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A Novel Microbial Biofilm Carrier for Wastewater Remediation

S K Al-Amshawee¹, M Y Yunus^{1,2*} and A A Azoddein¹

¹Faculty of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang, Gambang, 26300 Gambang, Pahang, Malaysia.

²Earth Resource and Sustainability Centre (ERAS), Universiti Malaysia Pahang, Gambang, 26300 Gambang, Pahang, Malaysia.

*yusri@ump.edu.my

Abstract. Biological wastewater treatment via biofilm colonies are still in their early stages of evolution. Solid carriers made of wide range of materials in different designs have been introduced to increase biofilm growth by delivering high surface area which expands microbes' attachment. It reduces 70-90% of total wastewater contamination (Based on the treatment circumstances and influent properties). In addition, it is considered a low-cost biological process and highly preferred by wastewater industries. Despite that, biofilm carriers failed to deliver a stable biotreatment. Unsteady bioremediation could occur because of using ineffective designed carrier which disturbs the microbial growth. Numerous biofilm carriers had been reviewed and mentioned in this paper like K1, AMT, BioBall, ...etc. Then, two carrier designs named as Ultra and Micro media were introduced to carry and protect biofilm and microbial colonies from being removed during the process. Its expected that the new biofilm carriers can improve moving bed biofilm reactor (MBBR) performance in terms of stability, biomass accumulation, clogging, and biofilm growth. At the end, unharmed wastewater can be discharged to the waterways or recycled back into the industry. Finally, this study suggests designing carriers having crimped surfaces to enhance the extracellular polymeric substance attachment.

1. Introduction

Every day, natural water resources suffer from releasing spectacular amounts of effluents having risky ranges of phosphorus, nitrogen, ammonia, chemical oxygen demand (COD), nitrate, and organic matters [1][2][3]. Hence, ammonia oxidizing bacteria consume oxygen, perform eutrophication and turn wastewater environment into anoxic and anaerobic which is considered fatal medium for aquatic life [4][5][6][7]. Mechanical, chemical, biological, and hybrid treatments have been proposed for pollutants removal, decomposition, and biodegradation. Currently, biotreatment or bioremediation is preferred among wastewater treatments because of being simple, ecofriendly, and cost effective [8][9][10]. Bioremediation process by microorganisms comprises from nitrification and denitrification. Nitrification process involves ammonia transformation into nitrite and nitrate via nitrite oxidizing bacteria (NOB), and ammonia oxidizing bacteria (AOB), respectively. Denitrification process uses organic compounds as electron donors to degrade ammonia to simple form (nitrogen gas) [11][12], as shown in Figure 1. Modified activated sludge processes and ponding systems have been chosen as traditional biotreatments to remove nutrients and reduce COD concentration. However, it still have



weaknesses such as biomass recycling, settling ability, retention time, ammonium nitrification, unstable performance, odors, emission of greenhouse gases, and occupying large area [13][14][15][16][17][18].

The nature of wastewater microbial cells tend them to attach and aggregate onto solid surfaces [19]. Besides, storing slow growing Nitrifiers colonies and biofilm in the bioreactor increases the bioremediation efficiency against wastewater contaminants (e.g., carbon, phosphorus, ammonia) [20][21]. Wide range of artificial solids (e.g., polyurethane, polypropylene, polyethylene) and natural solid materials like parts of plants, and stones have been investigated to colonize microbes without biomass recycling to increase solid retention and reduce required space area. Biofilm can be defined as a microbiological colony involve protozoa, fungi, and bacteria where they live together on a solid surface. These microbes produce extra cellular polymeric substances (EPS) to stabilise the microbial community and adsorb and accumulate organic and inorganic compounds (e.g., pesticides [22], chlorophenols, polyaromatic hydrocarbons, heavy metals [23]). The main components of EPS like Lipids, nucleic acids, proteins, and polysaccharides determine the metabolic activity, elasticity, strength, diffusivity, porosity, and density of a biofilm [24].

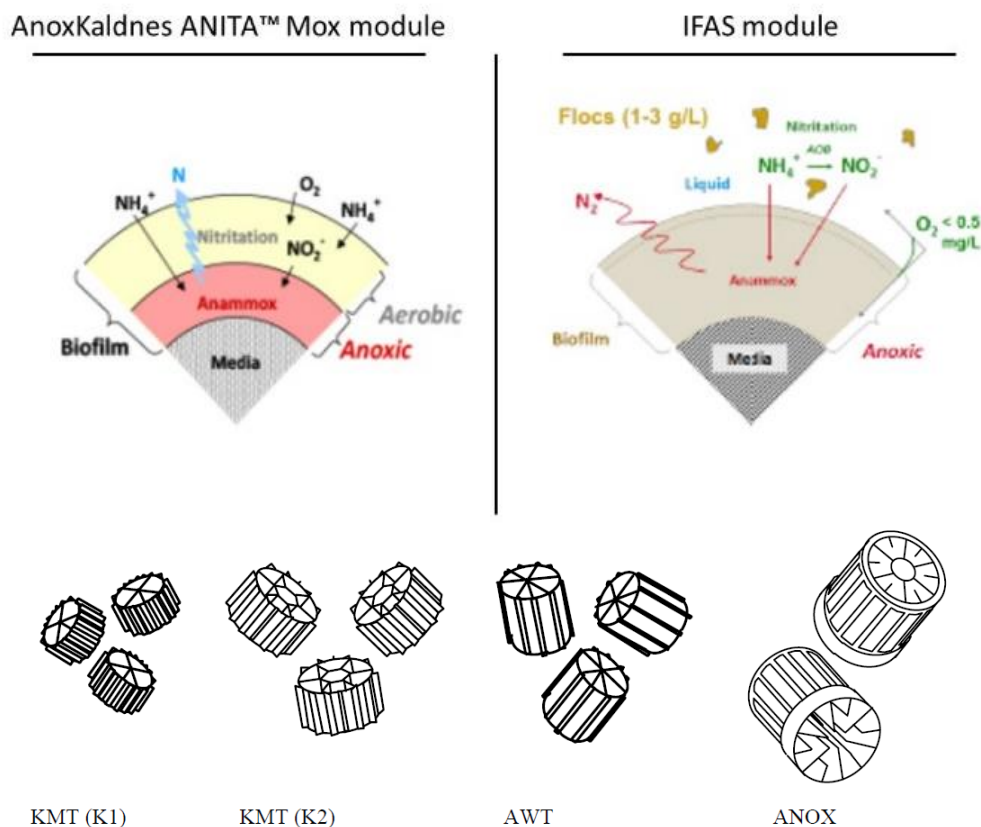


Figure 1. Illustration of biofilm growth on different carriers' surfaces. IFAS system is used for low influent concentration, while AnoxKaldnes Anita operation is employed for high strength wastewater.

Biofilms protect wastewater microbes from free radicals, UV light, and it's considered the cheapest and most used method for wastewater treatments. Since years, biofilm carriers are preferred over the activated sludge method to treat various types of wastewater (e.g., municipal wastewater, industrial wastewater). It's worth mentioning that it possesses resistance to water quality fluctuation (toxic compounds, pH, temperature), operational flexibility, good nutrients and organics removal efficiency, can manage high biomass concentration, and doesn't require filter bed channeling and low head loss or backwashing [25]. Although, wastewater microorganisms (AOB, NOB) show sensitivity to pH changes, toxic amounts, varying circumstances (aerobic, anaerobic, anoxic), temperature, biofilm carrier type,

and sun light intensity [26][27][28]. Other factors like sorption properties, percentage of the bed void, surface porosity and roughness, specific surface area, durability, and density are preferably to be analyzed and optimized.

Biofilm carriers are varied in their sizes, shapes, and materials and have been used to improve mixing between microorganisms and nutrients and enhancing oxygen transfer. It was successfully occupied for municipal wastewater, paper mill wastewater, and dairy wastewater [13][29][30]. In addition, it was integrated and involved with other methods such as suspended carrier biofilm reactors (SCBR), biological fluidized bed, aerated biofilters, fixed media reactors, rotating biological contactors (RBCs), integrated fixed film activated sludge (IFAS), and trickling filters [31][32][33][34][35]. Carrier pores clog is the main drawback of using biofilm technique. Clogging leads to backwashing, short circuit, and excess head loss. Fortunately, the main disadvantage was solved by circulating the biofilm bed such as moving bed biofilm reactor (MBBR) [17][29][36].

In the 1980s, MBBR was developed to perform as sludge process and biofilm reactor in one time for nitrogen and organic removal [17]. The MBBR acts as a house for the biofilm carriers providing the possible appropriate circumstances for mass transfer and metabolic processing of nitrogenous and carbonaceous constituents by microbes. The invention of MBBR was made to fulfill the requirements of high specific surface area for biofilm growth, low head loss, non-cloggable, and continuously operating [13]. It has been used for treating fish farms wastewater and producing potable water. It was reported that treatment efficiency is dependent on media size, shape, and specific surface area [37]. Media structure has the capability to change biofilm thickness, depth, and diffusion. Hence, using an appropriate carrier can cause turbulence which transport nutrients and dissolved oxygen to the attached colonies and retain a thin biofilm by the shear forces. Moreover, its preferred that the carrier possesses positive surface charge because the surface charge of the bacteria is negative. Other reports confirmed that the microbiology colony is negatively charged and looking to attach on cationic solid surfaces [38][39].

Researchers studied MBBR performance on nitrogen and carbon removal from urban synthetic wastewater [40]. The bioreactor was occupied for 5, and 7 hour of hydraulic retention time with 20, 30, and 40% packing rate of polyurethane foam (PUF). The study found that 20% packing rate delivers 37.4% ammonium removal, while 40% packing rate removes 96.3% at 5 h of HRT. In 2012, Martín-Pascual et al. investigated three different biofilm carriers (BIO-CONS, Kaldnes, Aqwise) inside MBBR for treating municipal wastewater. The experiment was operated under 20, 35, and 50 of filling ratio, and 5, 10, 15 h of HRT. The highest COD reduction was achieved under 15 hr of HRT, and 50% filling ratio. BIOCONS, Kaldnes, and Aqwise accomplished 46.13, 58.92, 56.97% of COD removal, respectively [41]. Another study confirms high media concentration produces denser, smoother and thinner biofilm. Thinner biofilm can possess high bacterial activity and deliver higher contaminates removal [1][11][12][14][15][16]. Nevertheless, very high filling ratio causes microorganisms detachment, and requires aeration system because of low oxygen diffusion, while low media concentration leads to low density, fluffy and rough biofilm [1][42]. It was found that 50% filling ratio is the optimum for biofilm growth, and bacterial activity with considering oxygen diffusion, while another researcher recommends using filling ratio less than 70% [37][43]. Further studies had occurred on different carrier materials such as dried-up pieces of stem of *Opuntia imbricata* cacti, strips of polyvinyl chloride sponge, natural rice husk, fireclay, coir geotextiles, fiber threads, bamboo charcoal, peach pit, and polyurethane spheres [44][45][46][47][48]. Additionally, carrier texture (surface) has major impact on the performance [33][49].

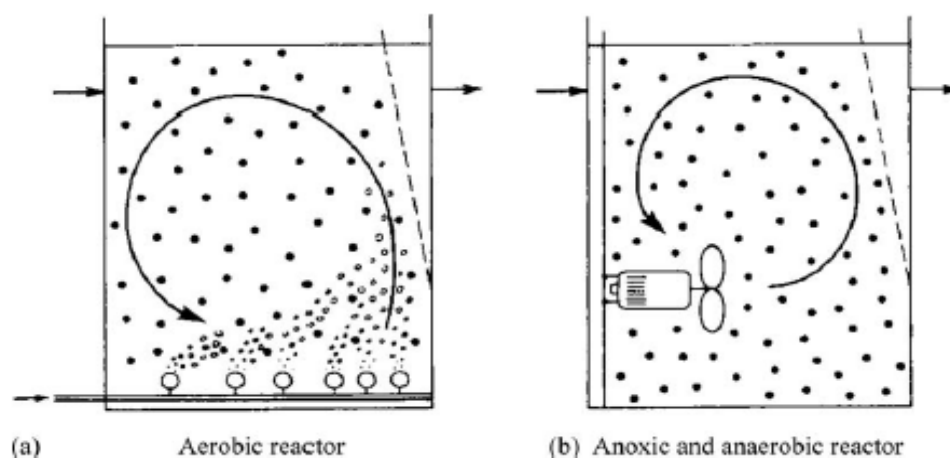


Figure 2. Drawing of moving bed biofilm reactor (MBBR) during aerobic and anaerobic conditions. Black dots represent biofilm carriers.

2. Recent studies

Waste tires are a global issue that threatens the environment because it requires large landfill and its almost non-degradable, and their improper disposal leads to spread of mosquito-borne disease. The current scrap tire recycling market (e.g., building materials, bumpers, protective cushions, roadway guard rails, rubber mats, roadway pavement material, fuel, reproduced tires) is insufficient to manage the 17 million tons of the global annual scrap tires [50][51][52]. Currently, incineration method of waste tires results in soil pollution and serious air and water contamination [53]. Therefore, it's important to promote new markets to re-use waste tires like producing biofilm carriers made of scrap tires. In 2017, Zahra Derakhshan et al. manufactured fixed bed biofilm carriers from reclaimed waste tires for wastewater treatment under different organic loading rates. The laboratory experiments showed that the carriers reduced higher amounts of sludge, COD, and total suspended solids (TSS) than having no carriers inside the sequencing batch reactor and the authors recommend apply the manufactured carriers to different wastewater applications [54].

Nacheva et al. (2008) studied seven types of carriers (polyurethane, polypropylene, cubes of polyethylene, grains of polyethylene, crushed tezontle, and ceramic spheres) placed inside continuous downflow reactors. Polyurethane carriers produced the best phosphorus and nitrogen removal, while polyethylene delivered the best COD removal [25]. In another research, 10% BioBall filling ratio was placed inside 15 L sequencing batch reactor for 72 days to treat synthetic wastewater having 8.7–12.0 of COD/N, and 32.4–54.6 of COD/TP [33]. It resulted $97.7 \pm 0.5\%$, $87.8 \pm 2.6\%$ and $94.3 \pm 1.3\%$ removal of COD, total nitrogen, and total phosphorus, respectively and achieved 96.5–99.7% of nitrification efficiency. Szilvia et al. (2013) studied biofilm growth on zeolite, Biolite™, and Perl™ in municipal wastewater in terms of nutrient removal efficiency and dehydrogenase enzyme activity (DHA) [55]. Perl™ and Biolite™ are manufactured carriers made from glass waste and ceramics. During the first 45 days, biofilms on Perl™ were very effective in removing organic matter, while Biolite™ had the best dehydrogenase enzyme activity. Moreover, the authors reported that the artificial carriers are more promising than natural zeolite for wastewater mediation. In 1990, Samuelsson and Kirchman et al. used carriers made from borosilicate glass and polyethylene to develop biofilm of *Pseudomonas* sp. strain S9 supplied with ribulose-1,5-bisphosphate carboxylase (RuBPCase) as a protein. They noticed that when the carriers are coated with the protein, more cells tended to attach, and the biofilm growth rose [56]. In 2016, Xinying Zhang et al. studied zeolite, sponge, and cermsite as biofilm carriers for domestic wastewater treatment. Proteobacteria, Bacteroidetes, Nitrospirae, Cyanobacteria and Actinobacteria had grown on the carrier surfaces. The sponge type carrier had the greatest amount of biomass due to the large surface area [57]. The findings of Xinying Zhang et al. agree

with Szilvia et al. (2013) that the zeolite type carrier doesn't show an outstanding performance towards biofilm growth or pollutants removal. Other researchers have been interested to examine zeolite because of its high ionic selectivity for NH_4^+ [58][59].

Nguyen et al. (2010) investigated different sizes of polyurethane sponges as biofilm carriers. The medium size had delivered the best pollutant removal efficiency (90% TP, 95% COD, 65% TN) and biomass growth under aerobic conditions [60]. In 2018, Chunyan Li et al. modified polyethylene carriers to have positive charged surfaces. It was used to promote bacterial adherence and biofilm growth inside a moving bed biofilm reactor. The process removed 392.6 mg/L of organic cyanide ions for 24 hours [61]. In 2012, Guozheng Li used a low temperature sintering method, the O method, to adhere TiO_2 on carriers surface made from sponge macroporous (BioCAP® purchased from Samsung Engineering Co., Ltd. Seoul, Korea) to grow biofilms at its interior while adhering photocatalyst on its exterior [62]. Furthermore, other researchers reported that high loading rate is responsible about the biofilm thickness at the carriers surfaces [1][63]. However, the ratio of high surface area to volume carriers can result in clogging which delivers poor solids settlement and low ammonia removal.

To the best of our knowledge, selection of a proper biofilm carrier is important factor because it determines the optimum biofilm thickness, biomass growth and efficiency of biodegradation. Moreover, Analysis of the microbiology community is a crucial factor because it helps to implement control strategies. Ødegaard et al. (2000) disagreed with that and reported that different designs of biofilm carrier have no effect on the MBBR performance towards COD removal, while having high surface area can manage high loading rates [37]. In 2016, Bradley Young et al. found limited studies on the effect of carrier type on MBBR performance [64]. We agree with Bradley Young et al. (2016) that the available knowledge about carrier type and design is critical and insufficient to develop and optimize MBBR system [64]. Bradley Young et al. (2016) concluded that the performance can't be predicted solely by finding biofilm mass and thickness. The scope of this study is to overcome biofilm carriers' disadvantages by finding advanced biofilm carrier. Innovative design can cooperate with wastewater microbes to process complete cycle of nitrification and denitrification. Hence, it can deliver organic and inorganic decomposition into simpler chemical shape and protects the healthy environment from H_2S , N_2O , and high strength effluents.

3. Methodology

In order to find an effective biofilm carrier design, the study had started by reviewing multiple kinds of carriers observing their material, design, specifications, and usage. Some of the carriers are presented in Figure 3 and their details are tabulated in Table 1. Auto Desk Auto Cad software was used to structure the new biofilm carrier. Important aspects have been considered during the designing step, such as thickness, texture, clogging, influent concentration, size, material, and biofilm retaining.



Figure 3. Multiple kinds of biofilm carriers.

Table 1. Kaldnes biofilm carriers' specifications.

Media	Material	Rooms number	Service life	Biofilm formation duration	Diameter vs height	Weight per M3	Valid Temp.
K1	Polyethylene	4	10 Years	5-15 days	12*9mm	120kg	-
K2	Polyethylene	4	10 Years	5-15 days	11*7mm	140kg	-
K3	Polyethylene	5	10 Years	5-15 days	11*7mm	150kg	-
K4	Polyethylene	6	10 Years	5-15 days	14.5*10mm	120kg	-
K5	Polyethylene	19	10 Years	5-15 days	20*12mm	95kg	-
K6	Polyethylene	19	>15	3-15 days	35*15mm	-	5-60°C
K7	Polyethylene	19	-	-	35*18mm	-	-
K8	Polyethylene	8	>15	3-15 days	5*10mm	-	5-60°C
K9	Polyethylene	40	>15	3-15 days	15*15mm	-	5-60°C
K10	Polyethylene	64	>15	3-15 days	25*4mm	-	5-60°C

4. Results and discussion

After two years of investigation about using an effective designed carrier for biological attachment to mediate wastewater, and gathering sufficient knowledge by reviewing numerous scientific papers, two carriers had been designed (see Figure 4). Designs are proposed after reviewing biofilm carriers like Kaldnes. It was noticed that all the reviewed biofilm carriers have weak design to protect the biofilm from being removed by turbulence. Hence, the outside surfaces of the new two carriers had been differently designed to accomplish an effective biofilm attachment protected from outside circumstances (e.g., wastewater flow, oxygen bubbles). Moreover, texture of the carrier surfaces can toughen or weaken the colonization attachment. Although, clogging made by high biofilm growth can cause inefficient treatment due low active surface area. Accordingly, two hollow cubes were designed with using Auto Desk Auto Cad software for high strength (e.g., palm oil mill effluent), and low strength wastewater (e.g., municipal), named as Ultra media and Micro media, respectively. Polyethylene or polypropylene are proposed as the main carrier material because it has 10-15 years of long lifetime and its considered lower cost in comparison to metals. Figure 4 shows the proposed designs for both high and low contaminated effluents, and the specifications are tabulated in Table 2.

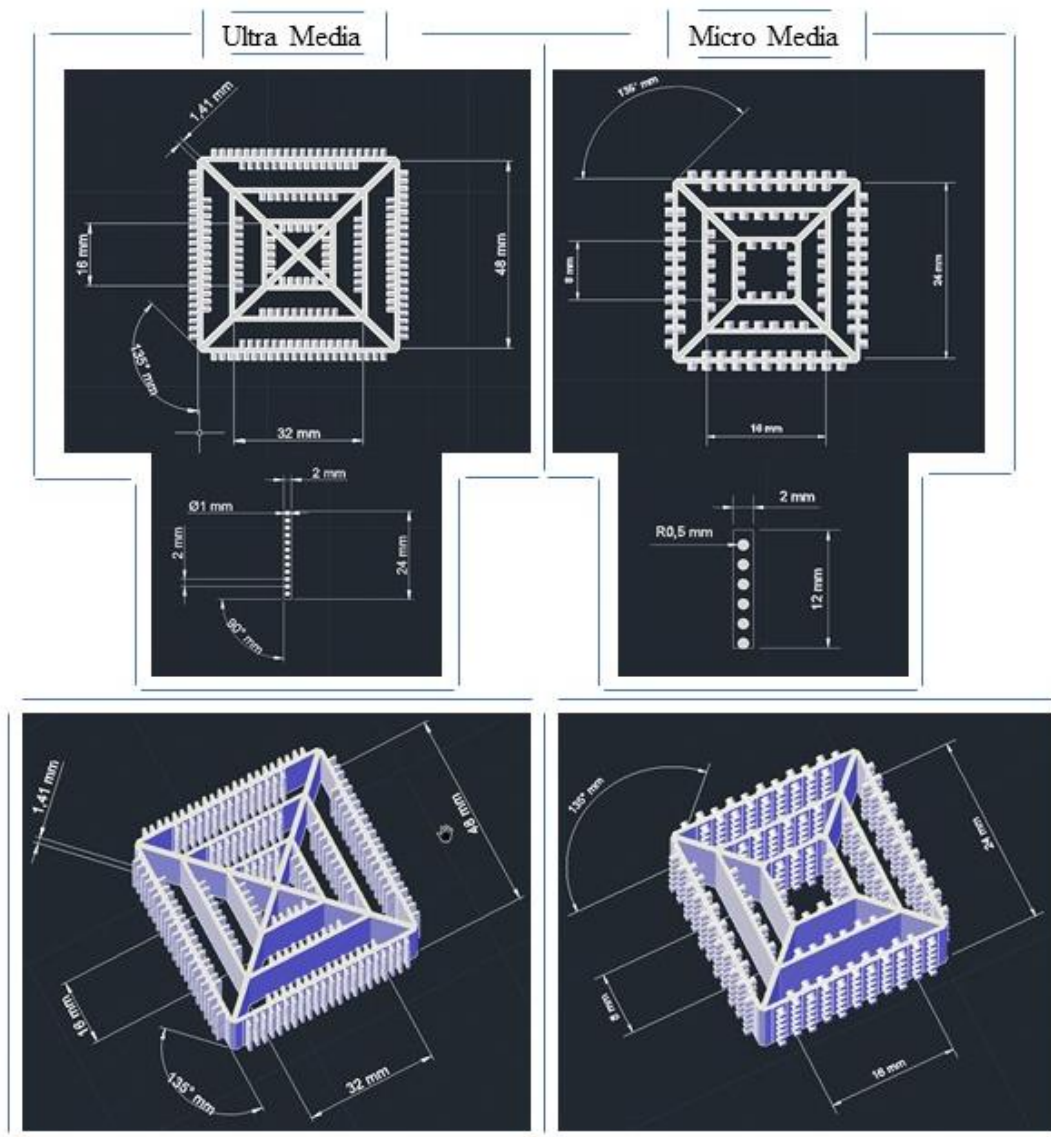
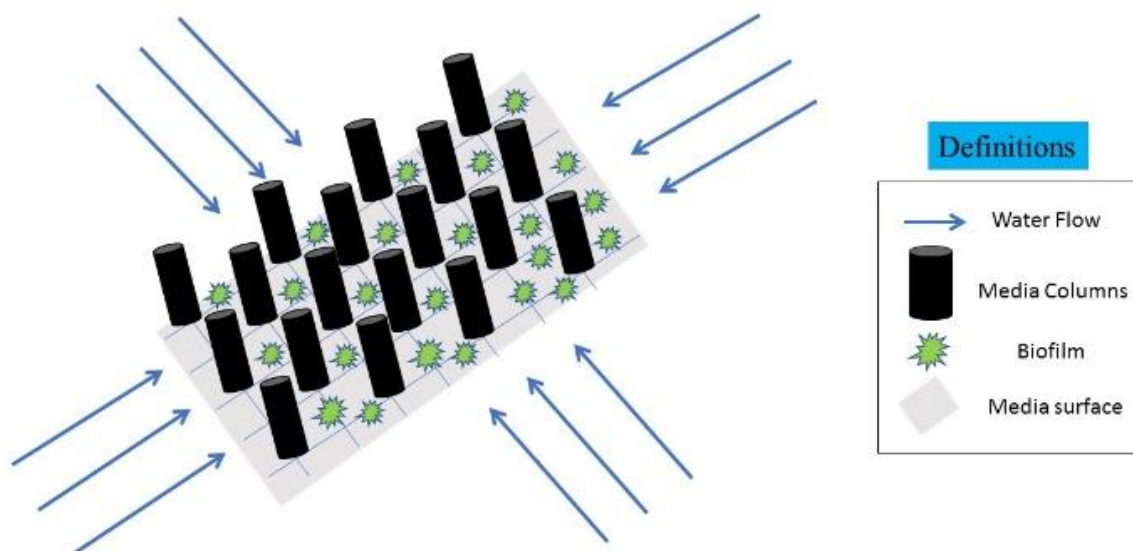


Figure 4. Structure of Ultra and Micro media.

Micro and Ultra media have number of tiny columns on their surfaces to reduce the impact of air bubbles and flow turbulence on the biofilm attachment. Therefore, carrier columns contribute in protecting the microbial colonies from removal and provide stable circumstances. Consequently, stabilization of biofilm attachment and growth removes higher ranges of COD concentration and requiring less maintenance and lower costs. Micro media has less columns than Ultra media to avoid excess biofilm formation since these media will be occupied for 10 to 15 years of wastewater bioremediation. Figure 5 demonstrates biofilm protection by carrier columns.

Table 2. Specifications of ultra and micro media.

Properties \ kind	Micro Biofilm Carrier (MBC)		Ultra Biofilm Carrier (UBC)		Units
Material	Polyethylene	Polypropylene	Polyethylene	Polypropylene	
Object surface area	168.3	168.3	583.6136	583.6136	mm ²
Column volume	0.7854	0.7854	1.5708	1.5708	mm ³
Object volume	2020.624	2020.624	14006.7264	14006.7264	mm ³
Density	0.9225	0.946	0.9225	0.946	gm/cm ³
Width	12	12	24	24	mm
Column NO. per line	6	6	12	12	columns
Weight wastewater	1.863	1.91	12.9	13.228	gm
	High strength	High strength	low strength	low strength	-
Filling ratio [1]	50-70%	50-70%	50-70%	50-70%	-

**Figure 5.** Biofilm protection mechanism.

Carriers having flat surfaces make biofilms easy to be removed because EPS is incapable from handling all kinds of outside stresses. Hence, biofilm colonies still can get destabilized and interrupted by liquid turbulent flow. So, biofilm carriers having crimped surfaces can increase stabilization and attachment of the biofilm as an effective factor with media columns to reach an optimized design for biofilm growth. Figure 6 presents possible texture for the carrier surface.

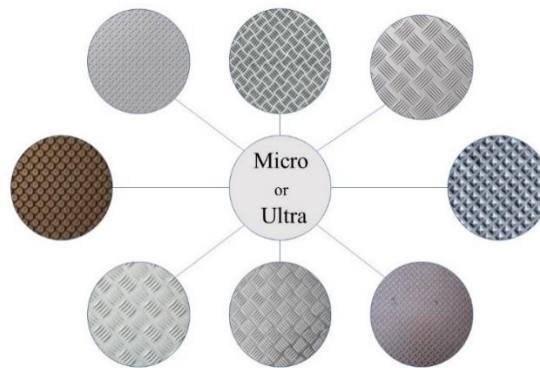


Figure 6. Carrier texture.

In comparison to the commercial biofilm carriers like Kaldnes, and Anox, Micro and Ultra media provide higher surface area for bacteria attachment and growth. Thus, wastewater bacteria increase their development by raising their colonies number and size, which significantly improves nitrification process, biodegradation, and mineralization. The manufacture of Mutag-biochip (biofilm carrier) had published an article discussing about choosing a suitable biofilm carrier. First of all, choosing a biofilm carrier is difficult because disadvantages are not well recognized [65]. Second, the properties of influent and effluent, contamination range, and operating temperature should be well known. Third, carrier design come with efficiencies (e.g., COD and Biochemical Oxygen Demand BOD removal) studied at different conditions that guide researchers and buyers to select a proper biofilm carrier. Despite that, studies and carrier manufactures have focused on developing biofilms in the interior of the carriers, while our study focused on all the surfaces (see Figure 7).



Figure 7. Shown biofilm growth at the inside layer of a commercial carrier [65].

Ultra and Micro media are expected to perform higher performance than the commercial biofilm carriers in process stability, continuous microbial evolution, no clogging, lower HRT, less biomass accumulation, larger biofilm growth, and possibly complete nitrification and denitrification. At the end, biodegraded, mineralized, and unarmful wastewater can be discharged to the waterways or recycled back into the industry.

5. Conclusion

Wastewater industries suffer from wastewater biotreatments because it's unstable process. Despite that, they are still using it because it's cheap compared to chemical and physical methods. Biofilm carriers

are essential parts of the MBBR to provide high surface area and stabilize the treatment performance. Its efficacy is showed by lasting for more than 10 years and delivering acceptable quality of treatment. Micro and Ultra Media are promising new biofilm carriers could achieve higher COD, BOD, TSS, mixed liquor suspended solids (MLSS), and ammonia removal than usual carrier. Their remarkable design is expected to deliver treatment stability, safe circumstances, and tougher biofilm attachment. Thus, slow growing colonies have sufficient time and safe environment to build their complex colonies, and implement nitrification, and denitrification processes.

6. Future trends and recommendations

Its predicated that biological treatments can lead wastewater mediation in the near future. It can produce valuable products like biomass and biogas. In addition, zero waste system is achievable and applicable on wastewater biotreatments. Thus, it can secure the healthy life from contamination, microbes' evolution (superbugs), climate destabilization (global warming), gas emission (N₂O, methane, CO₂, H₂S), and soil clogging (oxygen depletion). Although, biological treatments are still requiring research and development. Some tips are listed to advance wastewater treatment using biofilm carriers.

- Apply oxygen feed to accomplish nitrification (Oxygen feed should be calculated based on DO, and COD level).
- Slow oxygen feed (reaching saturation phase within 1-3 days).
- Use biofilm media that have curly surfaces.
- Employ closed system to stabilize the treatment climate (Temperature 35-40 °C).
- Utilize moving bed to increase bacterial contact with water contaminants.

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