose complex organic compounds through oxidation reaction and transform them into acceptable end products. Microorganisms use organic materials as an energy source to build new microbial cells [22-23] sCOD indicates the concentration of soluble biodegradable organic pollutants in wastewater which can be eliminated by biological wastewater treatment. The main significant advantages of biological wastewater treatment are its lower demand for capital investment, less environmental effects, and less operating costs in comparison with other treatment processes such as chemical and thermal oxidation. Biological treatment processes can be divided into three major types; suspended growth technology, attached growth (biofilm) system, and hybrid growth system.

2.2.1. Types of biological treatment processes

In suspended growth technology, the omnipresence of suspended microorganisms in mixing liquor phase leads to biodegradation of pollutants in wastewater. Activated sludge process is the most widely used technology in suspended growth process in which the solid particles of effluent are separated from the process effluent in a sedimentation tank (i.e., clarifier) and returned subsequently to the bioreactor [2]. In attached growth systems, the high microbial population can be accumulated in presence of support media and generate biofilm [24]. Bacteria cells in the population secrete a slime layer, made up of various organic polymers known as extracellular

polymeric substance (EPS) [25]. The structured community of bacterial cells enclosed in a self-produced EPS matrix forms biofilm. Existed EPS in the biofilm will entrap microbial cells in a three-dimensional matrix and enhance the settleability of bacterial colonies [26-27]. Moreover, EPS helps to increase biofilm sustainably against shear forces [26]. Excessive accumulation of microorganisms in the biofilm increases biomass concentration and sludge retention, which led to higher organic removal efficiency in attached growth systems in comparison with suspended growth systems [24]. In suspended growth systems, presence of secondary clarifier is necessary to return the activated biomass to the bioreactor. However, in attached growth systems increased concentration of immobilized biomass on the support media facilitates solid-liquid separation which would replaces the need of secondary clarifier and leads to a reduced volume of reactor. Other advantages of attached growth systems over suspended growth systems are higher volumetric degradation rate, higher loading capacity, lower sludge yield, and better tolerance of varying process conditions [28-30]. Suspended growth processes have some operational challenges such as sludge bulking. Sludge bulking is a result of wide range of filamentous type bacteria, which grow as a string of attached single cells and causes poor settling of sludge in activated sludge process with clarifiers. Poor settlement of microorganisms in clarifiers can results in high effluent suspended solids concentration and therefore less treatment performance. Hybrid growth system is biological wastewater treatment method which takes advantage of both attached growth and suspended growth systems for organic removal. In the hybrid system, the combination of biofilm attached to the support media and microorganisms in the suspended phase can maintain high biomass concentration which improves the performance of bioreactor in terms of organic removal efficiency [6].

In attached growth systems, the media or biofilm support can be immobilized (fixed bed biofilm reactor (FBBR)) [8] or mobilized in suspension (moving bed biofilm reactors (MBBR)) [7,31]. MBBR, with the movement of biofilm carriers through continuous mixing, would achieve better chance for bacteria to interact with suspended biodegradable organic matters than FBBRs. Moreover, the moving media in MBBR usually have large surface areas for bacterial colonization. In contrast, fixed media in FBBRs would have a portion of their surface areas unreachable by bacteria for their attachment.

2.2.2. Aerobic vs anaerobic processes

Depending on the presence of oxygen in the reaction, biological wastewater treatment can be classified into anaerobic and aerobic processes. Anaerobic treatment process, which employs microorganisms that function in the absence of oxygen, is effectively utilized for industrial wastewaters with high sCOD concentrations even at elevated temperatures. In contrast, aerobic wastewater treatment is a process in which bacteria utilizes oxygen to decompose organics and pollutants. It is commonly used to treat municipal wastewater with lower degradable COD concentration, higher effluent quality requirement, and nutrient removal at lower temperature [4].

2.2.3. Microorganisms' role in wastewater treatment

During the biological wastewater treatment, microorganisms biodegrade the organic matters found in wastewater. During this process, an oxidation-reduction reaction, which is also known as redox reaction, takes place simultaneously in the system. In the oxidation reaction, an electron donor substance will be oxidized by losing electrons; however, in the reduction reaction, an electron acceptor substance gains electrons from other materials and will be reduced. Redox reactions provide energy by transferring high energy electrons to low energy ones. In the overall

biological growth reactions, cell reaction occurs at the same time with redox reaction. Figure 2.1 shows the graphical overall reactions for biological growth. Figure 2.2 and Equation 2.1-2.4 show the overall reactions for biological growth.

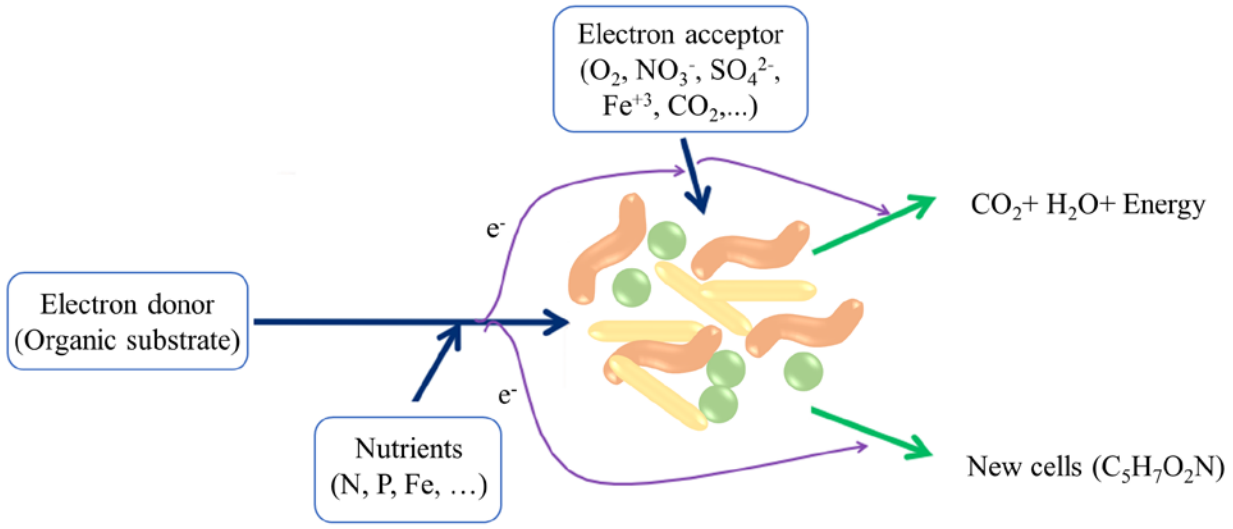

Figure 2.1. Graphical overall reactions for biological growth [4].

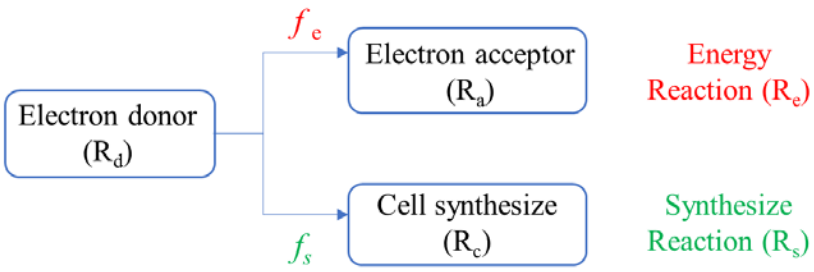

Figure 2.2. Overall reactions for biological growth

$$R = f_e \cdot R_e - f_s \cdot R_s \quad (2.1)$$

$$R_e = R_a - R_d \quad (2.2)$$

$$R_s = R_c - R_d \quad (2.3)$$

$$f_e + f_s = 1 \quad (2.4)$$

where; R_e, R_s, R_a, R_d, and R_c are energy reaction, synthesize reaction, electron acceptor reaction, electron donor reaction, and cell synthesize reaction respectively. Moreover, f_e and f_s are reaction factors for energy reaction and synthesize reaction respectively.

2.2.4. Biofilm

Biofilm is the term used to describe an assemblage of immobile microbial cells attached to a surface or to each other, enclosed in a matrix of self-produced EPS [32]. EPS, which along with microbial cells form biofilms, is the biofilm primary matrix material and contains between 50% up to 90% of biofilm total organic carbon [33]. Biofilm acts like a shelter for microorganisms and protects them from environmental stresses which increases their survival likelihood. Moreover, biofilm increases the microorganisms' resistance to disinfectants and antimicrobial treatments [32,34-35].

Different elements such as cell surface properties, substratum, interfaces between solid and liquid phases, and aqueous medium hydrodynamics affect the attachment and growth of microorganisms. Some papers suggest that ionic strength, pH, temperature, and nutrient levels of aqueous medium affect the attachment of microbes to the substratum. An experimental study revealed that the number of attached bacterial cells increases when nutrient concentrations augmented [36]. Moreover, the change in water temperature has been considered as the major probable reason for seasonal effects on the microbial attachment [37-38]. It has been shown that when the concentration of sodium, calcium, lanthanum, and ferric iron increases, the repulsive forces between bacterial cells and glass surface mitigate. As a result, a special kind of bacteria can attach to glass surfaces more easily [39-40].

The flow velocity of the aqueous medium has a critical effect on the attachment and detachment of the biofilm. When the boundary layer mitigates due to an increase in aqueous flow velocity, microorganisms will be subjected to more turbulence and mixing affects. If the flow velocity is high enough to apply more than sustainable shear forces to the microbial cell, the biofilm will be detached from the supporting surfaces [41-42].

2.2.4.1. EPS

A hydrated gel which is produced due to bacteria secretion is called extracellular polymeric substance (EPS). EPS is formed from the accumulation of large molecules such as proteins, nucleic acids, polysaccharides, and humic substances [43]. EPS plays a key role in physiochemical features of bacteria aggregation. EPS adheres to cells via interactions and this forms a net-like substance with water which prevents cell drainage [44]. Moreover, EPS can serve as a reliable source of carbon and energy when there is a lack of nutrient substances in the system [45]. Furthermore, their ability in binding cells to each other can act as a catalyst for the formation of bacteria aggregation [46].

Based on the solubility of EPS, two main categories are defined for them. The first category is bound EPS which attaches to cell walls and mainly consist of sheaths, loosely bound polymers, condensed gels, capsular polymers, and attached organic materials [47]. Nielsen et al. proposed a two-layer model for bound EPS in which the outer part is loose and without specific shape. However, the inner part of bound EPS which contains most of the bound EPS contents, is tight closely attached to the cell wall [25]. The other type of EPS is soluble EPS such as colloids, slimes, and some macromolecules which has limited interaction with cells [47].

There are many factors which affect determining the EPS compositions such as growth phase, bioreactor type, process parameter, and extraction methods [25]. However, many papers have proposed that the main compositions of EPS are proteins, carbohydrates, humid substances, uronic and nucleic acids, and lipids [19,48–50]. Although many papers reported a similar composition for EPS, it is believed that EPS structures do not follow any specific pattern and they have a heterogeneous structure [44].

2.2.4.2. Biofilm formation stages

Considering both the positive and negative effects of biofilms on human life, it is necessary to know how exactly they form. As it was mentioned earlier, the presence of microorganisms and surface is necessary for biofilm formation. At the first step, microorganisms associate with the surface through weak Van-der-Walls forces. Afterwards, the microorganisms grow and aggregate into microcolonies and secrete EPS. At the next step microorganisms develop a more complex community by building the biofilm in a mushroom shape [51-52]. The schematic biofilm formation stages can be seen in Figure 2.3.

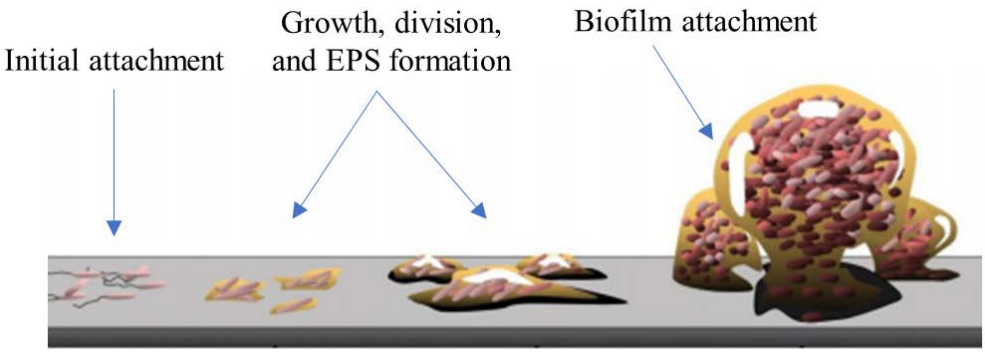

Figure 2.3. Biofilm formation stages. Adapted from [53] by permission from the publisher.

2.3. Biofilm carriers

The ability of a system to immobilize any biosystem depends on the characteristics of support media. In particular, the physical properties, surface area, roughness, and porosity of the media are governing factors in the design of the support media. Rough surfaces and porous materials would provide larger specific surface areas to facilitate the adhesion of microorganisms. Moreover, microbial cells located on rough surfaces or inside pores would experience suppressed shear force and be protected from detachment, which eventually promotes biofilm generation [32]. Bacteria with hydrophobic and hydrophilic properties tend to attach to hydrophobic and hydrophilic material surfaces, respectively. However, bacteria with hydrophobic properties have more potential to adhere to surfaces. Therefore, hydrophobic materials are more likely to promote biofilm formation than hydrophilic ones [32].

Different kinds of biofilm carriers have been tested in the attached growth systems by various researchers (Many researchers have tested different kinds of biofilm carriers in attached growth systems). Fibrous carriers [54-55] peat [56], polystyrene sheets [57], and polyurethane foam cubes [58] are some examples of support materials used for biofilm generation. Comparing to the existing biofilm carriers in the market, open-cell foams have remarkably larger specific surface area and higher surface roughness, which would promote biofilm adhesion to their surfaces [32,59].

In this work, outstanding attributes of PVDF and HDPE such as high mechanical strength, good thermal stability, superior chemical resistance, and hydrophobicity, made them a good candidate to be used as the polymer matrix for foam fabrication [60-61].

2.3.1. High Density Poly Ethylene

One of the most important thermoplastic foams is polyethylene (PE) foam. Chemically pure PE consist of alkanes with formula $C_{2n}H_{4n+2}$, where n is the degree of polymerization. Chemical structure of a PE chain is shown in Figure 2.4. There are many types of PE that have the same backbone and different branches or defects. In the solid states, branches and defects have lower crystallization ability, therefore, chains that have fewer defects have a higher degree of crystallization. As crystalline regions have a higher density than amorphous areas, the density of PE increases with the increment of the degree of crystallization.

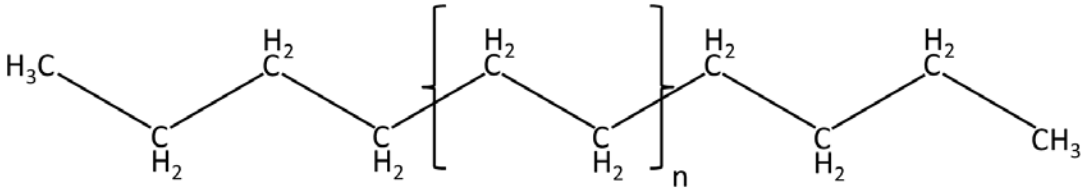

Figure 2.4. Chemical structure of PE [62]

Chemical structure of high density poly ethylene (HDPE) is the closest to pure PE. It possesses unbranched molecules with very few defects, therefore it has a high degree of crystallinity resulting in a high density. HDPE resins have typical densities in the range approximately 0.94-0.97 g/cm^3 [63].

HDPE used in this study (SCLAIR® 2710 Resin) was purchased from Nova Chemicals Company. It includes special attributes such as good organoleptic properties, natural color, excellent processability, exceptional warpage resistance, and product performance reliability. Moreover, HDPE is a low cost polymer with good mechanical strength, great chemical resistance, thermal stability, and inherent hydrophobicity [60-61]. Properties of this HDPE are presented in Table 2.2.

Table 2. 2.Properties of HDPE [64]

Property	Typical values	ASTM
Melt Index	17 g/10 min	D 1238
Density	0.951 g/cm³	D 792
Yield Strength	23 MPa	D 638
Elongation	530 %	D 638
Flexural Modulus	1,000 MPa	D 790
Hardness, Shore D	62	D 2240
Vicat Softening Point	120 °C	D 1525

2.3.2. Polyvinylidene Fluoride

Poly (vinylidene floride) (PVDF) is a semi crystalline polymer which is widely known for its extraordinary piezoelectric and pyroelectric properties. It also has other properties such as easy moldability, toughness and flexibility.

PVDF has four polymorphs, α, β, γ, and δ phase. α phase is obtained during crystallization of the polymer melt under moderate or high cooling rates and it is the most common polymorph of PVDF. There is no steric residence between fluorine atoms here and α-unit is non-polar due to the anti-parallel packing of chains. α phase is less compressible then PE possibly due to the electrostatic repulsions, come from the presence of florin atoms. β phase, obtained through mechanical deformation of melt-crystalized films, is mostly used in piezoelectric and pyroelectric applications. It has all-trans arrangement. Compressibility of the β-lattice is significantly less than α-lattice. γ phase is the other interesting polymorph of PVDF which is obtained during solution-crystallization from dimethyl formamide at high pressure. This conformation is intermediate between α and β phase and γ form can easily transform to the β form upon mechanical deformation. Detection of δ phase is the other important development during PVDF discovery. It has the same

unit-cell dimension and chain conformation with the α phase and their difference is in their interchain packing. Figure 2.5 shows chain conformation of all these polymorphs.

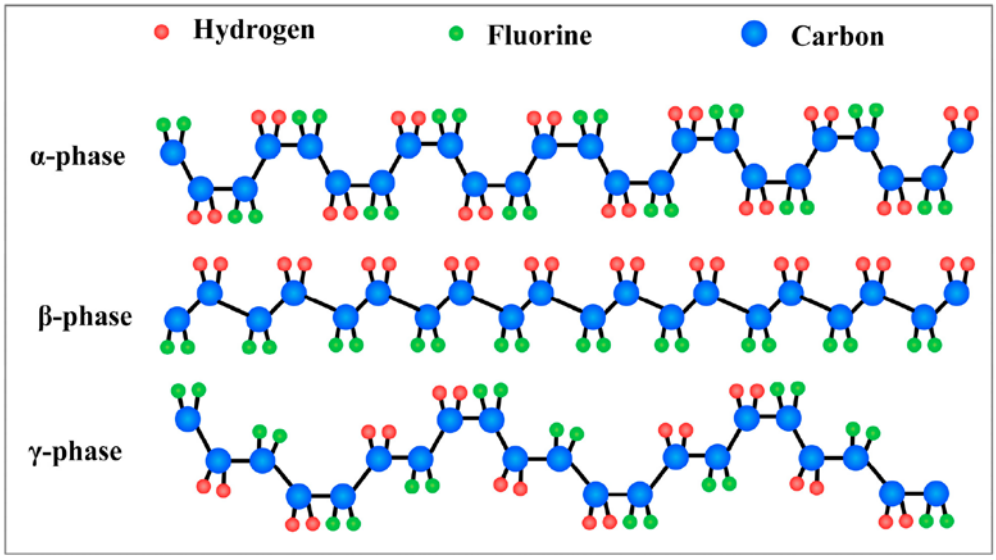

Figure 2.5. Chain conformation for the α, β and γ-phases of PVDF [65].

PVDF is also a very useful combination of mechanical strength, processability, and chemical resistance. Its physical properties place between PE and Polytetraflouroethylene (PTFE) and its mechanical properties are outstanding compared to other crystalline polymers such as polyethylene, polypropylene, nylons, PTFE, and polyoxymethylene.

In addition to the electrical application due to its excellent piezoelectric and pyroelectric properties, PVDF is widely used in other applications such as coatings, tubing, pipes, valves, containers, etc., due to its superior chemical resistance. Finally, some outstanding properties such as high mechanical strength, thermal stability, chemical resistance and high hydrophobicity have made PVDF a strong candidate for separation process in the form of foams [64-65]. Properties of this HDPE are presented in Table 2.3.

Table 2.3. Properties of PVDF [68]

Property	Value	Test Standard
Melt volume flow rate	1.13 $cm^3/10$ min	ISO 1133
Density	1780 kg/m^3	ISO 1183
Yield stress	50 MPa	ISO 527-1/-2
Yield strain	9 %	ISO 527-1/-2
Tensile modulus	2000 MPa	ISO 527-1/-2
Melting temperature	170 °C	ISO 11357-1/-3

In this chapter different biological wastewater treatment methods specially, attached growth systems were reviewed. Moreover, the role of biofilm carriers in the wastewater treatment plants for organic removal was discussed. In this context, desired characteristics of open-cell foams made them a potential habitat as biofilm carriers for microbial immobilization in wastewater treatment.

Chapter 3
Feasibility of Using Open-Cell Foams as Carriers to Promote Biofilm formation in attached growth systems

In this research study, open-cell polyvinylidene fluoride (PVDF) foams consisted of macroporous structures were designed and fabricated to be used as biofilm carriers for biological wastewater treatment. A manufacturing approach that integrated compression molding and particulate leaching was employed to fabricate the open-cell PVDF foams. PVDF foams with different porous structures and total protected surface areas were fabricated by different types (Sodium chloride (NaCl) and sodium acetate (NaOAc)) and different loadings (80 wt.% and 90 wt.%) of leaching agents. The fabricated carriers were used as moving bed biofilm reactors (MBBR) in batch aerobic and continuous aerobic flow wastewater treatment to evaluate their performance in terms of bacteria-to-carrier interaction and organic removal efficiency in biological wastewater treatment.

3.1. Experimental

3.1.1. Materials

Commercially available polyvinylidene fluoride (PVDF, Kynar 741, Arkema, with the molecular weight of 282 000 $g \cdot mol^{-1}$) was used as the polymer matrix to fabricate the open-cell foam carriers. Outstanding attributes of PVDF, such as high mechanical strength, good thermal stability, superior chemical resistance, and hydrophobicity, made it a good candidate to be used in this research [64-65]. Sodium chloride (NaCl, Windsor) and anhydrous sodium acetate (NaOAc, VWR) were used as leaching agents to produce open-cell structures. All materials are used as received without modifications. Table 3.1 summarizes the physical properties of the materials.

Table 3.1. Physical properties of PVDF, NaCl, and NaOAc

Material	Melting Temp. (°C)	Density (g/cm^3)
PVDF	170	1.78
NaCl	901	2.16
NaOAc	324	1.53

3.1.2. Preparation of open-cell PVDF biofilm carriers

PVDF powders were dry-blended with sieved NaCl or NaOAc particles with sizes ranging from 250 μm to 500 μm and smaller than 250 μm, respectively. Since NaOAc powders were very fine, it was not possible to obtain particles with the sizes larger than 250 μm. In this work the target was to fabricate open-cell foams with higher specific surface area, so media were fabricated by high salt contents (80 wt.% and 90 wt.%). Moreover, higher polymer contents in foam fabrication would adversely affect complete salt leaching process. The polymer-salt mixture loaded with 80 wt.% or 90 wt.% of leaching agents were molded by a compression molding machine (Craver Press, 4836 CH) into cylindrical samples (i.e., the diameter and height of each sample were 20 mm and 10 mm, respectively) by the following procedures:

STEP 1. PVDF-leaching agent mixtures were loaded into a four-cavity mold and subsequently transferred into the compression molding machine with a preset temperature of 185°C.

STEP 2. The mold was maintained in contact with the top and bottom heating platens for 5 minutes without increasing the pressure to completely melt the PVDF.

STEP 3. The samples and mold were pressurized to 5000 lbs-force for 5 minutes, and subsequently to 10 000 lbs-force for 10 minutes.

STEP 4. The mold was removed from the compression molding machine and was loaded into a cooling module with circulating water for 10 minutes to cool down the molded samples.

STEP 5. Each sample was immersed in 500 mL of deionized water for 72 hours to leach out NaCl or NaOAc from the PVDF matrix. Deionized water was replaced every 24 hours to avoid saturation of salt.

STEP 6. Samples were dried in an oven at 60°C for 24 hours.

3.1.3. Batch aerobic wastewater treatment

Experimental studies of batch aerobic wastewater treatment were conducted in a biological organic respirometric apparatus (BOD Trak II, HACH). Four 500 mL glass bottles were filled with 250 mL of return activated sludge (RAS) collected from Humber Wastewater Treatment Plant, 0.08 ml HACH BOD nutrient buffer, and 200 mL of sodium acetate solution as the feed. The volatile suspended solid (VSS) concentration in RAS and sCOD concentration of feed were 8000 mg/L and 10000 mg/L, respectively. Four bioreactors were set up. Each of them held a different PVDF foam sample made by either different salts or salt contents as summarized in Table 3.2 The feed-to-microorganism ratios were maintained to be unity. Aerated deionized water with dissolved oxygen (DO) concentration of 8 mg/L was used to prepare the feed solution in order to ensure sufficient oxygen supplied in bioreactors. In each bioreactor, a 0.25 g of potassium hydroxide pill was loaded in the cap without in contact with the content in the bioreactors. This was used to absorb the produced CO_2 during a biochemical reaction process during the degradation of organic materials. Cultures were mixed by a magnetic stirrer in a closed system condition to avoid biomass precipitation during the experiment, which were maintained at room temperature. Sensors were attached to the bioreactors to measure the consumed DO by microorganisms, which represented the microbial activity in each bioreactor, during the 5-day experiment. Upon the completion of the experiment, the suspended liquid phase was collected from each reactor to evaluate the performances of different carriers in sCOD removal.

Table 3.2. Samples tested in batch aerobic wastewater treatment experiments

Sample Name	Salt Type	Salt Content (wt.%)
$Foam_{NaCl,80}$	NaCl	80
$Foam_{NaCl,90}$	NaCl	90
$Foam_{NaOAc,80}$	NaOAc	80
$Foam_{NaOAc,90}$	NaOAc	90

3.1.4. Continuous aerobic flow wastewater treatment in MBBR

Continuous aerobic flow wastewater treatments in MBBR were conducted in two 1000 mL glass containers in order to evaluate the performance of open-cell PVDF carriers for sustainable biofilm growth and efficiency in biological wastewater treatment. Figure 3.1 illustrates a schematic of the MBBR experimental setup**.**

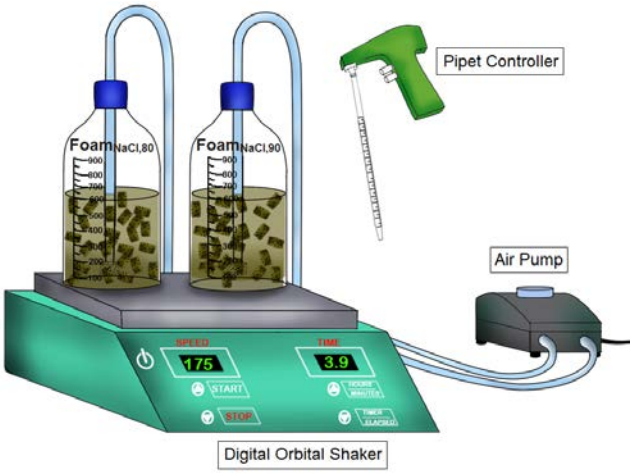

Figure 3.1. Schematic of moving bed bioreactor setup

Each bioreactor was loaded with 48 samples of $Foam_{NaCl,80}$, $Foam_{NaCl,90}$, and $Foam_{NaOAc,80}$. This represented a media filling ratio (i.e., the volume percent of the reactor being occupied by the carriers) of 30 vol.%. At the beginning of the test, each bioreactor was filled with 330 mL of RAS (i.e., 8400 mgVSS/L). An air pump, connected to air fine bubble diffusers, was used to supply

oxygen for microorganisms in the MBBR and to promote the uniform distribution of dissolved oxygen. Since bioreactors were planned to be moving bed, they were put in a digital orbital shaker, operated at 175 rpm, to induce the movement of PVDF open-cell carriers in the wastewater. Each bioreactor was fed by 300 mL of high concentration feed (i.e., 37200 mgsCOD/L) with a feed-to-microorganism ratio of unity. The microorganism content was determined based on the initial VSS concentration in the RAS. Table 3.3 summarizes the composition of the high concentration feed.

Table 3.3. The composition of high concentration feed

Component	**Concentration (g/L DI Water)**
CH_3COONa (NaOAc)	44.9
$NiCl_2 \cdot 6H_2O$	0.01
$CoCl_2 \cdot 6H_2O$	0.01
$CuCl_2$	0.04
$ZnCl_2$	0.02
$MnCl_2 \cdot 4H_2O$	0.25
$FeCl_2 \cdot 4H_2O$	0.15
$(NH_4)_6MO_7O_{24} \cdot 4H_2O$	0.04
H_3BO_3	0.02
$MgSO_4 \cdot 7H_2O$	2.8
$CaCl_2 \cdot 2H_2O$	1.33

The organic load was maintained at a high sCOD concentration in the first four days to accelerate the biofilm formation in the support media. For the remaining experiments, the organic load concentration was decreased by a factor of 100. Hydraulic retention time (HRT) was set to be 24 hr. In order to evaluate the performances of bioreactors, 20 mL of liquid samples were collected daily from the top segment of each bioreactor. The air pump and shaker were then turned off for two hours for biomass settlement. Consequently, 300 mL clean water at the top segment of the

bioreactors were replaced daily by 320 mL of fresh feed. The MBBR experiments were continuously operated for 110 days. sCOD in the suspended liquid phase was measured to evaluate the amount of soluble organic matters contributed by the added substrate. For sCOD measurement, collected water samples were filtered by a syringe filter with an average pore size of 0.45 μm to eliminate solid particles from the solution. The 2 mL filtered samples were then injected into COD vials and loaded into the digester for 2 hours at 150 °C. After the digestion of samples in the COD vials, the samples were cooled to the room temperature and sCOD was determined by COD analyzer (HACH, DR 3900) [69]. In addition to the sCOD measurement, the effectiveness of bacterial adhesion to the media in different bioreactors were compared by determining the VSS and the total suspended solid (TSS) in the water samples obtained from the bioreactors. VSS is a parameter used to monitor the biomass concentration in the suspended liquid phase of a bioreactor. Total suspended solid (TSS) represents the total amount of biomass in the system (i.e., VSS) and nonbiodegradable particles (i.e., fixed suspended solids (FSS)) in the bioreactors. In order to measure the amounts of VSS and TSS, a known volume (v) of water sample was injected into a pre-weighed glass microfibre filter with 1.2 μm particle retention and loaded onto an aluminium pan. The total mass of the dried sample, filter, and pan is denoted as m_1. They were then dried in an oven at a temperature of 105 °C for 2 hours. After cooling the sample, the mass (m_2) was measured at the room temperature. Then the aluminium pan, filter and the sample residue were burnt in a furnace at 550 °C for 20 minutes and their mass (m_3) was measured after cooling. Equations 3.1 and 3.2 were used to determine the *TSS* and *VSS* of the collected water samples [70]:

$$TSS = \frac{m_2 - m_1}{v} \tag{3.1}$$

$$VSS = \frac{m_2 - m_3}{v} \tag{3.2}$$

3.1.5. Characterization

Morphologies of the fabricated open-cell PVDF foams and the biofilm morphologies inside the carriers after subject to water treatment were characterized by using scanning electron microscopy (SEM, FEI Company, Quanta 3D FEG). To characterize the open-cell morphologies in PVDF foams, the cross-sections of PVDF open-cell foams (i.e., before culturing in the wastewater) were exposed by cryo-fracturing the samples under liquid nitrogen. The fractured surfaces were then sputter-coated with gold (Denton Vacuum, Desk V Sputter Coater). In order to analyze the biofilm morphologies inside the carriers after the water treatment experiments, samples were cut by a sharp blade to expose their cross-sections. Attached biofilm was extracted from support media by using a sonicator (VWR company, symphony^{TM}) continuously in 6 hrs with temperature control. Every 100 minutes the water in the sonicator was replaced by fresh water at room temperature to maintain the operational temperature bellow 40 °C. One media from each type was put inside a conical-bottom centrifuge tube which was filled with 50 mL deionized water and tightly wrapped by parafilm. After sonication, the suspended liquids were collected for further VSS measurement.

Open-cell content of each foam sample was analyzed by a pycnometer (Quantachrome, Micro-Ultrapyc 1200e). The open-cell content (i.e., porosity) of the sample is defined as the percentage of interconnected cells with respect to the geometric volume of the sample and can be calculated by Equation 3.3.

$$\text{Porosity } \% = \frac{V_g - V_p}{V_g} \times 100 \tag{3.3}$$

Where V_g and V_p are the geometric and pycnometric volume of the foam sample, respectively.

Particles size distribution (PSD) of NaCl and NaOAc were determined by a particle size analyzer (Beckman Coulter LS 13 320, Tornado DPS). By having the PSD of leaching agent particles and the known weight of leaching agents used for foam fabrication, the number of each particle with specific size would be found. The approximate total protected surface area available for biofilm formation in open-cell foams can be calculated according to Equation 3.4. It is worth mentioning that NaCl particles had a cubic shape. Moreover, due to the irregular shapes of NaOAc particles, these particles were considered as cubes. Real specific surface area of the open-cell foams prepared by NaOAc would be higher than what is calculated.

$$S_T = 6 \sum_{i=1}^{n} n_i L_i^2 \tag{3.4}$$

Where S_T is the total protected surface area for the foam samples and n_i is the number of salt particles with a size of L_i for NaCl or NaOAc particles.

3.2. Result and discussion

3.2.1. Effects of salt type and salt content on the structures of open-cell foams

Figure 3.2 plots the average porosities of open-cell foams prepared by using either different types or different loadings of salt. Experimental results revealed that, regardless of the salt type, the porosities of PVDF open-cell foams increased with salt loadings. Moreover, PVDF open-cell foams prepared by using NaOAc as the leaching agent had higher porosity than those prepared by using NaCl as the leaching agent. As NaOAc has a lower density than NaCl, the volume of NaOAc per unit mass is larger than that of NaCl. In other words, for open-cell samples prepared by the same loading (in wt.%) of NaOAc or NaCl, the total volume occupied by NaOAc would be larger

than that occupied by NaCl. As a result, PVDF open-cell foams prepared by NaOAc had higher open-cell contents than those prepared by NaCl.

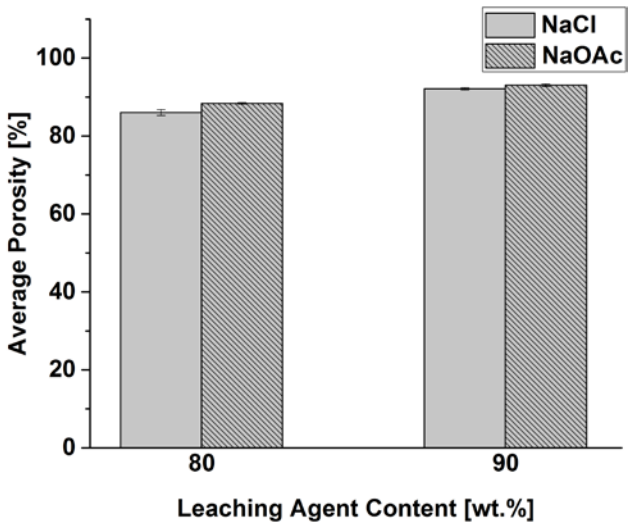

Figure 3.2. Average porosity of open-cell foams versus leaching agent content for samples with NaCl and NaOAc as leaching agents.

The PSD of NaCl and NaOAc were characterized by the particle size analyzer and the results are depicted in Figure 3.3. It can be observed that NaCl particles were larger than NaOAc particles. The PSD results indicated that over 85 vol.% of NaCl particles had sizes between 260 µm to 540 µm. In contrast, more than 87 vol.% of NaOAc particles had average sizes of 150 µm or smaller, and over 55 vol.% of the particles had sizes smaller than 90 µm.

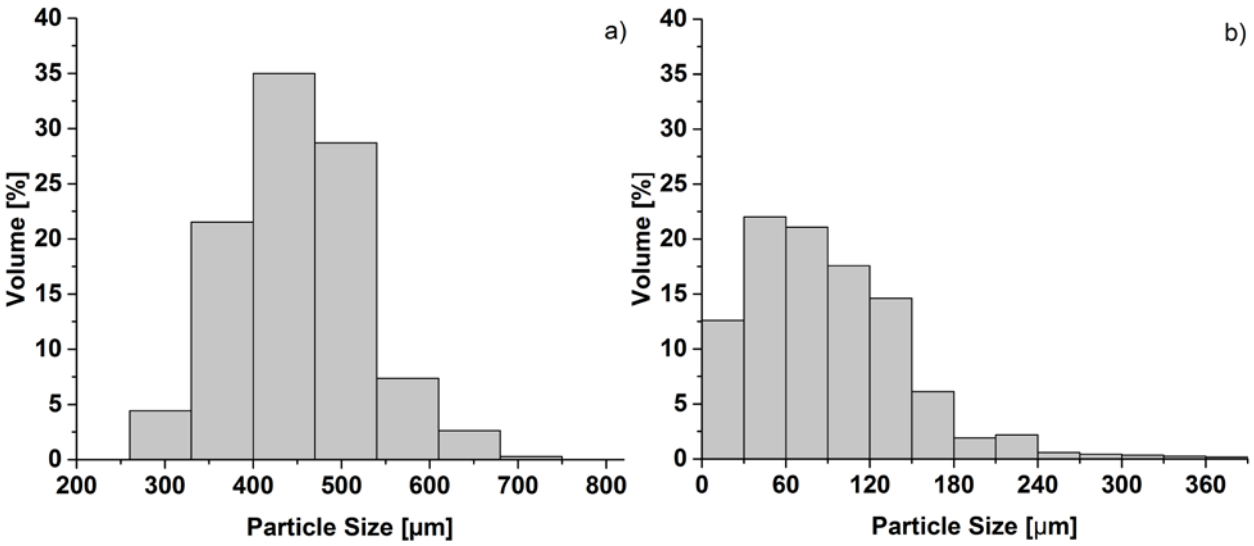

Figure 3.3. PSD of (a) NaCl, (b) NaOAc

Table 3.4. Protected surface area and specific surface area for open-cell foams [71]

Samples	**Specific surface area (m^2/m^3)**
$Foam_{NaCl,80}$	8,250+/-462
$Foam_{NaCl,90}$	9,780+/-126
$Foam_{NaOAc,80}$	93,300
$Foam_{NaOAc,90}$	105,600
AnoxKaldnes™ K1	500
ABC4™	600
ActiveCell™	450

By using PSD results and Equation 3.4, approximate specific surface areas of PVDF foams prepared by NaCl and NaOAc were calculated and summarized in Table 3.4. The results show that the fabricated open-cell foams had significantly larger specific surface areas than existing commercialized MBBR carriers [72]. This would provide more surface for biofilm adhesion and growth.

The effect of leaching agent content and particle sizes on the morphology of open-cell PVDF foams were analyzed by SEM. Figure 3.4 shows the SEM micrographs of the four open-cell PVDF foams which obtained at different magnifications. It can be observed that all foams had a large number of voids, which provided large specific surface areas for bacterial attachment. Furthermore, high interconnectivity among pores allowed bacteria to penetrate into the core of the foams and utilize all available surface areas within the macroporous structure. SEM micrographs in Figures 3.4(a) and 3.4(b) show that PVDF foams prepared by NaCl consisted of well-defined cubic pores. In contrast, Figures 3.4(c) and 3.4(d) reveal that PVDF foams prepared by NaOAc had irregular and finer pore geometries. Moreover, they contained a larger range of open-cell sizes than NaOAc particle sizes. The presence of tiny NaOAc particle sizes (i.e., smaller than 60 μm), resulted in their agglomeration during the compression molding process and caused larger voids in some parts of the foam prepared by NaOAc after salt leaching. Furthermore, due to the presence of very fine NaOAc particles, smaller pores could be observed on the cell walls of larger voids which can increase the foam fragility. However, according to Figure 3.3(a) the smallest particle sizes in NaCl are larger than 250 μm which formed large cell size distribution in the foams prepared by NaCl without extra small pores on their cell walls.

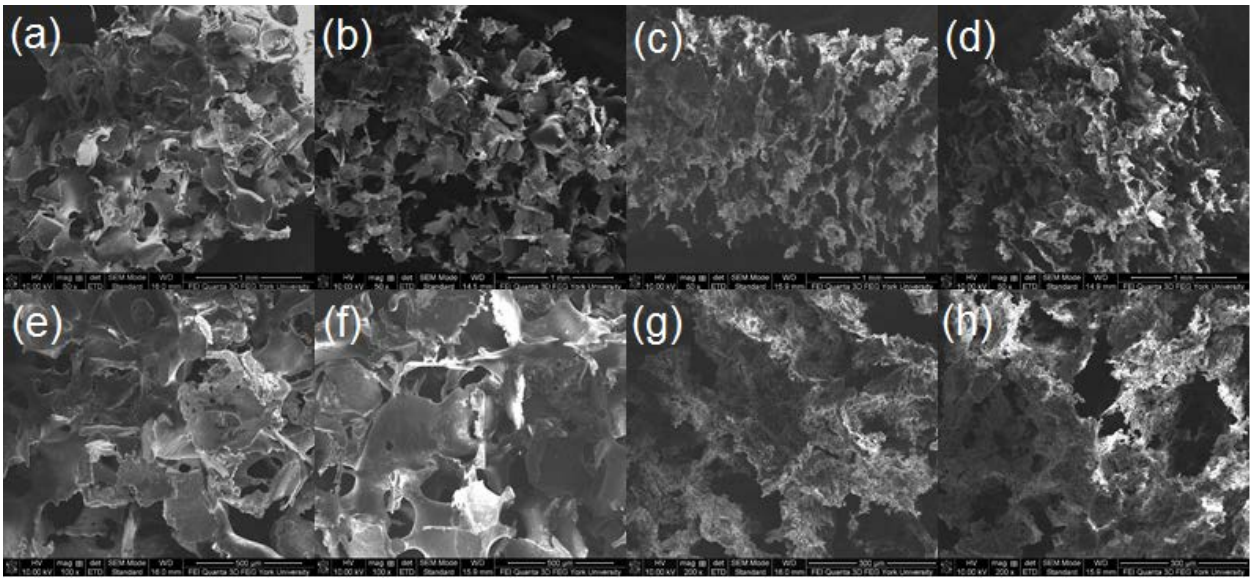

Figure 3.4. SEM micrographs at 50x for (a) $Foam_{NaCl,80}$, (b) $Foam_{NaCl,90}$, (c) $Foam_{NaOAc,80}$, (d) $Foam_{NaOAc,90}$, at 100x for (e) $Foam_{NaCl,80}$, (f) $Foam_{NaCl,90,}$ and 200x for (g) $Foam_{NaOAc,80}$, (h) $Foam_{NaOAc,90}$.

The loadings and particle sizes of leaching agent would affect the strength of the fabricated open-cell PVDF carriers. Higher loading of leaching agent in PVDF foams created higher porosity which led to thinner and weaker cell walls in the foam structure. In this context, PVDF foams prepared by NaOAc with initial leaching agent content of 90 wt.% were too fragile and completely broke down during the batch aerobic wastewater treatment.

3.2.2. Performance of batch aerobic wastewater treatment process

To evaluate the effect of open-cell foam carriers in organic removal from the bioreactors sCOD and biological oxygen demand (BOD) changes were investigated during the experiment. Figure 3.5(a) shows BOD change in bioreactors during the experimentation period. According to this figure, at the first phase of the experiment, BOD was increasing in all the bioreactors which indicates high oxygen uptake by microorganisms and thereby high microbial activity. However, in the second phase, BOD was gradually decreasing in all the bioreactors. Limited DO and

substrate inside the bioreactors restricted the microorganisms' activity and caused bacteria's decay.

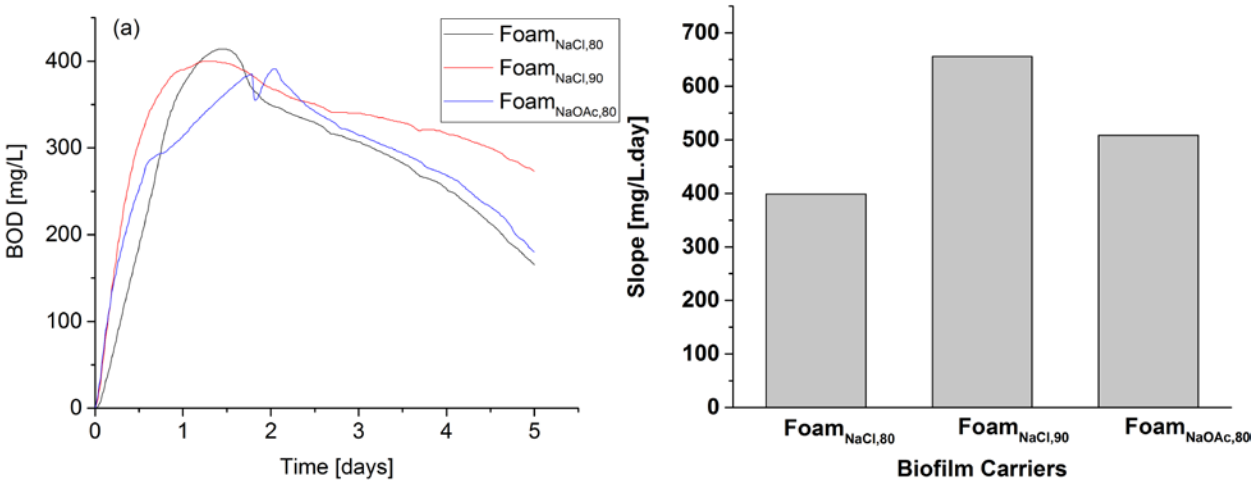

Figure 3.5. (a) BOD change with time for different bioreactors containing different biofilm carriers during the batch aerobic wastewater treatment process (b) Initial growth slope of microorganisms in bioreactors containing different biofilm carriers

The slope of the ascending part of the BOD graph shows the bacteria's oxygen uptake rate at the beginning of the experiment. Higher slope shows higher bacterial activity or bacterial growth which led to more DO consumption in bioreactors. Figure 3.5(b) illustrates that the bioreactor containing Foam$_{NaCl,90}$ had the highest initial rate of oxygen uptake compared to the other bioreactors. The higher rate of oxygen uptake in this bioreactor can be the result of Foam$_{NaCl,90}$ which accelerated bacterial activity and production.

According to Table 3.5 bioreactors had very low sCOD removal efficiency. The first reason for low sCOD removal efficiency would be microbial death which would release organic materials from cell tissues in bioreactors [48]. Furthermore, according to Figure 3.5(a) in all the bioreactors, BOD as the indicator of microorganisms' activity, was gradually decreasing which led to lower organic degradation. Besides, at the end of the experiment media were removed from the

bioreactors and cut to observe biofilm formation. Only the outer surfaces of the media were covered by biofilm while the inner parts were unreached. Therefore, 5 days of experimentation period did not give enough time to microorganisms for colonization and thereby the effect of biofilm was not completely observed in sCOD removal.

Table 3.5. sCOD removal efficiencies of bioreactors containing different open-cellular foams

Bioreactors	**sCOD Removal Efficiency (%)**
$Foam_{NaCl,80}$	0.77
$Foam_{NaCl,90}$	0.88
$Foam_{NaOAc,80}$	2.13

3.2.2. Performance of open-cell foams in continuous flow MBBR wastewater treatment process

In continuous aerobic flow wastewater treatment in MBBR which had higher shear stresses caused by the shaker, $Foam_{NaOAc,80}$ and $Foam_{NaOAc,90}$ were completely damaged in the first four days of the experiment. Therefore, only two bioreactors containing $Foam_{NaCl,80}$ and $Foam_{NaCl,90}$ were tested in this experiment. The continuous flow MBBR process was running for 110 days. The sCOD removal efficiency graphs of both bioreactors which consist of four different phases are depicted in Figure 3.6. The first phase of operation took two weeks for microorganisms' adaptation to the new habitat. At the second phase, which was during days 14-24, microorganisms in both bioreactors were attracted by a high concentration of fresh feed and could biodegrade organic materials with high efficiency. At the third phase of the process during days 24-46, which was biofilm stabilization time, microorganisms in both bioreactors were trying to get immobilized in their new support media and forming the mature biofilm attached to these carriers. Therefore, the VSS was dropped in the suspended phase and as a result microorganisms' organic

decomposing efficiency was reduced in bioreactors. However, from day 46 bioreactor containing $Foam_{NaCl,80}$ started to increase sCOD removal efficiency. The mature generated biofilm in this phase caused an improvement in the biodegradation of organic materials from third phase to the last phase.

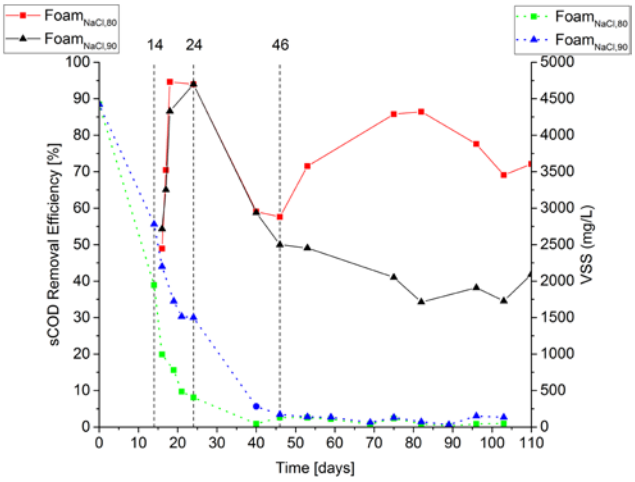

Figure 3.6. sCOD removal efficiency and VSS change by time in bioreactors containing $Foam_{NaCl,80}$ and $Foam_{NaCl,90}$ as biofilm carriers.

At the steady state,as show in Figure 3.7, sCOD removal efficiency in this bioreactor reached the average steady amount of 78%+/-7%. Adversely, in the bioreactor containing $Foam_{NaCl,90}$ the decreasing trend in sCOD removal efficiency continued after the third phase until the final days of experiment in which the average steady sCOD removal efficiency reached to 40%+/-2%.

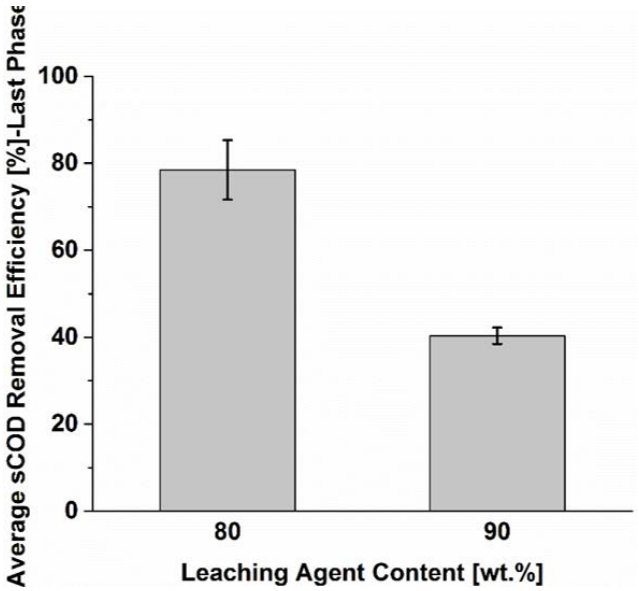

Figure 3.7. The average steady sCOD removal efficiency of bioreactors containing different biofilm carriers

Figure 3.8 shows $Foam_{NaCl,80}$ and $Foam_{NaCl,90}$ before and after being used in continuous aerobic flow wastewater treatment in MBBR. Images in Figure 3.8 show that during 110 days of biological process, $Foam_{NaCl,90}$ was deformed due to the high shear stresses created by the shaker and high retention time in the liquid phase. The occurred defects in these foams damaged open cells inside the foam and decreased available surface areas for bacteria to attach and generate biofilm. However, $Foam_{NaCl,80}$ had a stronger structure and retained its original shape and cell structure during the experiment. Lower available surface area for biofilm attachment in $Foam_{NaCl,90}$ carriers, explains lower sCOD removal efficiency in the bioreactor containing these carriers compared to the one containing $Foam_{NaCl,90}$.

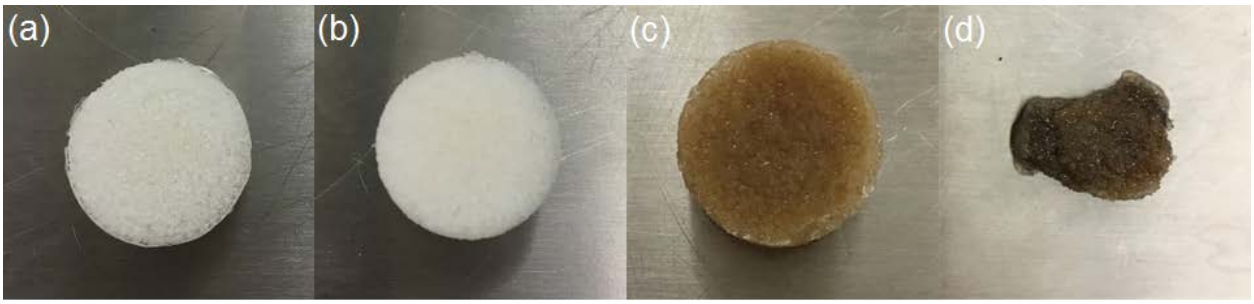

Figure 3.8. Open-cell foams before being used in biological process (a) $Foam_{NaCl,80}$, (b) $Foam_{NaCl,90}$ and after being used in biological process (c) $Foam_{NaCl,80}$, (d) $Foam_{NaCl,90}$.

Figure 3.9 shows the SEM micrographs of biofilm carriers which have been used for the biological process. These micrographs illustrate that both types of support media (e.g., $Foam_{NaCl,80,}$ $Foam_{NaCl,90}$) were completely covered by biofilm in all inner and outer surfaces. Existing biofilm on the media showed microorganisms' compatibility with new biofilm carriers. Moreover, the presence of biofilm in the core parts of the carriers revealed the ability of bacteria to penetrate into the foams and make biofilm in all available surface areas. Furthermore, penetration of bacteria to core parts of foams without clogging indicated that cell sizes in the support media were large enough for bacterial immobilization. It is important to note that the biofilm structure is different based on the types of foam.

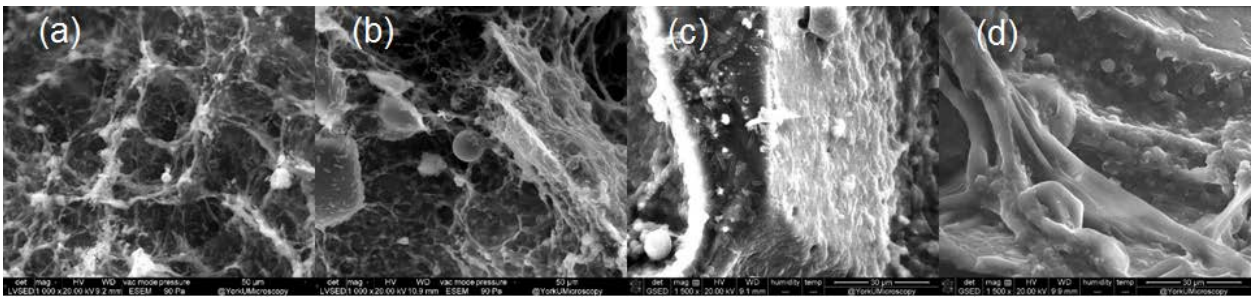

Figure 3.2. SEM micrographs of attached biofilm on (a) $Foam_{NaCl,80}$, (b) $Foam_{NaCl,90}$ at 1000x and (c) $Foam_{NaCl,80,}$ (d) $Foam_{NaCl,90}$ at 1500x

3.2.3. Attached biofilm measurement

Applying sonication, the average extracted biomass from $Foam_{NaCl,80}$ and $Foam_{NaCl,90}$ was 5.02 mg and 7.47 mg, respectively. Higher porosity in $Foam_{NaCl,90}$ provided higher interconnectivity among cells which would let the biofilm to be extracted from the media easier than $Foam_{NaCl,80}$. Meanwhile, after the sonication, still the biofilm was attached to the media to the large extent which meant that sonication could not completely detach biofilm from the supporting media. This confirms that open-cell foams are able to trap microorganisms inside their pores and protect biofilm. Since the biofilm detachment from the biofilm carriers at high shearing stresses is one drawback of existing carriers, this ability of open-cell foams in biofilm protection is an advantage over other carriers [32,73].

3.3. Conclusions

In this study, the effects of porosity and cell morphology of PVDF open-cell foams on biofilm formation and protection as well as organic removal efficiency was studied. SEM micrographs of open-cell foams showed that all PVDF carriers possessed a large number of voids and a high degree of interconnectivity among them. These resulted in the large specific surface area in the open-cell foams for biofilm formation (i.e., more than 10× to 20× of the area of commercialized carriers) and also facilitated microbial penetration through different parts of media. PVDF foams fabricated by NaCl as leaching agent had more sustainably than those prepared by NaOAc to be used for a long time in MBBRs with high shear forces. SEM images of PVDF foams prepared by NaCl showed that they were completely covered by biofilm in the exterior surfaces and the core regions of foams without clogging. Foams covered by biofilm confirmed microorganisms' compatibility with the PVDF open-cell as biofilm carriers. Foams made by 80 wt.% NaCl were stronger than those prepared by 90 wt.% NaCl and remained intact during the continuous flow

experiment and had average 78%+/-7% sCOD removal efficiency at the final days of the experiment. However, the sCOD removal efficiency for the carriers with 90 wt.% NaCl content reached to 40%+/-2%. Moreover, open-cell foam demonstrated high potential in biofilm protection. Biofilms were firmly attached to open-cell foams and sonication was not successful to detach total biofilm from open-cell foams which this high biofilm protection is an advantage over existing MBBR carriers.

Chapter 4
Effects of Foam Morphology on the Sustainability of Biofilms Performances in Wastewater Treatment

This chapter aims to develop innovative and cost-effective substratum in order to upgrade current biofilm carriers used in attached growth wastewater treatment systems. Open-cell foams have unique characteristics such as high levels of interconnectivity among their cells, high porosity and high surface roughness which make them a potential habitat for microbial immobilization as biofilm carrier [73]. The first phase of this study (chapter 3) showed that open-cell foams made by PVDF and 80 wt.% NaCl are in favor of microbial attachment with high organic removal efficiency and high sustainability against biofilm detachment under shear forces [2]. Outstanding attributes of PVDF such as high mechanical strength, thermal stability, chemical resistance, and hydrophobicity made it a good candidate to be used as the polymer matrix for foam fabrication. In the current chapter the effects of porosity and cell sizes of open-cell PVDF foams on biofilm formation, biofilm protection, and organic removal have been investigated. For this purpose, PVDF open-cell foams with different leaching agent loadings (80 wt.%, 85 wt.%, and 90 wt.%) and different particle sizes (ranging from 106 μm to larger than 500 μm) were manufactured. Moreover, HDPE was used as another polymer matrix to fabricate open-cell foams. Beside excellent properties of HDPE such as good mechanical strength, great chemical resistance, thermal stability, and inherent hydrophobicity, HDPE is a low cost polymer which makes it a good choice over other commercial polymers [60]. In the previous study PVDF open-cell foams with 80 wt.% leaching agent content and particle sizes in the range of 250 μm to 500 μm, achieved a good performance which led us to fabricate both PVDF and HDPE open-cell foams in these specific conditions to compare their performance in wastewater treatment.

4.1. Experimental

4.1.1. Materials and Methods

Commercially available polyvinylidene fluoride (PVDF, Kynar 741, Arkema) and high density polyethylene (HDPE, SCLAIR 2710 Resin, Nova Chemical) were used as the polymer matrix for foam fabrication. Sodium chloride (NaCl, Windsor) was used as a leaching agent to produce open-porous structures. All materials were used as received without modifications. Physical properties of materials are summarized in Table 4.1.

Table 4.1. Physical properties of PVDF, HDPE, and NaCl

Materials	Melting Temp. (°C)	Density (g/cm^3)
PVDF	170	1.78
HDPE	115-135	0.95
NaCl	901	2.16

4.1.2. Preparation of HDPE powder form from HDPE granules

Pre-weighed HDPE granules were poured inside grinding vials of freezer/mill machine (SPEX SamplePrep 6770). Liquid nitrogen was used to chill the samples in order to prepare them for pulverizing with a magnetically driven impactor. To reach to the fine HDPE powder, the freeze milling process was repeated for five runs and each run took 1 hour.

4.1.3. Preparation of open-cell biofilm carriers

PVDF and HDPE powders were dry-blended with sieved NaCl particles with sizes ranging from 106 μm to 250 μm (small), 250 μm to 500 μm (medium) and sizes larger than 500 μm (large). The polymer-salt mixtures, containing 80 wt.%, 85 wt.% or 90 wt.% of leaching agents, were molded by a compression molding machine (Craver Press, 4836 CH) into circular disc samples

(i.e., the diameter and height of each disc are 20 mm and 10 mm, respectively) with the same method that was explained in the chapter 3. In this work the target was to fabricate open-cell foams with higher specific surface area, so media were fabricated by high salt contents (80 wt.%, 85 wt.%, and 90 wt.%). Moreover, higher polymer contents in foam fabrication would adversely affect complete salt leaching process.

4.1.4. Experimental setup and operation

7 Polycarbonate Erlenmeyer flasks were used to set up the moving bed bioreactors. Air was supplied to the bioreactors using an air pump while one air fine bubble diffusor was placed at the bottom of each bioreactor to eject compressed air. There was a hole with 2 mm diameter in each bioreactor cap for air circulation. Bioreactors were attached to a shaker (Fisherbrand™ Wrist Motion) to provide homogenous circulation of media inside them. Figure 4.1 shows the experimental setup.

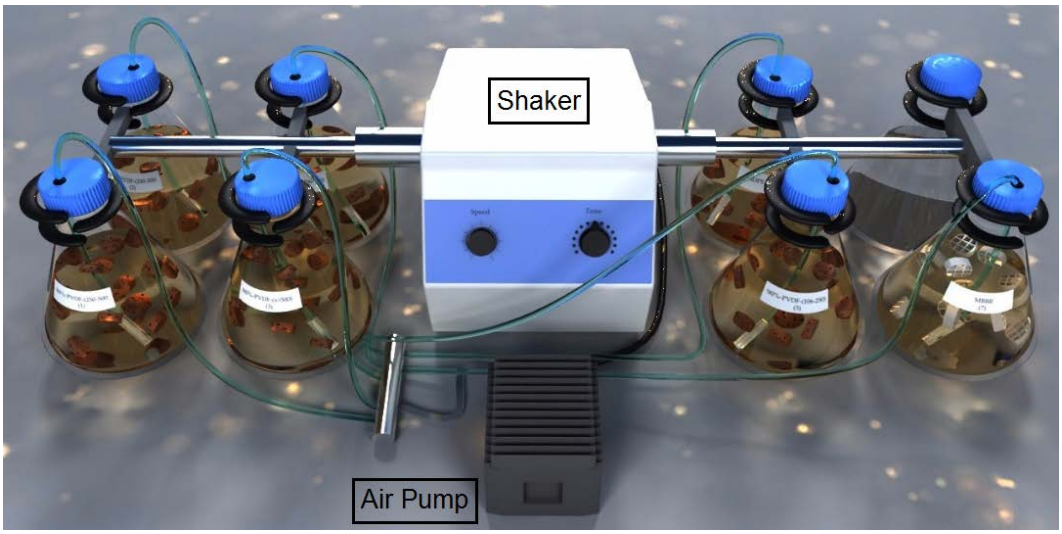

Figure 4.1. Schematic of moving bed bioreactors setup

Table 4.2. Open-cell foam samples tested in moving bed bioreactors

Sample Name	Polymer Type	Salt Content (wt.%)	Salt Particle Size (μm)
$PVDF_{80M}$	PVDF	80	250-500
$PVDF_{85M}$	PVDF	85	250-500
$PVDF_{80L}$	PVDF	80	>500
$PVDF_{80S}$	PVDF	80	106-250
$PVDF_{90S}$	PVDF	90	106-250
$HDPE_{80M}$	HDPE	80	250-500

Five bioreactors were filled with 20 media from each type of PVDF open-cell foams, one bioreactor contained 20 MBBR media, and one bioreactor contained 6 HDPE samples. Due to the time-consuming process of HDPE powder preparation, only 6 HDPE carriers were fabricated for this experiment. Each bioreactor containing open-cell foams held a different PVDF or HDPE foam sample made by either different salt contents or salt particle sizes as summarized in Table 4.2.

The effective volume of the bioreactors was 750 ml. As the initial start up, bioreactors were inoculated with RAS (i.e., 2360 mgVSS) collected from Humber Wastewater Treatment Plant. The first 2 weeks of the experiment were taken for microorganisms' acclimatization and thus facilitate biofilm formation on the biofilm carriers. Analysis of volatile suspended solids (VSS) changed in the suspended liquid phase of bioreactors evaluates early attachment of biofilm on the media. After the initial startup, bioreactors were fed by a synthetic substrate containing glucose and trace metals. Following composition of trace metals was used for the preparation of synthetic substrate (g/L): $NiCl_2 \cdot 6H_2O$ (0.01), $CoCl_2 \cdot 6H_2O$ (0.01), $CuCl_2$ (0.04), $ZnCl_2$ (0.02), $MnCl_2 \cdot 4H_2O$ (0.25), $FeCl_2 \cdot 4H_2O$ (0.15), $(NH_4)_6MO_7O_{24} \cdot 4H_2O$ (0.04), H_3BO_3 (0.02), $MgSO_4 \cdot 7H_2O$ (2.8), $CaCl_2 \cdot 2H_2O$ (1.33). The trace metals proportion to the synthetic substrate was 2:1000 mL. To evaluate the performance of bioreactors once a week 10 mL liquid samples were collected from

the top segment of bioreactors. In order to feed the system, everyday, pumps and the shaker were turned off for 2 hours for biomass settlement. Consequently, 20 mL of suspension at the very top segment of the bioreactors were replaced by 20 mL of fresh feed. This procedure was done until the 50th day of the experiment. Afterwards organic loading and injection flow rate increased to 40 ml with skipping settlement process to accelerate microorganisms discharge from the bioreactors and evaluate the effect of biofilm in sCOD removal.

4.1.5. Characterization

sCOD of the suspended liquid phase in bioreactors was measured by COD analyzer (HACH, DR 3900) [69] as a representation of soluble organic materials inside bioreactors coming from the substrate added to the bioreactors. Rather than sCOD measurement, another important analysis is determining the concentration of biomass inside the suspended liquid phase of bioreactors which shows bacterial growth and attachment to the media. For VSS measuremernt, a specific volume of suspended phase sample was first injected into a pre-weighed glass microfibre filter, with 1.2μm particle retention, and then it was dried in the oven at 105 °C for 2 hours. Afterwards, the filter and the sample residue were ignited in a furnace at 550 °C for 20 minutes. The weight difference before and after the ignition shows the VSS of the sample.

Scanning electron microscopy (SEM) (FEI Company, Quanta 3D FEG) was used to characterize open-cell foams and biofilm structure. To prepare open-cell foams for microscopy cross-sections of samples were exposed by cryo-fracturing them under liquid nitrogen and the fractured surfaces were then sputter-coated with gold (Denton Vacuum, Desk V Sputter Coater). Moreover, after biological process, in order to analyze biofilm in different positions of biofilm

carriers, 1/8 of each media were cut by the sharp blade and five different positions of the media were observed by SEM according to the Figure 4.2.

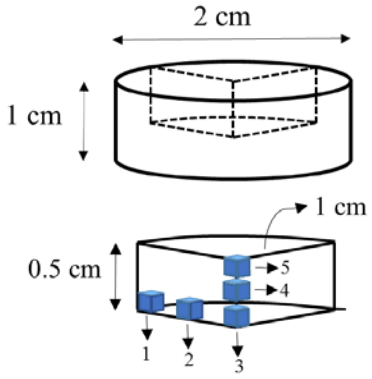

Figure 4.2. Specific positions of biofilm carriers observed in SEM

Open-cell content (porosity) of foams was analyzed by pycnometer (Quantachrome, Micro-Ultrapyc 1200e). The open-cell content is defined as the percentage of interconnected cells with respect to the geometric volume of the sample. Particle size analyzer (Beckman Coulter LS 13 320, Tornado DPS) was used to determine the PSD of NaCl particles as a leaching agent.

The available surface area in foam samples for microorganisms' attachment was calculated according to Equation 4.1. By having the PSD of leaching agent particles and the known weight of leaching agent used for foam fabrication, the number of each particle with specific size would be found. Moreover, it is worth mentioning that NaCl particles had a cubic shape.

$$S_T = 6 \sum_{i=1}^{n} n_i L_i^{\,2} \tag{4.1}$$

Where S_T is the total protected surface area for the foam samples and n_i is the number of NaCl particles with a size of L_i.

In order to measure the amount of biomass attached to each biofilm carrier, one media of each type was put in a conical-bottom centrifuge tube which was filled with 50 mL deionized water and tightly wrapped by parafilm. The tubes then were put in the sonicator device (VWR company, symphony^{TM}) to extract the biofilm from the media. The process was done for three runs and each run took 6hrs continuously with temperature control. Every 100 minutes the water in the sonicator chamber was replaced by fresh water at room temperature to maintain the operational temperature bellow 40 $^{\circ}$ C. After each run the suspended liquid was collected from the tubes and replaced with fresh DI water. Collected suspended liquids were further used for VSS measurement.

4.2. Results and discussion

4.2.1. Effects of salt size and salt content on the structures of open-cell foams

Salt size and salt content are the governing factor which can affect the open-cell foam structure. Pycnometer was used to measure the porosity (open-cell content) of different fabricated carriers. Porosity of open-cell foams are shown in Figure 4.3. Pycnometer results confirmed that PVDF open-cell foams which had higher leaching agent contents during their fabrication process, had higher porosities. Moreover, Figure 4.3 showed that different cell sizes of the foams did not affect the porosity of the media to large extent. Comparing the samples weight before and after salt leaching process showed that almost all the salt which was used for foam fabrication had been leached out from the PVDF samples. This showed that PVDF foams had a complete salt leaching process and the porosity of the samples only relied on the initial leaching agent content used for foam fabrication. However, open-cell foams prepared by HDPE were not completely able to remove all the salt from the media during the leaching process. The experimental data showed that between 13 wt.% to 17 wt.% of HDPE open-cell foams contained salt residue which led these samples to have less porosity comparing to the PVDF open-cell foams prepared by the same

leaching agent content. According to PSD results, PVDF powders had smaller and finer particle sizes (less than 40 µm) than HDPE powders (200 µm to 2000 µm). Bigger sizes of HDPE powders caused uneven polymer distribution during the compression molding process which could trap some part of NaCl inside the foam and prevent them from complete salt leaching. As a result, $HDPE_{80M}$ had lower porosity than other media prepared by PVDF (e.g., $PVDF_{80M}$, $PVDF_{80L}$, and $PVDF_{80S}$) at the same leaching agent content.

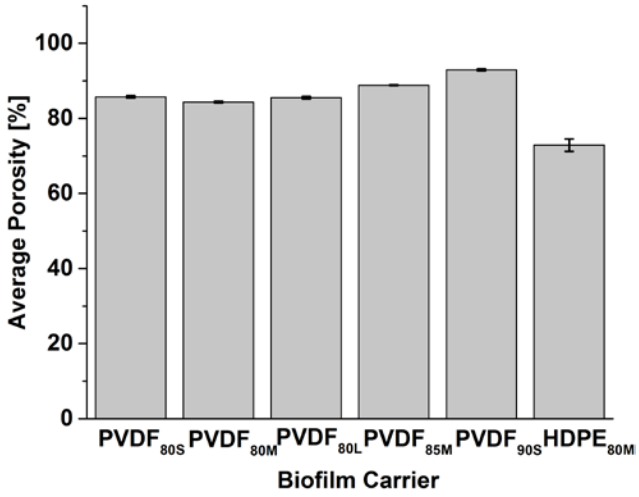

Figure 4.3. Average porosity of open-cell foams versus leaching agent content for samples prepared by PVDF and HDPE as the polymer matrix and NaCl as a leaching agent.

NaCl particles before the open-cell foam fabrication were sieved and classified into three ranges; 106 µm-250 µm, 250 µm-500 µm, and sizes larger than 500 µm. However, in order to calculate the protected surface area of the open-cell foams, the exact particle size distribution of NaCl was found by particle size analyzer. The PSD results for the three different size ranges of NaCl are shown in Figure 4.4.

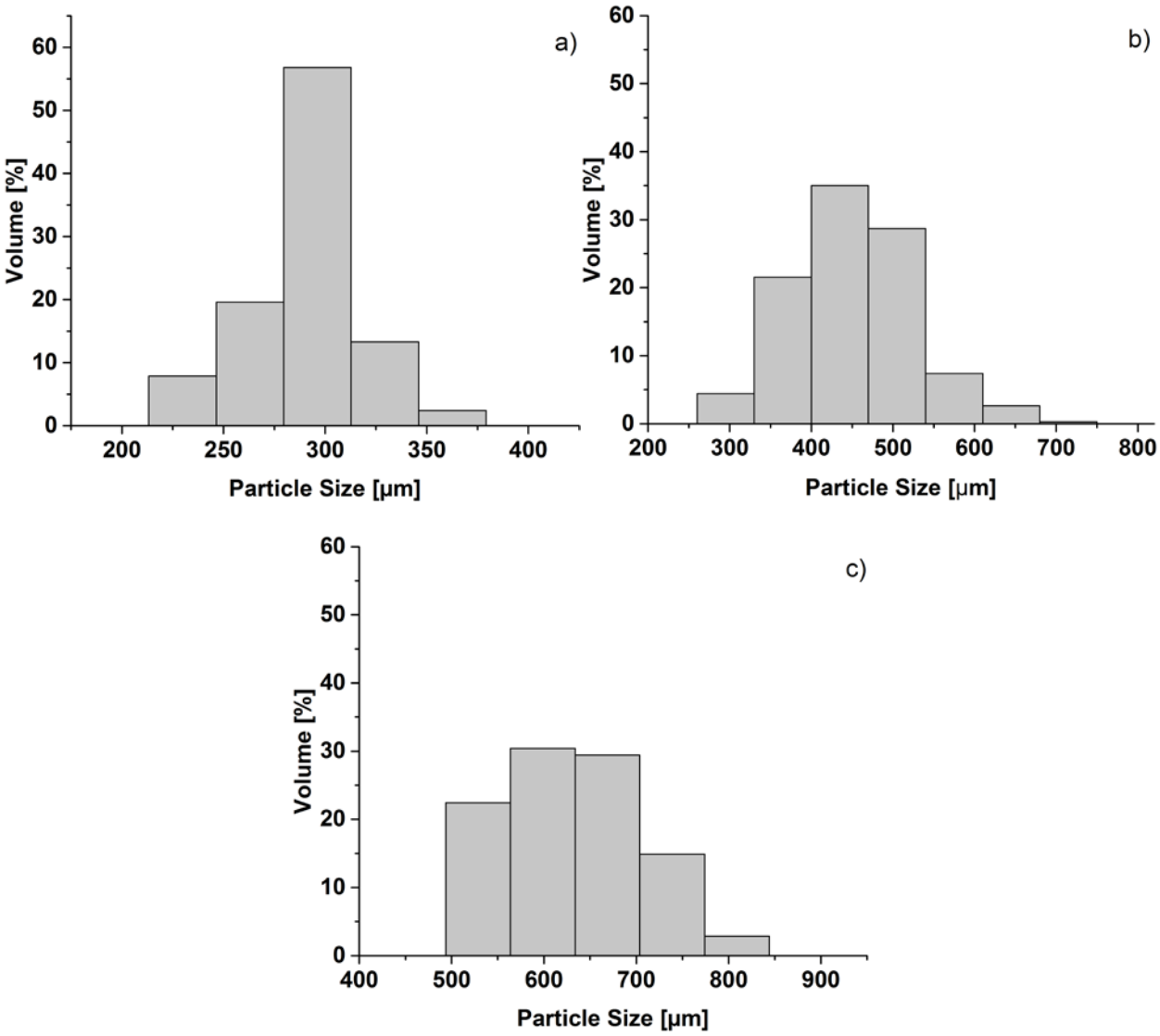

Figure 4.4. PSD of NaCl at size ranges of a) 106 µm-250 µm, b) 250 µm-500 µm, and c) larger than 500 µm

By using PSD results and Equation 4.1, also assuming that open-cell foams had complete leaching agent process, approximate specific surface areas of samples were calculated and summarized in Table 4.3. Moreover, this table shows the specific surface area of some carriers currently in the market.

Table 4.3. Specific surface area of different biofilm carriers [71]

Samples	Specific surface area (m^2/m^3)
$PVDF_{80M}$	8,250+/-462
$PVDF_{85M}$	7,780+/-761
$PVDF_{80L}$	5,580+/-40
$PVDF_{80S}$	12,350+/-507
$PVDF_{90S}$	15,000+/-617
$HDPE_{80M}$	6,550+/-114
AnoxKaldnes™ K1	500
ABC4™	600
ActiveCell™	450

Comparing the specific surface area of the open-cell foams and other biofilm carriers in the market, open-cell foams have a much higher specific surface area (e.g., 10× to 20×) which is the great advantages over other biofilm carriers.

Figure 4.5 shows SEM micrographs of 6 open-cell foams prepared by PVDF or HDPE. The effect of leaching agent content and particle sizes on the morphology of open-cell PVDF foams were analyzed by SEM. It can be observed that all foams had a large number of voids, which provided large specific surface areas for bacterial attachment. Furthermore, high interconnectivity among pores allowed bacteria to penetrate into the core of the foams and utilized all available surface areas within the macroporous structure. SEM micrographs in all open-cell foams consisted of well-defined cubic pores.

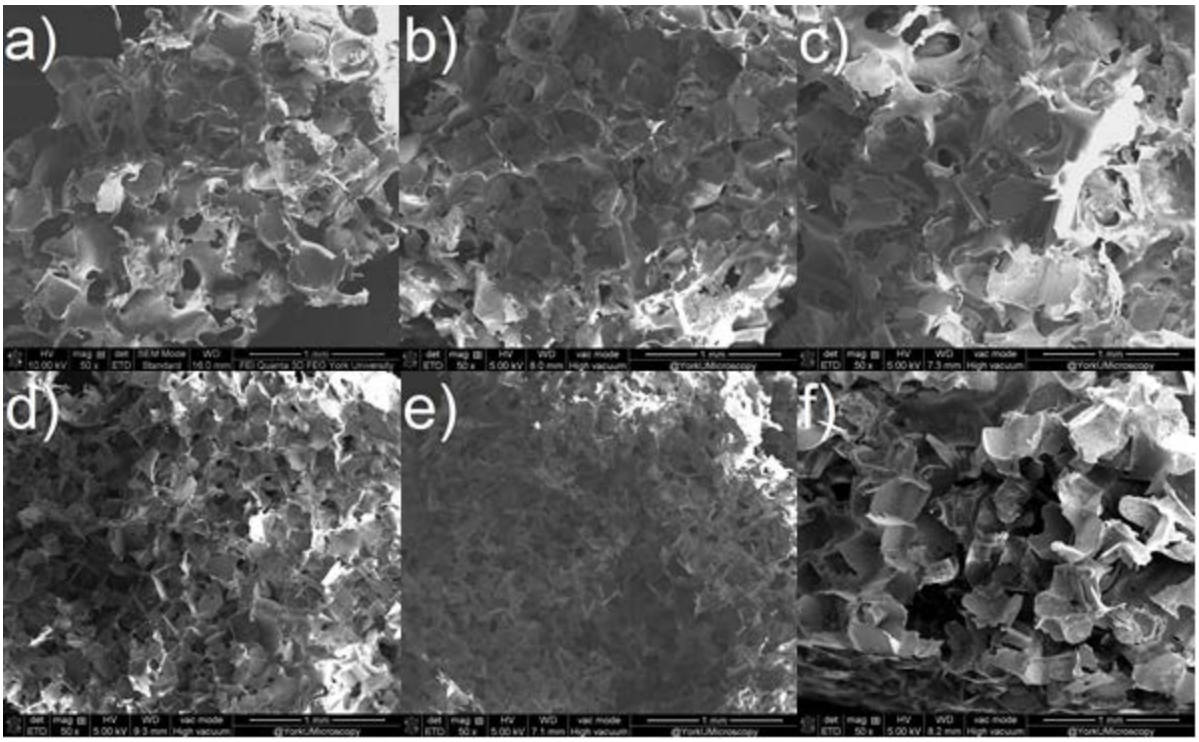

Figure 4.5. SEM micrographs of clean biofilm carriers at 50x for a) $PVDF_{80M}$, b) $PVDF_{85M}$, c) $PVDF_{80L}$, d) $PVDF_{80S}$, e) $PVDF_{90S}$, f) $HDPE_{80M}$

Figure 4.6 shows biofilm carriers before being used in the biological process.

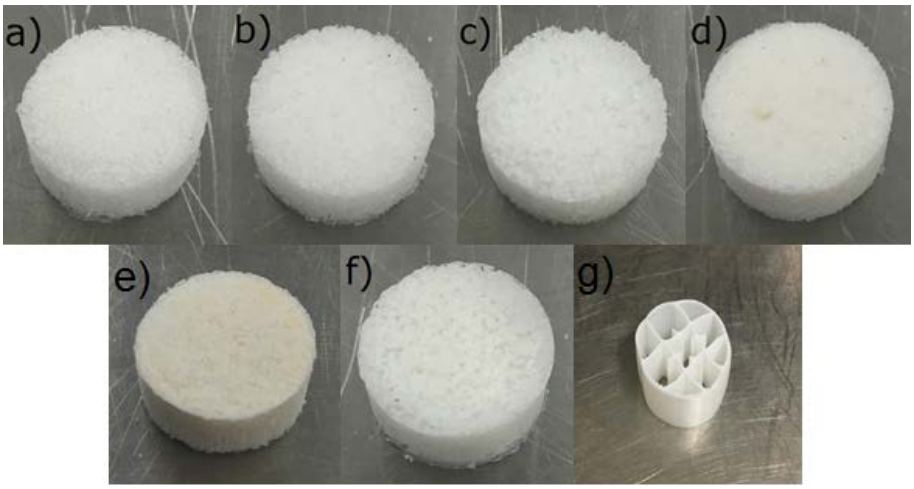

Figure 4.6. Biofilm carriers before biological process a) $PVDF_{80M}$, b) $PVDF_{85M}$, c) $PVDF_{80L}$, d) $PVDF_{80S}$, e) $PVDF_{90S}$, f) $HDPE_{80M}$ g) MBBR

Figure 4.7 shows biofilm carriers after being used in continuous aerobic flow wastewater treatment in MBBR. The image in Figure 4.7(e) shows that during the biological process $PVDF_{90S}$ media was deformed due to the high shear stresses of the shaker and high retention time in the liquid phase. The loadings and particle sizes of leaching agent would affect the strength of the fabricated open-cell PVDF carriers. Higher loadings of leaching agent in the open-cell foams led to thinner and weaker cell walls in the foam structure. Foams with thinner cell walls have lower mechanical strength and are more fragile under harsh conditions. This explains the fragility of the open-cell foam made by 90 wt.% leaching agent. However, rest of open-cell foams had a stronger structure and retained their original shape and cell structure during the experiment. MBBR media had a strong structure and no damage occurred to it during the experiment.

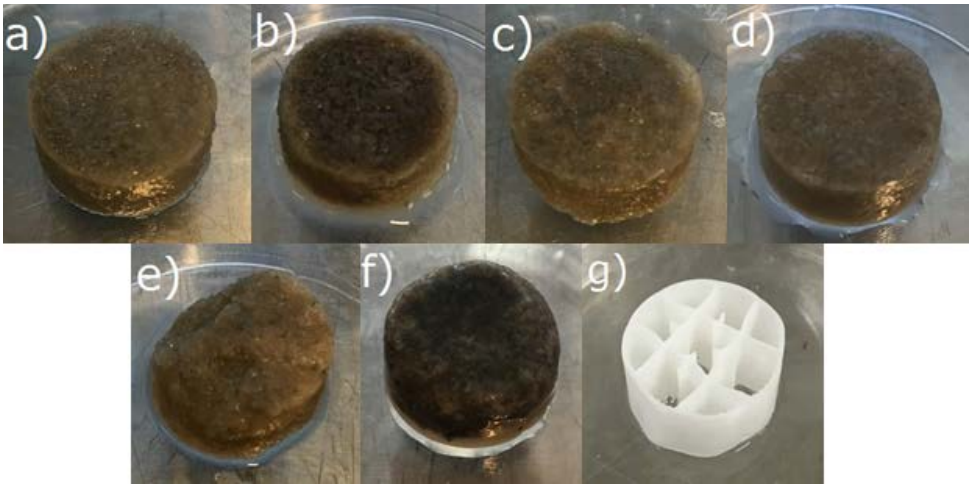

Figure 4.7. Biofilm carriers after biological process a) $PVDF_{80M}$, b) $PVDF_{85M}$, c) $PVDF_{80L}$, d) $PVDF_{80S}$, e) $PVDF_{90S}$, f) $HDPE_{80M}$ g) MBBR

4.2.2. Performance of biofilm carriers in continuous flow MBBR wastewater treatment process

Over the experimentation period the amount of microorganisms inside the bioreactors was changing. Initially, bioreactors were filled with RAS which had a specific amount of VSS. The first 2 weeks of the experiment were taken for microorganisms' acclimatization. The reduction in

the VSS in this phase of experiment can be caused by the early microbial attachment to the biofilm carriers. After the 2 weeks, in order to increase the bacterial growth inside the bioreactors to facilitate biofilm attachment, systems were feeding by synthetic substrate. Gradually, the substrate concentration was increasing until the 3rd week of the experiment which the feed to microorganisms ratio reached 1 gCOD/gVSS. This phase, in which bioreactors contained a high amount of VSS, was called biofilm development phase. In order to statistically compare the organic removal efficiency of different bioreactors, TTEST as one type of inferential statistics was used. This test is used to determine whether there is a significant difference between the means of two groups. In this test P value shows that how confident do we want to be about the statement that there is a significantly difference between the groups of the data (e.g., $P<0.05$ shows 95% confidence and $P<0.01$ shows 90% confidence). As shown in figure 4.8(a), due to the high amount of VSS inside the bioreactors at the biofilm development phase, sCOD removal efficiency at this phase was high and statistically the same (TTEST: $P>0.05$) for all the bioreactors.

Afterwards, in order to investigate the effect of attached biomass to the biofilm carriers on the organic removal efficiency of the bioreactors, the VSS concentration in the suspension was targeted to be reduced. The substrate concentration was decreasing to eliminate the VSS growth inside the bioreactors. So, the food to microorganisms ratio was gradually decrease and finally fixed at 0.4 gCOD/gVSS for all the bioreactors. Moreover, organic loading and injection flow rate increased to 40 ml (with skipping the settlement process) which accelerated microorganisms discharge from the bioreactors. Besides, at this phase, microorganisms were trying to get immobilized in their new support media and forming the mature biofilm attached to these carriers. Therefore, the VSS was dropped in the suspended phase. As a result, microorganisms' organic decomposing efficiency was reduced in all the bioreactors. This phase of the experiment was called

biofilm enriched phase where the effect of biofilms on organic removal efficiency of the systems was noticed. Figure 4.8(b) shows the effect of hybrid system (both suspended VSS and attached biomass) on the organic removal efficiency of the bioreactors at biofilm enriched phase. This figure shows that all the bioreactors containing PVDF carriers statistically had the same organic removal efficiency (TTEST: P>0.05). Moreover, Figure 4.9(b) shows that the effect of porosity and cell sizes of PVDF open-cell foams on the organic removal efficiency of the bioreactors containing these foams were negligible. Moreover, both bioreactors containing HDPE open-cell foams and MBBR media had statistically the same organic removal efficiency (TTEST: P>0.05) and lower than other bioreactors.

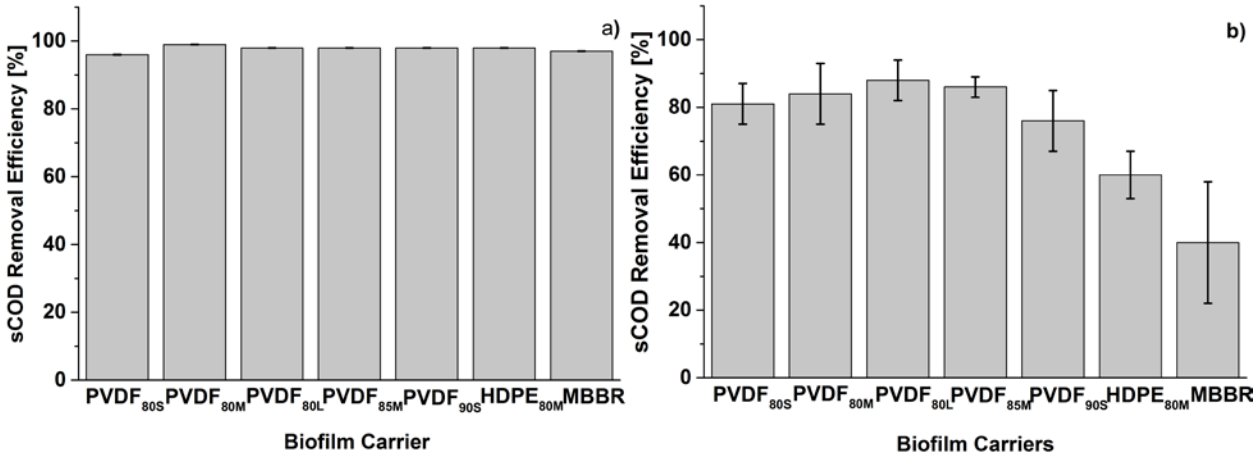

Figure 4.8. sCOD removal efficiency of bioreactors containing different biofilm carriers at a) biofilm development phase b) enriched biofilm phase

Figure 4.9 shows the SEM micrographs of biofilm carriers which have been in the biological environment until the end of the experimentation period. he SEM micrographs show lower biofilm density at the areas close to the outer surfaces (P1 and P5) of the biofilm carriers. The outer surfaces of the media are supposed to be subjected by higher shear forces inside the bioreactors, created by the shaker, which led to the lower capability of biofilm formation or higher biofilm detachments

in these areas compared to the inner parts. However, due to the high porosity and roughness of open cell foams, shear forces would be diminished in the inner sides of the open-cell foams.

Moreover, SEM micrographs in figure 4.9 show that for most of the biofilm carriers in the positions of P2 and P4, they have significantly higher biofilm aggregation comparing to the outer surfaces. However, for some of the biofilm carriers the core part of the media (P3) have less microbial attachment comparing to the P2 and P4 positions which can be the result of limited oxygen and substrate diffusion to these areas.

Furthermore, these micrographs show that media with higher porosity ($PVDF_{90S}$ and $PVDF_{85M}$) had higher microbial accumulation. Higher porosity of the biofilm carriers led to higher available surface area for biofilm formation. Moreover, higher porosity of the open-cell foams created higher interconnectivity among cells which would develop oxygen and substrate diffusion in the media and promote biofilm formation. Although the results showed that open-cell foams prepared by HDPE had low biofilm accumulation, less filling factor in the bioreactor containing these media, due to the difficulty of manufacturing of HDPE carriers, can be a reason for the low organic removal efficiency in this bioreactor.

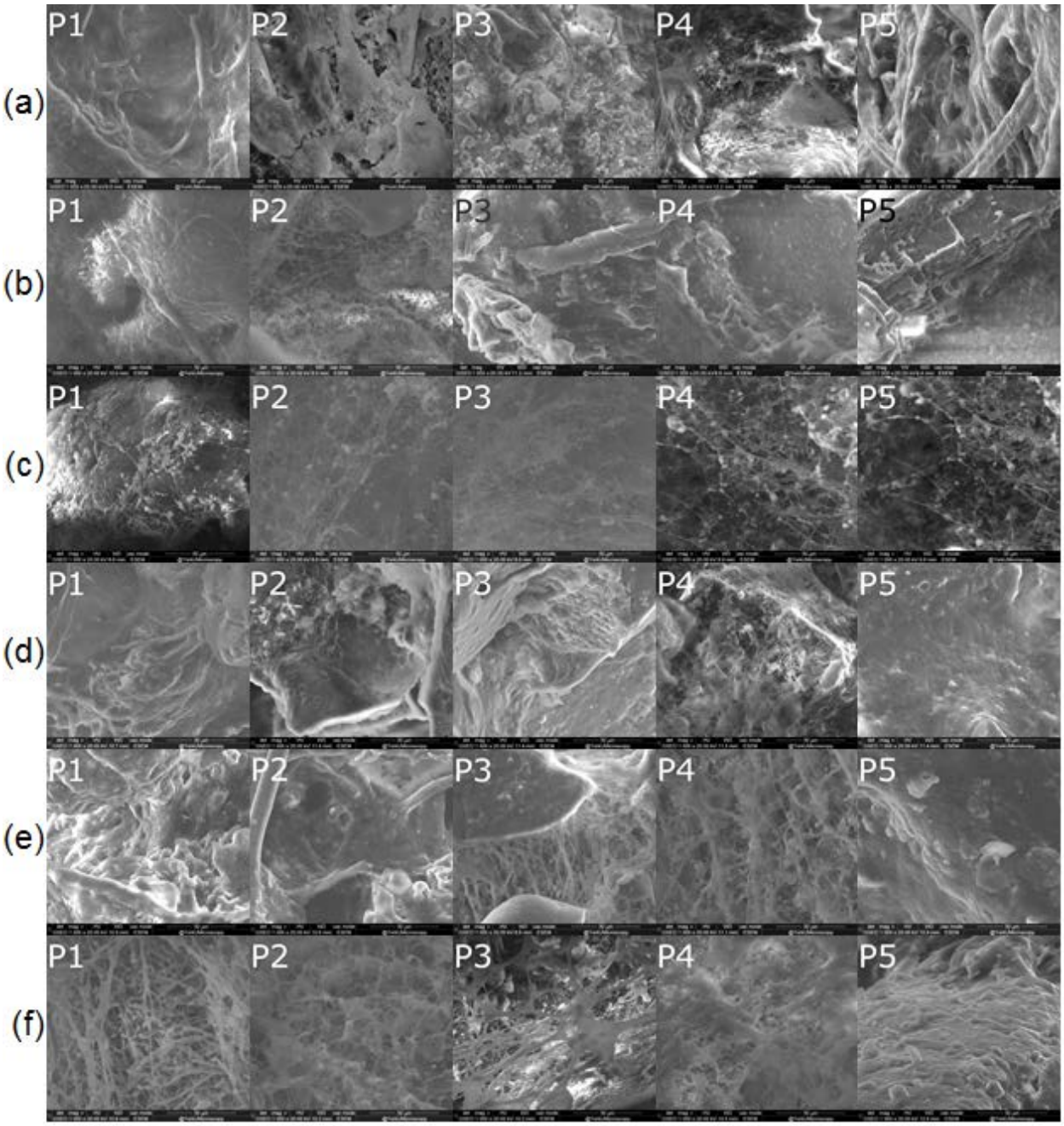

Figure 4.9. SEM micrographs of biofilm carriers covered by biofilm from different positions of samples (P1 to P5) a) $PVDF_{80M}$, b) $PVDF_{85M}$, c) $PVDF_{80L}$, d) $PVDF_{80S}$, e) $PVDF_{90S}$, f) $HDPE_{80M}$

Figure 4.10 shows the SEM micrograph of three different parts of MBBR media which is commercially used in the wastewater treatment plants. The micrographs show that MBBR media was not successful in biofilm formation. High shear forces inside the bioreactors can affect low

attachment density in this media. Open-cell foams provided a much larger protected surface area for biofilm formation in the bioreactor. Moreover, high roughness and a large number of voids inside the foams eliminated shear forces applied to the media which consequently promoted biofilm formation in these samples [25,32]. Low attached biomass to the MBBR media explains the lower sCOD removal efficiency of the bioreactor containing these media than other bioreactors filling with PVDF open-cell foams.

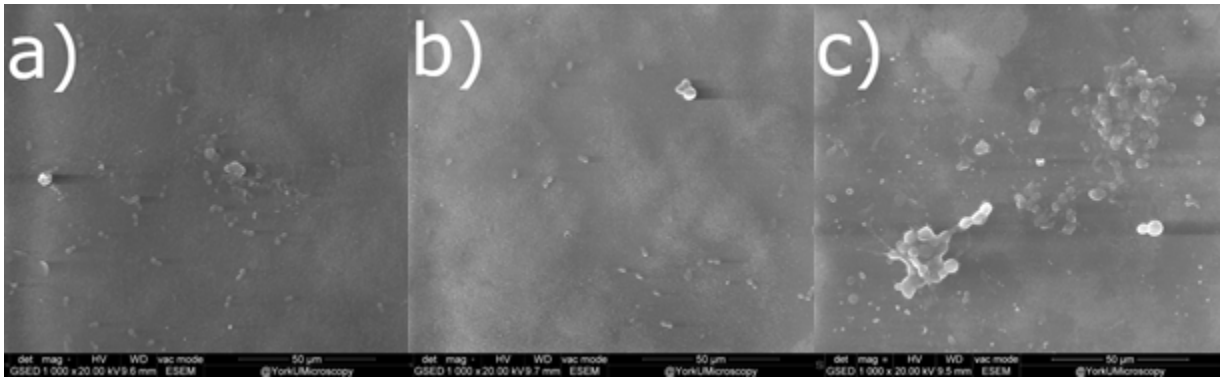

Figure 4.10. SEM micrographs of biofilm attached to MBBR media

The amounts of attached biomass per gram of biofilm carriers which have been detached using sonication, are shown in Figure 4.11. The results show that media with higher porosities ($PVDF_{90S}$ and $PVDF_{85M}$) have more attached biomass per gram of media up to 91 mgVSS/g media. Open-cell foams with higher porosities provided a larger protected surface area which increased the chance of biofilm formation. Moreover, higher interconnectivity among the cells facilitates oxygen and substrate diffusion which led to enhance biofilm formation. Due to the lower porosity of media fabricated by HDPE and available surface area, the biofilm formation was lower by $HDPE_{80M}$ compare to $PVDF_{90S}$. Moreover, as shown in Figure 4.12, the attached biomass to MBBR media was very low (8 mgVSS/g media) due the limited protected area and low surface area.

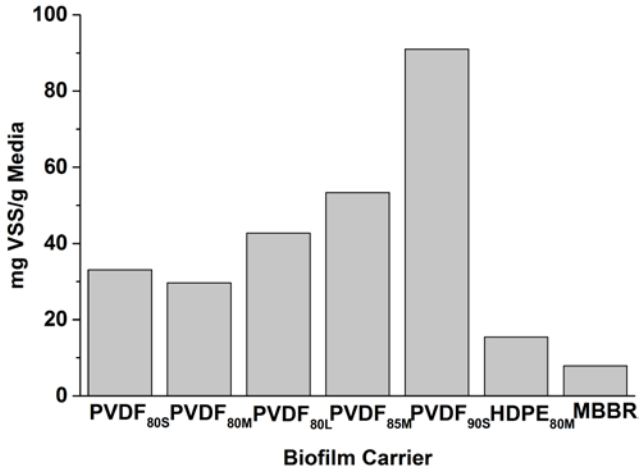

Figure 4.11. Amount of detached biomass in different biofilm carriers

Meanwhile, after 24 hrs of sonication all the open-cell foam samples were covered by biofilm which meant that sonication could not completely detach biofilm from the supporting media. This shows high ability of open-cell foams in trapping microorganisms inside their pores which diminished biofilm detachment at high shear forces in MBBRs.

4.3. Conclusions

In this study, PVDF and HDPE open-cell foams with different porosities and cell sizes were fabricated. SEM micrographs of open-cell foams showed that all PVDF carriers possessed a large number of voids and a high degree of interconnectivity among their cells. These resulted in the large specific surface area in the open-cell foams for biofilm formation. Comparing to the existing biofilm carriers in the market, open-cell foams had more than 10× to 20× of the specific surface area of the commercialized ones.

The effects of different biofilm carriers on organic removal efficiency of the bioreactors were investigated. The experimental data showed that all the open-cell foams prepared by PVDF which possessed more than 80% porosity, statistically had the same organic removal efficiency at the last

phase (enriched biofilm) of the experiment. These data showed that the amount of attached biomass to these media was not significantly different from each other to have an effect on the organic removal efficiency of the bioreactors containing them. Moreover, all the bioreactors containing PVDF carriers had much higher organic removal efficiency than both bioreactors containing HDPE carriers and MBBR media. Low attached biomass to the MBBR media explains the lower performance of the bioreactor containing these media comparing to the bioreactors filling with PVDF open-cell foams. Although the results showed that open-cell foam prepared by HDPE had low biofilm accumulation, less filling factor in the bioreactor containing these media can be a reason for the low organic removal efficiency in this bioreactor.

Moreover, SEM micrographs of attached biofilm to the media showed that biofilm carriers with higher porosity had more biofilm accumulation on their surfaces. The amount of attached biomass to the biofilm carriers which were extracted by using sonication, confirmed the results from SEM. The experimental data for the attached biomass measurement showed that the media with higher porosity had higher attached biomass. On the others side, both SEM micrographs and attached biomass measurement showed that MBBR media were not able to form a biofilm on their surfaces. High shear forces inside the bioreactors can affect low attachment density in these media (8 mgVSS/g media). Moreover, high roughness and a large number of voids inside the open-cell foams eliminated shear forces applied to the media which consequently promoted biofilm formation in these samples. Furthermore, MBBR media had significantly smaller specific surface area than open-cell foams which decreased their probability of biofilm accumulation. Due to the lower porosity of media fabricated by HDPE and presence of closed-cells in these media, available surface area for their biofilm formation was lower than other open-cell foams. As a result,

$HDPE_{80M}$ had a lower amount of attached biomass (15 mgVSS/g media) to their surfaces compared to media fabricated by PVDF.

Open-cell foams with 90 wt.% leaching agent content had up to 91 mgVSS/g media attached biomass per gram of media and were able to promote biofilm formation on their surfaces more than the other biofilm carriers with less porosities. However, media with 90% porosity did not sustain high shear forces created by the shaker in the bioreactors and was damaged over time. These media can be useful in the bioreactors which have a low shear forces environment (anoxic systems). Economically, in the MBBRs, it is a governing factor that biofilm carriers have high sustainability over time to reduce the cost of exchanging the media. In conclusion, open-cell foam with the maximum leaching agent content of 85 wt.% can be applicable in wastewater treatment plants. Moreover, at high porosities the effect of cell sizes (ranging from 106 µm to sizes larger than 500 µm) on biofilm carriers' performance in wastewater treatment is negligible.

Chapter 5
Conclusion and Recommendations

5.1. Conclusion

In recent years, considerable attention has been given towards the development of biological wastewater treatment methods especially, attached growth bioreactor systems. This research aimed to design and develop a novel biofilm carrier by using the open-cell foams as protective structures for biofilm formation. High biomass attachment to the open-cell foams can develop organic treatment process and eliminate the need for secondary clarifiers in the wastewater treatment plants.

The first phase of this research demonstrated open-cell foams feasibility as biofilm carriers to promote the biofilm growth and protection. In this phase, PVDF open-cell foams were fabricated by manufacturing approach that integrated compression molding and particulate leaching methods. SEM micrographs revealed that all open-cell foams fabricated by both leaching agent types (e.g., sodium chloride (NaCl) and sodium acetate (NaOAc)) and contents (e.g., 80 wt.% and 90 wt.%) possessed a large number of voids and a high degree of interconnectivity among them. These resulted in a large specific surface area in the open-cell foams (i.e., more than 10× to 20× of the area of commercialized carriers). High specific surface area of the open-cell foams was an advantage to facilitate microbial attachment to these media. Experimental data showed that both the leaching agent type and salt particle sizes were governing factors which effected the mechanical strength of open-cell foams. Both open-cell foams which were fabricated by NaOAc as the leaching agent could not sustain the shear forces inside the bioreactors and were completely damaged by being used in the biological process. Open-cell foams prepared by 90 wt.% NaCl were

partiality damaged in the biological process, however, those prepared by 80 wt.% NaCl had a stronger structure and remained intact during the continuous flow experiment. SEM micrographs revealed that PVDF carriers used in the continuous aerobic flow wastewater treatment experiments were completely covered by biofilm in the exterior surfaces and the core regions of foams without clogging. This provides strong evidence of the bacterial compatibility of the fabricated open-cell PVDF carriers. Moreover, porous structure of the open-cell foams assisted biofilm protection at high shear forces, while biofilm detachment is one the commercialized carriers' drawbacks.

At the second phase, 6 types of open-cell foams were fabricated by the same method as the first phase. PVDF and HDPE were used as polymer matrixes and NaCl was used as the leaching agent for the foam fabrication. Open-cell foams were used as biofilm carriers in the continuous aerobic flow wastewater treatment to investigate the effects of porosity, cell morphology, and surface properties of the open-cell foams on the sustainability of biofilm and their performances in wastewater treatment. Moreover, MBBR media were used in one of the bioreactors as the control system to compare their performance with open-cell foams.

Both SEM micrographs and the experimental data from the attached biomass measurement revealed that biofilm carriers with higher porosities had more biofilm accumulation on their surfaces. On the others side, SEM micrographs and data from measuring the attached biomass showed that MBBR media were not able to form a biofilm on their surfaces. High shear forces inside the bioreactors and significantly smaller specific surface area of the MBBR media than open-cell foams would cause low attachment density in these media. However, high roughness and a large number of voids inside the open-cell foams eliminated shear forces applied to the media which consequently promoted biofilm formation in these samples. Open-cell foams prepared by

HDPE had a lower porosity which led to a smaller available surface area for biofilm attachment. As a result, $HDPE_{80M}$ had a lower amount of attached biomass to their surfaces compared to biofilm carriers fabricated by PVDF.

At high porosities (more than 80%) the effect of cell sizes (ranging from 106 µm to sizes larger than 500 µm) on biofilm carriers' organic removal efficiency in wastewater treatment was negligible. At the last phase of continuous flow experiment, which was the biofilm enriched phase, all the bioreactors containing PVDF open-cell foams reached to more than 80% organic removal efficiency. Moreover, all the bioreactors containing PVDF carriers had much higher organic removal efficiency than both bioreactors which contained MBBR and HDPE carriers. Low attached biomass to the MBBR media explains the lower performance of the bioreactor containing these media comparing to the bioreactors filling with PVDF open-cell foams. The Bioreactor containing HDPE carriers had less filling factor than other bioreactors which beside low biofilm accumulation on these media, can explain low organic removal efficiency in this bioreactor.

Economically, biofilm carriers should have high mechanical strength to sustain shear forces inside the MBBRs over time. The experimental data showed that media with 90 wt.% leaching agent content cannot sustain high shear forces created by the shaker in the bioreactors and would be damaged over time. In conclusion, open-cell foams with the maximum leaching agent content of 85 wt.% can be applicable in wastewater treatment plants. Moreover,

5.2. Recommendation for future work

This research was focused on the application of open-cell foams as biofilm carriers to develop organic removal treatment in the attached growth systems. Open-cell foams with different cell structures may affect the biofilm formation in MBBRs. To create open-cell foams with larger

specific surface areas, one can use NaCl with smaller particles sizes. Open-cell foams fabricated by salt particle sizes in the range of 106 µm to 250 µm were successful in biofilm formation without clogging; therefore, the carriers with smaller cell sizes may be applicable as biofilm carriers. Furthermore, in the future one can prepare finer HDPE powder which increases the HDPE open-cell foams porosity and may affect their performance in wastewater treatment. Also, open-cell foams with smaller thickness can be fabricated in the future. Media with smaller thicknesses have better oxygen and substrate diffusion in their core sections comparing to the open-cell foams with larger thicknesses. This will facilitate biofilm formation in the core parts of the carriers and thereby will be more economically efficient. Moreover, for the future experimental setup, one can use larger bioreactors with higher oxygen loadings to mobilize the carriers and eliminate the shaker. Eliminating the shaker would reduce the shear forces inside the bioreactor which may affect the carriers' performance in biofilm formation.

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