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### Experimental Evaluation of Bio-Carrier Morphology for Improved Agricultural Wastewater Treatment in Attached Growth Systems

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#### ARTICLE INFO

#### ABSTRACT

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#### 1. Main text

The demand for efficient, economical, and sustainable solutions is an ongoing endeavor. An emerging avenue in this field is the adoption of attached growth systems [1], marking a departure from conventional methods [2], [3] by leveraging the capabilities of microbial communities immobilized on diverse substrates. These systems present a versatile approach to addressing a wide array of wastewater treatment challenges, spanning from municipal sewage to industrial effluents, and agricultural runoff [4].

Attached growth systems [5], also known as biofilm reactors, exploit microorganisms' intrinsic ability to

In the field of wastewater treatment, the need for efficient and sustainable solutions is perpetual. This study delves into the efficacy of attached growth systems, a burgeoning approach that capitalizes on microbial communities immobilized on various substrates, in addressing diverse wastewater treatment challenges. Biofilm reactors natural tendency to form structured communities on solid surfaces. By investigating the geometry of biocarriers in these systems, this study evaluates the factors influencing treatment efficiency. We employed experimental trials, focusing on media length and surface area, utilizing highdensity polyethylene (HDPE) tubing sections of varying dimensions. Results indicate that perforated biocarriers outperform hollow biocarriers, showcasing superior organic matter removal efficiencies, attributed to their larger surface area and enhanced mass transfer capabilities. Additionally, biofilm growth was found to correlate directly with surface area, as greater attachment sites foster denser and more resilient biofilms. The concentration of microbial activity towards the edges of the media suggests their pivotal role in nutrient removal. Overall, this study underscores the significance of biocarrier geometry in enhancing treatment performance and advancing the sustainability of attached growth-based wastewater treatment processes.

> form organized communities on solid surfaces. Unlike suspended growth systems [1] where microorganisms remain in suspension, attached growth systems promote the development of biofilms on specially engineered supports. This approach offers numerous benefits, including heightened treatment efficiency, increased biomass retention, and enhanced resilience to shocks and operational variations. Within attached growth systems, the biocarrier assumes a pivotal role in providing a substrate for microbial attachment and biofilm formation [6], [7].

> Biocarriers typically consist of inert materials boasting high surface area-to-volume ratios, designed to support microbial growth while facilitating

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efficient wastewater treatment processes. These carriers come in diverse shapes, sizes, and compositions, each tailored to optimize microbial colonization, substrate utilization, and treatment efficacy [8].

A primary function of biocarriers is to foster an environment conducive to microbial attachment and biofilm development. The surface characteristics of biocarriers, encompassing factors such as texture, roughness, and porosity, influence initial microbial adhesion and subsequent biofilm formation [9]. Additionally, structural attributes such as pore size distribution and interconnectivity govern the diffusion of essential nutrients, oxygen, and metabolic byproducts within the biofilm matrix, critical for sustaining microbial activity and overall treatment performance. Biocarriers commonly employ materials like plastic media [8], [10] (e.g., polyethylene, polypropylene, PVC) [11] and natural substances (e.g., gravel, rocks, ceramics) [12], offering advantages such as mechanical robustness, chemical inertness, and prolonged service life, rendering them suitable for various wastewater treatment applications.

The selection of biocarriers is contingent upon several factors. including specific treatment objectives, system hydraulics, microbial requirements, and cost considerations [6], [13]. Engineers and researchers continuously explore innovative biocarrier designs and materials to enhance performance and address emerging challenges in wastewater treatment, such as the removal of emerging contaminants and the mitigation of fouling and biofilm detachment [14], [15].

In wastewater treatment technologies such as Moving Bed Biofilm Reactors (MBBRs)[16], [17], the presence of floating media in the aeration zone offer an additional advantage represented in the need absence of recycling activated sludge in to the aeration tank due to the retention of biofilm on freefloating media [18], [19] as shown in Figure (1).



#### Fig. 1: Difference between activated sludge process and Moving bed biofilm reactors

The geometry of biocarriers in attached growth systems significantly impacts wastewater treatment efficiency [9], [20]. Parameters like surface area, porosity, interconnectivity, shape, arrangement, and material properties play crucial roles. Biocarriers with larger surface areas and higher porosity facilitate better microbial attachment and nutrient diffusion, leading to improved pollutant removal. Wellconnected pores ensure uniform flow distribution, minimizing channeling risks. Biocarrier shape and arrangement influence flow patterns and biofilm distribution, while material properties affect adhesion and durability. Optimization of biocarrier geometry enhances treatment performance, achieving better pollutant removal and system reliability [8], [20]. Figure (2) shows different geometries of biocarriers used in attached growth systems.



Fig. 2: MBBRs carriers

Overall, biocarriers play a crucial role in attached growth systems by providing a platform for microbial attachment, biofilm formation, and efficient pollutant removal. Their design, characteristics, and optimization are integral to maximizing the effectiveness and sustainability of attached growthbased wastewater treatment processes.

#### 2. Methodology

#### 3. 2.1. Agricultural Wastewater Collection Samples

Agricultural wastewater was gathered from Abu al-Akhdar drain located in Sharqia Governorate as shown in Figure (3), Egypt. A plastic container was employed to procure the agricultural wastewater sample, which was filled via a plastic vessel. These containers were readied for transportation to the workplace for subsequent treatment of the collected samples. A total of 25 plastic containers, each with a capacity of 20 liters, were utilized for sample collection. Table (1) shows the physiochemical characteristics of agricultural wastewater collected from Abu al-Akhdar drain



Fig. 3 Wastewater Samples Collection from Abu al-Akhdar drain

Parameters	Units	Average Values	Standard Deviation
Chemical oxygen demand (COD)	mg/l	403.85	21.45
Biological oxygen demand (BOD)	mg/l	182.45	10.9
PH		7.7	
Total Suspended Solids (TSS)	mg/l	132.875	8.78
Total Kjeldahl Nitrogen (TKN)	mg/l	78.25	3
Total phosphorus (TP)	mg/l	36.84	2.97

#### 2.2. Experimental Model Setup

A mini batch model of 15 liters volume for each tank was carried out in the laboratory of environmental engineering, Faculty of engineering, Zagazig University to perform four experimental trials based on the variation of bio-carrier geometry. The model used in this study represents the simulation of MBBRs as wastewater passes first through a primary sedimentation tank, then the aeration reactor, and finally settled in the final settling tank as shown in Figure (4). Biocarriers packed in the reactors at each laboratory run was of a filling ratio up to 30 %. The reactor was equipped with an air blower with coarse bubbles to enable the media to rotate and mix within the reactor in order to form the biofilm required for the organic matter degradation. wastewater was remained in the MBBR reactor for 24 hours to give the microorganisms especially the slow-growing nitrifying bacteria the required time for organic matter, and nutrients removal.



Aeration Tank

Fig. 4: Experimental Model Setup

#### 2.3. Media Preparation

To investigate the impact of geometry of biocarrier such media length and surface area on biofilm growth and activity, a specialized media was created by cutting high-density polyethylene (HDPE) tubing into sections measuring 5 mm representing short media and 20 mm representing long media respectively as shown in Figure (5, a). The raw material used in MBBR media from Al-Andulas Bimex company , located in Al-Obour city, Cairo, Egypt. Besides investigation the effect of media length, also the surface area was studied by using the previous media (Short and long ones) with different media geometry as shown in Figure (5,b). Mostafa E. Y. Ahmed, et.al / Experimental Evaluation of Bio-Carrier Morphology for Improved Agricultural Wastewater Treatment in Attached Growth Systems



Fig. 5: MBBRs Biocarriers

Each reactor was equipped with media of a filling ratios (30%). In Multistage Biofilm Reactor (MBBR) systems, it is customary to consider the internal surface area as the biologically active area, as the external surfaces are subject to scouring by collisions with other media. The specific biologically active surface area in each reactor was determined as shown in Table (2). Also, Figure (6) represents a flow chart of the experimental trials

Table 2: The biocarriers characteristics

Parameters	Hollow Bio- carrier	Perforated Bio-carrier
Material	HDPE	HDPE
Outer Diameter	8 mm	8 mm
Inner diameter	6 mm	
Voids Ratio	75 %	50 %
Surface Area (m <sup>2</sup> /m <sup>3</sup> )	0.41	0.44



Fig. 6: Flow Chart of laboratory work

#### 2.4. Steps of Experimental Work

The experimental procedure involved the separate inoculation of both carrier types in 10 L of fresh activated sludge obtained from a nearby wastewater treatment plant, maintained at room temperature with coarse-bubble mixing for a duration of 3 days. Subsequently, following the inoculation period, the media solution within each Moving Bed Biofilm Reactor (MBBR) was substituted with 5 L of settled activated sludge and 10 L of fresh primary effluent, with the continuous feed being introduced to the Plug-Flow Sequencing Batch Reactor (PST). To determine biofilm growth on the reactor walls, daily brushing was conducted, with each reactor utilizing a coarse bubble aerator. Various experimental trials were conducted, each with a distinct filling ratio as detailed in Table 3, employing both bio-carriers. Post-treatment stages, wastewater samples were collected physicochemical to analyze their characteristics.

Table 3: List of experimental Trials

Trial serial	Description
Trial (1)	Short Hollow Media
Trial (2)	Long Hollow Media
Trial (3)	Short Perforated Media
Trial (4)	Long Perforated Media

#### 4. Results and Discussion

## 4.1. Performance of Media length on ammonia (NH4) Uptake, and Nitrate Removal

Both media configurations (Long & Short) exhibited comparable levels of ammonia uptake, calculated as the difference between influent and effluent ammonia concentrations, throughout the duration of the study, as illustrated in Figure 7 & 8. However, the Long media consistently demonstrated higher nitrate concentrations and lower nitrite concentrations compared to the Short one, particularly evident from approximately days 50 to 100.

Additionally, during periods of 50 to 100 days, the long media achieved greater ammonia uptake, indicating a potentially more active or tolerant community of Ammonia-Oxidizing Bacteria (AOB) than the short reactor. By day 100 of continuous operation, both Moving Bed Biofilm Reactors (MBBRs) achieved maximum ammonia uptake rates of approximately 180-190 mg NH4-N/L. Furthermore, after 100 days, the ammonia uptake, as well as nitrite and nitrate production, reached steady states and exhibited minimal differences between the two reactors.



Fig. 7: shows the concentrations of effluent ammonia for both Long and short Hollow Media.



Fig. 8: shows the ammonia Uptake for both Long and short Hollow Media.

# 4.2. Effect of Biocarrier Geometry on the Biofilm Growth

The growth of biocarrier growth was measured by volatile biofilm solids (VBS) concentrations localized on the biocarrier. From the experimental observations, it was clear that the more biocarrier length, the more VBS concentrations as illustrated in Figure (9). The long hollow biocarrier achieved VBS concentrations of 184 mg/L, although short hollow biocarrier achieved 75 mg/L. Also, the effect of geometry of biocarrier such as the surface area of

media affect mainly the biofilm growth. As a result, the perforated media which has a surface area of 0.44  $m^2/m^3$  achieved a higher VBS concentrations than Hollow media that has a surface area of 0.41  $m^2/m^3$ .



Fig. 9: VBS concentrations for Hollow, and Perforated biocarriers

Based on these results, it was clear that the increase in surface area promotes enhanced biofilm growth due to the availability of additional attachment sites for microbial colonization [21]. As the surface area increases, more microorganisms can adhere to the substrate, forming a denser and more robust biofilm. This phenomenon occurs because microorganisms require a solid surface to attach to and grow, and a greater surface area provides more opportunities for attachment [22]. Additionally, increased surface area allows for greater retention of nutrients and metabolic by products within the biofilm, creating a favourable environment for microbial growth and activity. Therefore, an increase in surface area directly correlates with an increase in biofilm growth by facilitating greater microbial attachment, nutrient availability, and metabolic activity within the biofilm structure [23].

## 4.3. Performance of Surface area on Organic matter removal

As shown in Figure (10) and Table (4), a removal efficiency comparison between the four MBBR biocarrier (Short-Hollow, Long-Hollow, Short-Perforated, and Long-Perforated) was performed in terms of COD, BOD, TKN, and TP concentrations after each experimental trial.

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# Fig. 10: Removal efficiency of each Media configuration

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	Parameters	Units	RAW	P.S.T	A.T	F.S.T
	COD	mg/l	428	298	174	115.90
	BOD	mg/l	192	124	64.5	47.20
Short	PH		7.7	7.7	8.1	8.00
Hollow	TSS	mg/l	144	48	28.8	18.70
	TKN	mg/l	80	57.7	40.3	22.30
	TP	mg/l	37	28.3	22	12.80
	COD	mg/l	412	281	152	105.7
	BOD	mg/l	167.2	122	57.4	43.00
Long	PH		7.7	7.7	8.1	8.10
Hollow	TSS	mg/l	123.2	42.6	26.2	15.80
	TKN	mg/l	74.4	58.3	38.9	21.50
	TP	mg/l	38.56	28.15	21.4	9.90
Long Perforated	COD	mg/l	398	302	137.3	100.3
	BOD	mg/l	188.3	127.2	51.9	42.40
	PH		7.7	7.7	7.8	7.90
	TSS	mg/l	134.7	51	24.6	18.20
	TKN	mg/l	77.4	52.3	36.4	19.70
	TP	mg/l	39.2	26.9	20.73	9.30
Short Perforated	COD	mg/l	377.4	297.3	158.4	108.85
	BOD	mg/l	182.3	122.6	61.9	44.30
	PH		7.7	7.7	7.9	7.80
	TSS	mg/l	129.6	48.8	27.5	18.50
	TKN	mg/l	81.2	58.3	38.4	21.60
	TP	mg/l	32.6	28.1	21.4	9.82

Perforated biocarriers outperform hollow biocarriers in removing organic matter, like COD and BOD, due to several key reasons. Firstly, the presence of perforations in biocarriers offers a larger surface area for microbes to attach to, promoting the formation of a robust biofilm and increasing microbial activity [24]. This allows for more efficient breakdown and utilization of organic matter in the wastewater. Moreover, perforated biocarriers facilitate better movement of oxygen, nutrients, and waste products within the biofilm, optimizing microbial metabolism and substrate utilization [25].

The hydraulic properties of perforated biocarriers ensure even flow distribution, reducing the risk of uneven flow patterns and ensuring effective contact between the wastewater and the biofilm. Additionally, the structure of perforated biocarriers encourages the development of stable biofilms less prone to detachment, ensuring sustained microbial activity and efficient removal of organic matter.

Overall, the combination of increased surface area, enhanced mass transfer, improved hydraulic properties, and biofilm stability in perforated biocarriers contributes to their superior performance in organic matter removal compared to hollow biocarriers. Furthermore, the concentration of microbial activity towards the ends of the media, particularly in thicker edge biofilms, suggests that these areas are predominantly responsible for nutrient removal. While the activity of ammonia-oxidizing bacteria is evenly distributed along the length of the longer media, nitrate-oxidizing bacteria activity is more significant in the edge biofilm, although the interior biofilm still exhibits comparable activity rates [25].

#### 5. Reusability of Plastic media

The resilience and durability of plastic media enables it to endure the demanding conditions typically encountered in wastewater treatment processes, including fluctuations in temperature, pH, and exposure chemicals. Regular maintenance to practices, such as routine cleaning to prevent fouling and the accumulation of biofilm, can prolong the lifespan of plastic media and ensure optimal performance of the attached growth system. Previous studies have assessed the durability of different types of plastic media in a Moving Bed Biofilm Reactor (MBBR) system over a span of 10 years. Despite some degradation in physical properties over time, researchers observed that the plastic media remained functional and proficient for microbial attachment and biofilm formation throughout the duration of the study.

Once its lifespan, which can extend beyond 10 years, is reached, plastic media can be recycled. Recycling plastic media involves repurposing it for secondary treatment, experimental research, composite material production, and local initiatives. Through the reuse of plastic media at various stages of the treatment process and collaboration with recycling facilities, wastewater treatment plants can mitigate waste generation and advance sustainability efforts.

#### 6. Conclusion

In conclusion, the experimental study on bio-carrier treatment geometry's impact on wastewater efficiency highlights the crucial role of perforated biocarriers in enhancing organic matter removal. Through careful investigation of biocarrier shape and surface area, we found that perforated biocarriers outperformed hollow ones, attributed to their larger surface area and improved mass transfer capabilities. Moreover, the direct correlation between biofilm growth and surface area underscores the importance of optimizing carrier geometry for maximizing treatment performance. The concentration of microbial activity towards the edges of the media further emphasizes the significance of biocarrier design in facilitating nutrient removal. plastic media used in attached growth systems for wastewater treatment offer high reusability and long lifespan when properly maintained. Research studies and practical experience highlight the durability and effectiveness of plastic media in facilitating microbial attachment and biofilm formation, making them a valuable component of wastewater treatment processes.

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#### **Conflict of interest**

The authors declare that they have no conflicts of interest regarding the publication of this paper.

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